

**ENDOPARASITES OF WORKING DONKEYS IN ETHIOPIA:
EPIDEMIOLOGICAL STUDY AND MATHEMATICAL
MODELLING.**

BY

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ABSTRACT

Gastrointestinal parasites have been found to be the major contributors to the short life span of working donkeys in developing countries. However, epidemiological studies of donkey parasites in the world in general and in Ethiopia, in particular, are fragmentary and not readily available. Given this paucity of information, the present work is aimed at the study of gastrointestinal parasites of working donkeys of Ethiopia with particular reference to the cyathostomins and the non-strongyles: cestodes, trematodes, ascarids and gasterophilids, using various epidemiological techniques: coprology, post-mortem examination, immuno-diagnosis and mathematical modelling.

A total of 803 and 797 donkeys from four different geographical regions, and 112 donkeys were examined by coprology, serology and post-mortem, respectively. Both coprological survey and post-mortem findings have shown not only high infection prevalences but also the infection of donkeys with multiple helminths and arthropods. The finding of high sero-prevalence of cestode infection, which is consistent with the results of coprological and post-mortem findings clearly indicates that cestodosis is one of the major parasitic problems in the donkey population of Ethiopia. The potential pathogenicity of cestodes, ascarid, flukes and gastrophilus larvae, and their effects on working performance and reproduction of donkeys are described.

Regional variation of infection prevalences of the studied parasites was observed; in particular cestode and fluke infections. The highland region of Bereh had the highest risk of cestodosis and fasciolosis compared to the other regions. This significant variation indicates that general agro-ecological differences exist that are important for the development and survival of parasites and their intermediate hosts.

The high efficacy of praziquantel (Equitape, Fort Dodge) was also demonstrated in reducing cestode faecal egg counts and serum antibody levels.

A mathematical approach was used to model seasonal variation of cyathostomin faecal egg output, and in simulating anthelmintic control strategy. The model is based on parameters of biological development of cyathostomins and climatic data. A good fit of the model prediction to the field data was obtained after some parameter adjustments. The development rate of ingested larvae to egg laying adults, survival time of adults and the assumption made in modelling the peak pasture larval availability were the main driving forces for the model prediction to fit to the observed data. The apparent fit of the model prediction to the field data obtained after parameter adjustment generally indicated some major differences between donkeys and horse in their reaction to the parasite and /or between cyathostomins of donkeys and horses. The results of the simulation of the effect of various protocols for the timing and frequency of anthelmintic treatment on the adult cyathostomin worm burden have shown that treating donkeys only once in a year or a combination of once in a year followed by every two or even four years can substantially reduces and maintains the parasite burden far below the pre-treatment level for many years.

Generally the study made has revealed that the non-strongyle gastrointestinal parasites of donkeys are highly prevalent and have a high pathogenic potential, and the findings of cestodes and trematodes are not accidental or unusual, as previously suggested.

DECLARATION

I, Getachew Mulugeta, declare that the work presented in this thesis is original and has not been submitted for another degree. The work is carried out by the author, except where collaboration and assistance with others has been acknowledged.

Getachew Mulugeta

Parts of this thesis have been published or accepted for publication elsewhere:

Abstracts

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TABLE OF CONTENTS

ABSTRACT II

DECLARATION III

ACKNOWLEDGMENTS IV

LIST OF TABLES XI

LIST OF FIGURES.....XIV

LIST OF ABBREVIATIONSXVII

CHAPTER ONE 1

GENERAL INTRODUCTION AND LITERATURE REVIEW..... 1

1.1. Introduction.....1

1.2 General objectives of the study4

1.3. Health and management problems of working donkeys5

1.4 Gastrointestinal parasites of working donkeys7

 1.4.1. Strongyles (Strongylidae) 11

 1.4.1.1. Large strongyles..... 11

 1.4.1.2. Cyathostomins (small strongyles)..... 18

 1.4.2. Gasterophilids.24

 1.4.3. Equine ascariasis26

 1.4.3.1 Ascarid infection in donkeys29

 1.4.4. Equine tapeworms.....31

 1.4.4.1 Diagnosis of cestode infection in equids35

 1.4.4.2 Cestodes infection in donkeys37

 1.4.5. *Fasciola*.....38

 1.4.5.1. Fasciolosis in Ethiopia41

 1.4.5.2. Equine trematodes.....41

1.5 Mathematical modelling.....45

 1.5.1 Types of mathematical models47

 1.5.2 Mathematical modelling in veterinary medicine.50

 1.5.2.1 Mathematical modelling in veterinary parasitology.51

 1.5.2.2 Mathematical modelling in equine health problems53

 1.5.2.3. Mathematical modelling in equine gastrointestinal parasites54

1.6. Population dynamics of equine strongyles.....55

1.6.1 Seasonal pattern of egg hatching and larval development of equine strongyles	56
1.6.2 Longevity or survival of infective larvae of strongyles on pasture	59
1.6.3 Larval translations and pasture larval recovery	61
1.6.4 Larval distribution and ingestion rate of infective strongyle larvae	63
1.6.5 Establishment of infective larvae in the host	65
1.6.6 The pre-patent period (PPP) of cyathostomins	66
1.6.7 Fecundity of equine cyathostomins	67
1.6.8 Cyathostomin burden of horses and donkeys	69
1.6.9 Life expectancy or longevity of adult cyathostomins	70
CHAPTER TWO.....	72
GENERAL MATERIALS AND METHODS.....	72
2.1 Study country- Ethiopia	72
2.2 Description of the study regions	74
2.3. Study animals	79
2.4 Sample size	79
2.5 Sampling procedures	81
2.5.1 Peasant associations (PAs).....	81
2.5.2 Donkeys	81
2.6. Data management and analysis	82
2.7 Faecal sample collection and laboratory processing	83
2.7.1. Cestodes	83
2.7.2. Trematodes (<i>Fasciola</i> and <i>Gastrodiscus</i>)	85
2.7.3. <i>Parascaris equorum</i>	86
2.8. Retrospective data on post mortem worm recovery and identification	87
2.8.1. Gasterophilus larvae.....	88
2.8.1.1. <i>Gastrophilus</i> larval identification	88
2.8.2. <i>Parascaris equorum</i> , <i>Anoplocephala magna</i> and <i>Anoplocephaloides mamillana</i> ...	88
2.8.3. <i>Anoplocephala perfoliata</i> and <i>Gastrodiscus aegyptiacus</i>	89
2.8.4. Trematodes (<i>Fasciola</i> spp).....	89
2.9 Enzyme-linked immunosorbent assay (ELISA) for the detection of antibody to <i>A. perfoliata</i> in donkey sera.	90
2.9.1 Antigen.....	90
2.9.2 Serum collection and ELISA	90
2.9.3. Reagent recipes used for the tapeworm ELISA (Diagnosteq, equine division, University of Liverpool):	91
2.10. Determining efficacy of praziquantel against cestode and assessing serum antibody level of <i>A. perfoliata</i> after treatment.	92

CHAPTER THREE	94
CROSS-SECTIONAL COPROLOGICAL SURVEY OF CESTODE, TREMATODE AND ASCARID INFECTION IN DONKEYS, ETHIOPIA.	94
3.1. Introduction	94
3.2. Materials and methods.....	95
3.2.2. Data analysis	96
3.3. Results.....	97
3.3.1 Cestodes	97
3.3.2. <i>Parascaris equorum</i>	102
3.3.3. Trematodes.....	104
3.4. Discussion	113
3.4.1. Cestodes	113
3.4.2. <i>Parascaris equorum</i>	118
3.4.3 <i>Fasciola</i>	122
3.4.4 <i>Gastrodiscus</i>	125
3.4.5 <i>Ascaris</i> and <i>Trichuris</i>	126
3.4.6 <i>Oxyuris equi</i>	127
CHAPTER FOUR.....	129
THE PREVALENCE AND ABUNDANCE OF GASTROINTESTINAL PARASITES OF WORKING DONKEYS IN ETHIOPIA: A RETROSPECTIVE STUDY OF CESTODES, TREMATODES, ASCARIDS AND ARTHROPODS.....	129
4.1 Introduction	129
4.2. Materials and methods.....	130
4.2.1. Animals	130
4.2.2. Statistical analysis	132
4.3 Results	132
4.3.1. Anoplocephalids.	133
4.3.2 Ascarids.....	135
4.3.3 Fasciolids	136
4.3.4. Paramphistomatids	139
4.3.5. Gasterophilids (bot larvae).....	140
4.4 Discussion.....	143
4.4.1 Anoplocephalids	144
4.4.2. Ascarids.....	145
4.4.3 Fasciolids	147

4.4.4 Paramphistomatids	151
4.4.5. Gasterophilids	153
4.4.6. Overview.....	157
CHAPTER FIVE	159
A SERO-EPIDEMIOLOGICAL STUDY OF EQUINE CESTODOSIS	159
5.1 Introduction	159
5.2. Materials and Methods.....	160
5.2.1 Animals	160
5.2.2 Blood sample collection and preparation of sera	161
5.2.3 Enzyme-linked immunosorbent assay (ELISA)	161
5.2.4 Interpretation of the results	162
5.2.5 Statistical analysis	163
5.3. Results.....	164
5.3.1. Serological responses.....	164
5.3.2. Relationship between serological and coprological assays	166
5.3.3 Age dependency of serologically-determined infection intensity	167
5.3.4 Temporal variation in serological responses.....	168
5.4 Discussion.....	169
5.4.1 Serological responses.....	169
5.4.2. Relationship between serological and coprological assay	171
5.4.3 Age dependency of serologically-determined infection intensity	173
5.4.4 Temporal variation in antibody response.....	175
CHAPTER 6	178
FIELD EFFICACY OF PRAZIQUANTEL ORAL PASTE AGAINST	
NATURALLY ACQUIRED CESTODES OF DONKEYS IN ETHIOPIA.	
.....	178
6.1 Introduction	178
6.2. Materials and methods.....	179
6.2.1 Animals	179
6.2.2 Faecal and blood sample collection and analysis	180
6.2.3 Statistical analysis	181
6.3. Results.....	181
6.4 Discussion.....	186
CHAPTER SEVEN.....	190

MATHEMATICAL APPROACH IN MODELLING SEASONAL VARIATION OF STRONGYLE FAECAL EGG COUNTS UNDER TROPICAL WEATHER CONDITION IN DONKEYS, ETHIOPIA. 190

7.1. Introduction 190

7.1.1. Basic life cycle of cyathostomin 191

7.2 Parameters, their definition and functional forms used in the model. 194

7.2.1 Development of eggs to infective larvae (L3) 196

7.2.2 Rainfall as a determinant factor for larval translation to pasture 196

7.2.3 Survival and mortality rate of L3 on pasture 199

7.2.4 Larval ingestion rate (β) 200

7.2.5 Establishment rate of the ingested infective larvae (s) 201

7.2.6 Development rate of L3 to egg laying adults (m_2) 202

7.2.7 Survival rate of adult cyathostomins (μ_2) 203

7.2.8 Fecundity (f) 203

7.3. Materials and methods 205

7.3.1 Meteorological data 205

7.3.2 Faecal worm egg count 205

7.4. Detailed model description 205

7.4.1 Deferential equations used to describe the rates of change with time in the constructed model 208

7.4.1.1 The rate of change of infective larvae on pasture (L3). 209

7.4.1.2 The rate of change of adult cyathostomins in the lumen of large intestine (A) 209

7.4.1.3 The rate of change of egg production (E) 209

7.4.2 Initial conditions and sequence of events in the model 210

7.4.3 Model run 210

7.4.4 Standardisation of output data values and model validation 211

7.5. Simulation of anthelmintic control strategy 212

7.6. Results and model tuning 212

7.6.1 Effect of varying some model parameter values on the prediction of peak faecal egg counts and pasture larval contamination. 214

7.6.2 Simulated effect of anthelmintic control strategy 216

7.7. Discussion 218

7.7.1 Predicted seasonal pattern of faecal egg count and infective larvae on pasture. 218

7.7.2 Simulation of anthelmintic control strategy 223

CHAPTER EIGHT 226

GENERAL DISCUSSION 226

APPENDICES 241

REFERENCES 258

LIST OF TABLES

Table 1.1. Species of non-strongyle helminths described in donkeys and their prevalence (%) 9

Table 1.2. Species of large strongyles described infecting donkeys and their prevalence (%). 14

Table 1.3 The prevalence and intensity of *S. vulgaris* infection in donkeys. 16

Table 1.4. Species of cyathostomins described in donkeys and their prevalence (%). 21

Table 1.5. Species of *Gasterophilus* larvae described in donkeys and their prevalence (%). 25

Table 1.6. Infection of *Parascaris equorum* in donkeys in various regions. 30

Table 1.7. Species of tapeworm described and their prevalence (%) in donkeys. 37

Table 1.8 Nematode egg development to infective larvae under tropical and subtropical conditions. 57

Table 1.9 Larval yields of strongyles in different seasons of tropical and subtropical conditions 7 days after faecal deposition on pasture. 58

Table 1.10 Survival of nematode infective larvae on pasture under tropical and subtropical conditions 61

Table 1.11. Pasture strongyle larval recovery under optimum weather conditions 62

Table 1.12 Pre-patent periods (ppp) of cyathostomins of horses and ponies 67

Table 1.13. Gastrointestinal adult cyathostomin burden of horses and donkeys. 69

Table 2.1 Equine population of the study areas. 76

Table 2.2. Climatic data and agro-ecological zone of the study areas. 77

Table 2.3. Monthly Rainfall (mm) distribution of the study areas (2003/2004). 77

Table 2.4 Mean daily maximum and (minimum) temperature of the study areas (°C) for each month during the study period (2003/2004). 78

Table 2.5. Peasant associations randomly selected from each region. 81

Table 2.6. Estimated number of donkeys to be sampled from each study region 82

Table 3.1. The prevalence of non-strongyle polyparasitism determined by coprological examination in donkeys, Ethiopia (n=803). 99

Table 3.2. The prevalence (%) and the overall mean faecal egg counts of equine tapeworm in four study regions, Ethiopia. 100

Table 3.3. The prevalence (%) and the overall mean faecal egg counts of *P. equorum* in four study regions, Ethiopia. 103

Table 3.4. The prevalence (%) and the overall mean faecal egg counts of <i>Fasciola</i> spp in four study regions, Ethiopia.....	105
Table 3.5. The prevalence (%) and the overall mean faecal egg counts of <i>Gastrodiscus</i> in four study regions, Ethiopia.....	107
Table 3.6. The overall mean faecal egg counts (epg) calculated fitted to the negative binomial distribution, and the prevalences of the four helminth parasites in four age (years) categories of donkeys, Ethiopia.	110
Table 3.7 The overall mean faecal egg count (epg) calculated fitted to the negative binomial distribution, and the prevalence of four helminth parasites in each sex groups of donkeys, Ethiopia.....	111
Table 3.8 The overall faecal egg counts (epg) calculated fitted to the negative binomial distribution, and the prevalence of the four helminth parasites in the three body condition score (BCS) categories of donkeys, Ethiopia.	112
Table 4.1 Prevalence, mean intensity and range of the different species of cestodes, trematodes, ascarid and bot larvae recovered from donkeys at necropsy, Ethiopia (n=112).....	133
Table 4.2. Number and proportion of infected donkeys with <i>P. equorum</i> in different age groups.....	136
Table 4.3 The infection levels of flukes in 112 autopsied donkeys, Ethiopia.	137
Table 4.4 The infection levels of <i>G. aegyptiacus</i> in 112 autopsied donkeys, Ethiopia.....	140
Table 4.5. Frequency distribution of the number of bot larvae in infected donkeys	142
Table 5.1 Number of donkeys examined and the proportion with different levels of infection intensities of <i>A. perfoliata</i> in different regions, Ethiopia.....	165
Table 5.2 Chi-square test in the analysis of the frequencies of infection intensities of <i>A. perfoliata</i> in different regions, Ethiopia.....	166
Table 6.1 The mean and median faecal cestode egg count and percentage efficacy of praziquantel administered to donkeys infected with cestodes.	182
Table 6.2 Summary statistics for ELISA OD values and predicted odds ratio for the risk of having colic in 22 praziquantel treated and 16 control donkeys infected with tapeworms.	184
Table 7.1 Model parameters and functional forms (all rates per capita instantaneous daily rates).....	195
Table 7.2. Rainfall data and herbage coverage for the study year (1996) and its interpretation in the model.....	199

Table 7.3. State variables used in the model.....207

Table 7.4. Development time delays and their definitions207

Table 7.5 Effect of varying the values of parameters, ingested infective larval development rate and the survival rate of adults, as measured by sum of squares of residuals, in fitting model prediction to the observed data.214

LIST OF FIGURES

Fig. 2.1 Country location map of Ethiopia (MOA, 2000)	73
Fig. 2.2 Location map of study sites: Ada, Akaki, Bereh and Boset.	75
Fig 3.1 Seasonal prevalence of cestode infection of donkeys in the study regions, Ethiopia.	101
Fig.3.2 Seasonal distribution of the overall mean faecal egg counts of tapeworm of donkeys, Ethiopia. Error bars indicate mean \pm se. The mean is a fitted value for the negative binomial distribution linked with identity function.....	101
Fig. 3.3. Monthly rainfall distribution of each region, Ethiopia.	102
Fig 3.4 Seasonal prevalence of <i>P. equorum</i> of donkeys in the study regions, Ethiopia.....	104
Fig.3.5 Seasonal distribution of the overall mean faecal egg counts of <i>P. equorum</i> of donkeys, Ethiopia. Error bars indicate mean \pm se. The mean is a fitted value for the negative binomial distribution linked with identity function	104
Fig 3.6 Seasonal prevalence of <i>Fasciola</i> spp of donkeys in the study regions, Ethiopia	106
Fig. 3.7 Seasonal distribution of the overall mean faecal egg counts of <i>Fasciola</i> spp of donkeys, Ethiopia. Error bars indicate mean \pm se. The mean is a fitted value for the negative binomial distribution linked with identity function.....	106
Fig 3.8 Seasonal prevalence of <i>Gastrodiscus</i> of donkeys in the study regions, Ethiopia.....	108
Fig 3.9. Seasonal distribution of the overall mean faecal egg counts of <i>Gastrodiscus</i> of donkeys, Ethiopia. Error bars indicate mean \pm se. The mean is a fitted value for the negative binomial distribution linked with identity function.	108
Fig 3.10. Eggs of ascarids (white arrow) and <i>Trichuris</i> (thin black arrow) recovered from donkey faeces: The thick black arrow shows <i>P. equorum</i> egg.	109
Fig. 4.1 Frequency distribution of the number of <i>A. perfoliata</i> in 112 donkeys autopsied, Ethiopia.....	134
Fig. 4.2. Clumps of <i>A. perfoliata</i> attached around the ileocaecal junction of a donkey, Ethiopia. Note the ulceration and diphtheritic membrane after detachment of the tapeworm (black arrow) and the hyperaemic and oedematous ileocaecal valve (white arrow).....	135

Fig.4.3. Mature <i>P. equorum</i> in a four-year-old donkey, which died of colic after anthelmintic treatment. Most of the worms were found blocking the ileocaecal junction (ICJ), in the caecum (CM) and few in the small intestine.	136
Fig. 4.4. The mean infection intensity of flukes in different age categories of the 112 autopsied donkeys, Ethiopia.	137
Fig. 4.5. Hyperplasia (thickening) of the main bile duct due to fluke infection.	138
Fig 4.6. Brownish grey nodular or patches obstructing branches of the main bile duct (arrows).	139
Fig. 4.7. Large masses of <i>G. nasalis</i> attached to the pylorus (thick black arrow) and <i>G. intestinalis</i> along the margo plicatus (thick white arrow). Note the pyloric opening (thin white arrow) completely surrounded by the larvae.	141
Fig 4.8 Prolapsed rectal mucosa due to irritation and tenesmus from the gasterophilus larvae in donkeys: a) swollen and oedematous mucosa. b) extensively oedematous and ruptured rectal mucosa with the larvae attached to it.	143
Fig 5.1 The distribution of ELISA OD to anti-12/13 kDa IgG(T) of <i>A. perfoliata</i> in donkeys (n=797).	164
Fig. 5.2. Scatter diagram of the relationship between ELISA OD and McMaster cestode faecal egg count.	167
Fig. 5.3 IgG(T) response to 12/13 kDa antigen as a measure of infection intensity in different age groups. ELISA results are expressed as median optical density (405nm) for each age group in each region.	168
Fig. 5.4. Seasonal variations in the median OD of serum antibody level to a 12/13 kDa antigen of <i>A. perfoliata</i> in naturally infected donkeys of different regions, Ethiopia.	169
Fig 5.5 Monthly rainfall distribution of each region, Ethiopia.	169
Fig 6.1. The mean faecal egg counts in the treated and control groups at each sampling period. Week-0 is before treatment and Week-2 to weeks-16 after treatment. Error bars indicate mean + se (standard error).	182
Fig. 6.2 ELISA OD values of the 22 donkeys before, 8 and 16 weeks after treatment with praziquantel. Each number on the x-axis represents an individual donkey.	183
Fig. 6.3. Box and whiskers plot of serum antibody levels of <i>A. perfoliata</i> infected donkeys before and 8 and 16 weeks after treatment with praziquantel. TR0 and CON0 treatment and control groups before treatment; TR8 and CON8 treatment and control	

groups after 8 weeks treatment; TR16 and CON16 treatment and control groups after 16 weeks of treatment, respectively.....	185
Fig. 6.4. The predicted OR for the risk of spasmodic colic before, and 8 and 16 weeks post-treatment in treated and control groups. TR0 and CON0 treatment and control groups before treatment; TR8 and CON8 treatment and control groups after 8 weeks treatment; TR16 and CON16 treatment and control groups after 16 weeks of treatment, respectively (Odds ratio = $e^{2.756 \cdot \text{ELISA OD}}$).	185
F.g.7.1. The life cycle of cyathostomins in equids. Arrested larval development is not considered here.	192
F.g.7.2. A network representation of a simplified lifecycle of cyathostomins. E, number of eggs, L3, number of infective larvae, and A is number of adult parasites. y , β and f are larval yield, ingestion rate and rate of egg production, respectively.	193
Fig.7.3. Network representation of the basic architecture of the simulation model constructed in ModelMaker.	206
Fig. 7.4 Observed and predicted monthly faecal worm egg counts of cyathostomins of donkeys in the mid-lowland regions, Ada and Akaki, Ethiopia. The sum of squares of residuals is 32.9.	213
Fig. 7.5 Predicted peaks of infective larvae on pasture before adjustment was made for development time of ingested infective larvae to adult and survival time of adults.	213
Fig. 7.6 Observed and predicted monthly faecal worm egg counts of cyathostomins of donkeys in the mid-lowland regions, Ada and Akaki, Ethiopia, after the development rate of ingested infective larvae and survival rate of adults were adjusted. The sum of squares of residuals is =5.2.....	215
Fig.7.7 Peak predicted infective larvae on pasture before and after adjustment was made for development time of ingested infective larvae to adult and survival time of adults.	215
Fig. 7.8. Effect of a single anthelmintic dose on the level of worm burden administered at the end of dry season, main rainy season and at both times compared to the uncontrolled system.	216
Fig. 7.9 Effect of annual dosing on cyathostomin worm burden in donkeys, Ethiopia.....	217
Fig. 7.10. Effect of biennial dosing on the cyathostomin worm burden in donkeys, Ethiopia.	218
Fig 7.11. Effect of dosing every four-year on the cyathostomin worm burden in donkeys, Ethiopia.	218

LIST OF ABBREVIATIONS

ABTS	azino-bits (3-ethylbenzthiazoline 6-sulphuric acid)
C	centigrade
CI	confidence interval
ENMSA	Ethiopian national meteorological service agency
df	degrees of freedom
Epg	eggs per gram
EL3	early third stage larvae
ELISA	enzyme linked immunosorbent assay
E/S	excretory/secretory
FAO	Food and agricultural organization
g	gravity
gm	gram
Ig	immunoglobulin
KDa	kilo Dalton
Kg	kilogram
L1	first stage larvae
L2	second stage larvae
L3	third stage (infective) larvae
L4	fourth stage larvae
L5	fifth stage larvae
M	Molar
Mab	monoclonal antibody
masl	metre above seal level
mbsl	metre below seal level
mg	milligram
ml	millilitre
mm	millimetre
MOA	Ministry of agriculture
µg	microgram
µl	microlitre
µm	micrometre
NCP	nitrocellulose paper
nm	nanometre
OD	optical density
OR	odds ratio
PAGE	polyacrylamide gel electrophoresis
P	probability
PBS	phosphate buffered saline
r_s	Spearman's rank correlation coefficient
rpm	revolution per minute

sd	standard deviation
SDS	sodium dodecyl sulphate
se	standard error
sg	specific gravity
SMP	skimmed milk powder
Spp	species
w/v	weight for volume

CHAPTER ONE

GENERAL INTRODUCTION AND LITERATURE REVIEW

1.1. Introduction

The donkey (*Equus asinus*), ass or burro (the term commonly used in the countries of the western and southern hemispheres) is a separate species from the horse (*Equus caballus*). Both species, however, have been subjected to similar evolutionary processes and so their bodies are built on similar lines. Donkeys and horses are close enough genetically to produce viable, but sterile hybrid offsprings, mules and hinnies. The Donkey has 62 chromosomes while the horse has 64 (Lasley, 1978). The long evolutionary processes and a variety of uses by man have produced great variety in the type of donkeys, from the wild Somali ass (*Equus asinus somaliensis*) to the Sicilian or Miniature donkey and the Mammoth ass.

In many countries, particularly in developing countries, equine population statistics are estimates and extrapolations. Substantial donkey populations occur in remote rural areas of developing countries where it is difficult or impractical to survey them accurately (Starkey and Starkey, 1997). Most of the data on the equine population have been collected by the Food and Agriculture Organization (FAO) and are based on estimates submitted by national agricultural ministries (FAO, 2004). Today, the world equine population is estimated at over 113 million with over 1 billion people directly dependent upon them (Prentis, 1994). More than eighty five percent of all equids, 97% of all donkeys, 98% of all mules and 74% of all horses are found in developing countries. Furthermore, 37.3% of the world equine population is made up of donkeys. When the global trend in the use of equine species is examined over the last four decades, the population of horses has been static, that of the mules has risen while the donkey has declined. However, in most developing countries, particularly in Africa, the picture is different; the earliest FAO estimate of the world donkey

population is from 1961 when there were an estimated 37 million donkeys. Since then, the world donkey population has increased steadily and today it is estimated to be about 43 million, an increase of approximately 19% (FAO, 2004). Almost half of the world donkey population is found in Asia, over one quarter in Africa and the rest mainly in Latin America.

Ethiopia has long had a large donkey population, which has increased from 3 million in 1949 to 5.2 million today (FAO, 2004). This is equivalent to 12.1% of the world and 33.5% of the African donkey population. It is now not only the biggest in Africa, but also the second largest in the world after China. Although they are found in almost all ecological zones of the country, the majority are located in the highland plateaus (Admassie et al., 1983; Feseha et al., 1997) where 90% of the people live (Admassie et al., 1983; Gizaw 1987). Ninety seven percent of the donkey population is found in three regions: 44% in Oromia, 34% in Amhara and 19% in Tigray (Central Statistics, 1995), with the density of donkeys being highest in Tigray, Arsi and Shewa. It is difficult, at present, to suggest the critical minimum donkey density per unit of population. However, according to FAO (1989), there were 27 donkeys per 100 people in Ethiopia, placing the country amongst the first twenty with sizable densities in the world. There are more donkeys than either horses or mules in all regions, except Arsi, Ilubabor, and Sidamo (Wilson, 1991).

Donkeys provide valuable service in many parts of the world. They are mostly used for transport, tillage and other agricultural activities. Despite the increased use of fossil fuel, animals appear to be a continued source of assistance and one, which plays a significant role in the economy of most developing countries (Ebenezer, 1991). It is important to realise that the great majority of donkeys in the world, over 97%, are kept specifically for work (Wilson, 1990; Ebenezer, 1991; Iversen, 1991; Prentis, 1994; Starkey, 1994a; Fernando, 1997; Fielding and Krause, 1998).

In Ethiopia, donkeys have been used as beasts of burden for a long time and still render their services as pack animals throughout the country. This is mainly in rural and peri-urban areas where modern means of transportation are absent, unaffordable or unsuitable (Wilson, 1990, 1991; Feseha, 1991, 1997; Getachew, 1999). Donkeys are still used even where modern transport is available in cities and towns (Sisay, 1997). They have been found to provide pack services carrying over fifteen kinds of commodity weighing 60-100 kg and covering distances of 15-30 km for durations up to 4-5 hours (Feseha et al., 1997). Donkeys as draught power, for tillage purposes and other agricultural activities are not popular in many parts of Ethiopia and the use of donkeys for agricultural operations is minimal (Feseha et al., 1997). However, in some regions, particularly in the rift valley areas, donkeys are used to pull carts to transport different commodities and for tillage purposes, in association with oxen (Zelalem et al., 1997).

Contrary to the size of the world donkey population and the service they provide to society and the national economies, particularly in developing countries, the level of care given to these animals, both by society and government, is low. Although it ranks among the first species to be domesticated by humans and despite its immense contribution to the development of civilisation, the donkey has been neglected as a subject of scientific inquiry. Compared to other equidae research on the donkey, particularly, work done on gastrointestinal parasites, which are known to be the major cause of early demise of donkeys, is limited, an issue identified by several people writing about or working on promoting the case of donkeys (Svendsen, 1991; Starkey 1997; Feseha et al., 1997; Getachew, 1999). They are perhaps, the most neglected animals among livestock.

1.2 General objectives of the study

Given the paucity of information specifically dedicated to parasitic infections of donkeys, the present work was intended:

- to study the epidemiology of gastrointestinal parasites of working donkeys of Ethiopia with particular reference to cyathostomins, cestodes, trematodes, ascarids and gasterophilids.
- to develop a simple mathematical modelling approach in the study of seasonal variation and population dynamics of cyathostomins.

1.2.1 Specific objectives:

- Cross-sectional coprological survey of cestodes, trematodes, and ascarids
- Retrospective post-mortem study on the prevalence and abundance of internal parasites with particular reference to cestodes, trematodes, ascarids and gasterophilids
- Sero-epidemiological study of *Anoplocephala perfoliata* infection in donkeys.
- Field efficacy study of praziquantel against naturally acquired cestodes of donkeys.
- To produce a mathematical model for the seasonal variation of cyathostomin faecal egg output and infective larvae on pasture, and to simulate the rational anthelmintic control strategies under the mid-lowland tropical weather condition of Ethiopia.

In order to lay a foundation for the relevant studies undertaken based on the objectives set above, a review of the present knowledge covering the different health and management problems of donkeys, particularly gastrointestinal parasites, are presented in this chapter.

1.3. Health and management problems of working donkeys

It is natural for many people in developed countries to assume that the care of equids in general, and donkeys in particular, in developing countries is commensurate with the contribution and worth of the animal. This is seldom the case, and health care is almost non-existent for donkeys (Svendsen, 1991; Green, 1994; Getachew, 1999). Many reports suggest that there is a belief that donkeys do not get sick and hence do not need treatment (Bakkoury and Belemlih, 1991; De Aluja et al., 1991; Pradhan, et al., 1991; Upadhyay, 1991; Svendsen, 1991; Starkey, 1995a; Feseha, 1997; Itepu, 1997; Twerda et al., 1997; Wells and Krecek., 1997). Management and care for donkeys seems to many people to be unnecessary as donkeys are one of the few domesticated animals that appear to do rather well with minimal management (Pearson et al., 1997) and appear to be less affected or resistant or tolerant to most of the diseases that affect other animals, notably trypanosomosis (Connor, 1994; Barrowman, 1991) and African Horse Sickness (Coetzer and Erasmus, 1994; Brown and Dardiri, 1990). However, it has been shown that donkeys are potentially susceptible to almost all diseases that affect other equids even though the degree of susceptibility varies (Howell, 1963; Uppal, 1991; De Wall et al., 1994; Pandey, et al. 1994; Feseha, 1997; Wells Krecek, 1997; Getachew, 1999).

Donkeys, in addition to zebras and other wild animals, may be more resistant to helminth parasites than other equids, and infections may appear to cause less severe clinical disease (Malan, et al. 1997). As donkeys are commonly infected with several species of parasites, often with heavy internal parasite burden, there is a strong reason to believe that they would suffer from the pathogenic effect of parasitic infections. Moreover, in many developing countries, donkeys are used for work, undernourished, often maintained on low-quality diet and highly stressed from over-working and other disease conditions (Svendsen, 1997a b; Feseha, 1998; Pearson et al., 1999; Krecek and Guthrie, 1999; Getachew, 1999), which make

them susceptible to parasitic infections. Some studies have shown that parasites are the major contributors to the short life span of donkeys in Africa, Asia and Latin America (Pandey et al, 1994; Feseha, 1998; Svendsen, 1997a b). It has also been demonstrated that strategic worming programmes can not only increase the general health of the donkey, thereby allowing it to maximize the use of the sparse food allocated to it, but also can lead to improved body condition and increased the life span compared to animals not wormed (Urch and Allen, 1980; Bliss et al., 1985; Yilma et al., 1989; Feseha et al., 1991; Feseha, 1998; Khallaayoune, 1991; Svendsen, 1997a; b Wells, 1997). The control of parasitic infection may well be one of the keys to the well being of working donkeys.

Except in a very few special cases, little that would be termed ‘animal management’ in the modern sense is practiced for Ethiopian donkeys. In general, the problems of working donkeys are often more severe in areas where modern life and technology impinge upon the traditional life style (Prentis, 1994). The majority of problems of working donkeys are due to mis-management, neglect and cruelty from ignorance (Belemlih, 1991; De Aluja et al., 1991; Feseha et al., 1997; Jones, 1991; Pradhan et al., 1991; Svendsen, 1991; MacGregor, 1994; Starkey, 1995a; Getachew, 1999). The major reasons for mis-management, ill or poor treatment of donkeys are complex and include the poor economy of the country, lack of education and training, lack of materials, equipment, essential basic drugs and professional advice and basic lack of understanding by the people of the potential productivity of their animals, given the correct care (MacGregor, 1994). It seems that deliberate maltreatment is rare, and health problems are more likely to be due to ignorance and the ubiquitous nature of disease results in the animals’ owners becoming indifferent or being unaware that anything is wrong (Dorman, 1994).

1.4 Gastrointestinal parasites of working donkeys

Studies of the helminth prevalence and burden in working donkeys kept in various regions have shown that the average faecal nematode egg count is quite high and the prevalence of infection is 100% (Khallaayoune, 1991; Selim et al., 1994; Feseha et al., 1991; Demir et al., 1995; Arslan et al., 1998; Wells et al., 1998; Getachew, 1999). Studies by Khallaayoune (1991) and Wells et al. (1998) estimated the average faecal nematode egg counts at 2000 and 1800 epg (eggs per gram of faeces), respectively. Similar studies in Ethiopia (Feseha et al., 1991; Getachew, 1999) demonstrated an average of 1440 to 2500 epg depending on the season. An epg of 42,650 was recorded in donkeys (Khallaayoune, 1991). According to Soulsby (1982), an epg of 500 suggests mild infection, 800-1000 a moderate infection and above 1500 a severe infection. Applying these ideas, it would therefore appear that most working donkeys in Ethiopia are severely infected with nematodes.

Although the faecal egg count of an individual animal does not correlate well with the severity of its parasitic infection/burden, herd average egg counts actually reflect the rate at which animals acquire infections and the degree to which they are contaminating their environment with eggs (Uhlinger, 1991). Working donkeys do not receive treatment and hence shed high numbers of eggs. Some studies have shown that helminths in working donkeys are not only highly prevalent but the infection intensity or worm burden is also very high (Pandey and Eysker, 1988, 1990; Eysker and Pandey, 1989; Eysker, 1987; Pandey, 1980, 1981; Getachew, 1999; Matthee et al., 2000; Matthee et al., 2002).

According to Lichtenfels (1975), helminths of equids include more than 75 species in 28 genera of nematodes, five species of trematodes and four species of cestodes. Studies conducted in identifying the species of helminths in working donkeys not only showed that there is an overall similarity in the type of parasites with other equids but also a similarity in

the species of parasites, particularly helminths, present in a wide range of climatic zones (Tables 1.1-1.5). This similarity in species of helminth parasites contrasts markedly with the situation in ruminants in which there is a clear difference in the genera of the helminths which predominate in areas where the main season for acquiring the infection is cool, with temperature 15-20°C as against those where the equivalent season is warmer (Hammond and Sewell, 1990). The complete and thorough studies carried out to date in identifying the gastrointestinal helminth parasites of working donkeys are those conducted by Matthee et al. (2000) and Matthee et al. (2002) in South Africa, Getachew (1999) in Ethiopia, Eysker and Pandey (1989) in Zimbabwe and Burgu et al. (1995) in Turkey (Tables 1.1-1.5).

Table 1.1. Species of non-strongyle helminths described in donkeys and their prevalence (%)

	Countries						
	B. Faso ¹	Chad ²	Ethiopia ³	Kenya ⁴	Morocco ⁵	S. Africa ⁶	Zimbabwe ⁷
Number examined	30	183	13	14	782	16	14
Recovery	pwr	pwr	pwr	pwr	pwr	pwr	pwr**
Helminths							
<i>Strongyloides westeri</i>	+	-	+	-	1(597)*	+	7
<i>Trichostrongylus axei</i>	+	-	100	17.0 (6)	100(597)	6.3	64
<i>Dyctiocaulus arnfieldi</i>	-	-	46.2	100(6)	48.0(597)	6.3	-
<i>Probystmayria vivipara</i>	-	-	100	-	-	12.5	29
<i>Oxyuris equi</i>	30	57	46.2	12.5(8)	13.0(597)	43.6	43
<i>Habronema muscae</i>	90	20	53.8	67.5(8)	89.9	75.0	86
<i>H. majus</i>	93	15	+	-	85.0	75.0	64
<i>D. megastoma</i>	43	54	53.8	-	1.0	50.0	79
<i>Gastrodiscus aegyptiacus</i>	57	34	+	-	-	62.5	14
<i>Setaria equina</i>	-	89	46.2	25.0(8)	29.0(765)	31.3	29
<i>Trichuris</i> spp.	-	-	+	-	-	-	-
<i>Dicrocoelium dendriticum</i>	-	-	+	-	-	-	-

* Numbers in parentheses show number of donkeys examined when different from total. **. pwr- post-mortem worm recovery. + Described

1.Vercruysse et al., 1986; 2.Graber, 1970; 3. Getachew, 1999, Feseha et al., 1991; 4. Lewa et al., 1998, Karanja et al., 1994; 5. Khallaayoune, 1991, Pandey, 1980, Pandey et al. 1992a, Pandey & Cabaret, 1993; 6. Matthee et al., 2000, Matthee et al., 2002; 7. Pandey & Eysker, 1988, 1990, 1991.

Table 1.1.continued.....

	Countries							
	Germany ¹	Greece ²	India ³	Italy ⁴	Iraq ⁵	Mexico ⁶	Turkey ⁷	USA ⁸
Number examined	106	37	71	36	12	?	110	8
Recovery	cos**	cos	cos	pwr	pwr	?	cos/pwr	pwr
Helminths								
<i>strongyloides westeri</i>	17.0	5.4	-	-	-	+	4.6	-
<i>Trichostrongylus axei</i>	77.4	-	+	-	-	+	30.0	100
<i>Dyctiocaulus arnfieldi</i>	45.3	2.7	-	-	+	-	14.6(82)*	+
<i>Probystmayria vivipara</i>	-	-	-	+	-	-	77.0	+
<i>Oxyuris equi</i>	1.9	-	+	-	+	-	30.0	25.0
<i>Habronema muscae</i>	-	-	-	+	-	+	64.5	75.0
<i>H. majus</i>	-	-	-	+	-	+	55.5	-
<i>D. megastoma</i>	-	-	-	+	-	-	-	25.0
<i>Gastrodiscus aegyptiacus</i>	-	-	+	-	-	-	-	-
<i>Setaria equina</i>	-	-	-	+	-	-	-	+
<i>Trichuris</i> spps.	-	-	-	-	-	-	+	-
<i>Dicrocoelium dendriticum</i>	-	-	-	-	-	-	+	-

* Numbers in parentheses show number of donkeys examined when different from total. ** cos-coprology. ?,- No data

1.Beelitz et al., 1996; 2.Sotiraki et al., 1997; 3. Sengupta and Yadav, 1998; 4. Ricci and Abatini, 1992; 5. Daoud and Al-Alousi, 1995; 6. de Aluja et al., 1990, Rodriguez-Maldonado, 1991; 7. Burgu et al., 1995, Demir et al., 1995, Arslan and Umur, 1998, Gonenc, 1999; 8. Tolliver et al., 1985

1.4.1. Strongyles (Strongylidae)

Strongyles live as adults in the large intestine of equids (horses, donkeys, zebras, wild asses and their hybrids) and are commonly categorized as large and small strongyles. The strongyles as a group comprise almost one-half of the over 100 species of internal parasites found in horses (Krecek et al., 1987).

Strongyles have a direct life cycle. They lay eggs that are passed in the faeces of the host to the environment. Under favorable environmental conditions, optimum temperature and humidity, the embryo develops within the egg to the first stage larvae (L1), which hatches. Further development occurs to the second stage (L2) and third stage (L3) on the pasture. The rate of development and survival of the free-living stages depends on the surrounding temperature and humidity or rainfall (Ogbourne, 1972; English, 1979a; Mfitilodze and Hutchinson, 1988; Soulsby, 1982; Urquhart et al., 1996). The third stage larva (L3) is the infective stage, and infects equids if ingested. In the host, development continuous with advancement to the fourth stage (L4) and the fifth stage (L5) or adult. The prepatent period varies between the large and the small strongyles and among the species. It varies from 6-12 months for large strongyles (Soulsby, 1982; Urquhart et al., 1996; Duncan and Pirie, 1972). Although the prepatent period of individual small strongyles has not been studied, some studies have shown that it is in the range of 5-18 weeks (Soulsby, 1982; Urquhart et al., 1996; Duncan and Pirie, 1972; Love and Duncan, 1992; Smith, 1978; Reinemeyer, 1988). Whether the prepatent periods of strongyles in donkeys are similar or different from that in horses is unknown.

1.4.1.1. Large strongyles.

Large strongyles encompass the genera *Strongylus*, *Craterostomum*, *Oesophagodontus*, *Triodontophorus* and *Bidentostomum* (Lichtenfels, 1975; Lichtenfels, 1998). The commonly known large strongyle species infecting equids are *Strongylus vulgaris*, *S. edentatus*, *S.*

equines, Triodontophorus brevicauda, T. serratus, T. minor, T. tenuicollis, T. nipponicus, Craterostomum acuticaudatum, C. tenicauda and Oesophagodontus robustus (Soulsby, 1982; Reinecke, 1983; Urquhart et al., 1996).

Work done to identify the species of large strongyles infecting working donkeys in various regions have revealed similar species to those described in horses (Table 1.2). *Strongylus asini* has been described in donkeys and other wild equids (Boulanger, 1920). *Strongylus* and *Triodontophorus* species are considered the most pathogenic of the large strongyles in horses (Duncan, 1973; Slocombe, 1985; Austin, 1994; Proudman and Matthews, 2002). However, it is the epidemiology and pathogenic effect of *S. vulgaris* that has been well studied in horses in the northern hemisphere under temperate climate (Duncan, 1973. 1974; Ogbourne and Duncan, 1985) because of the potential severe tissue damage, including occlusion of the blood vessels (cranial mesenteric arteries and its branches) during parenteral larval migration which often results in colic and sometimes death (Duncan, 1973; Drudge and Lyons, 1977). Although variations were seen, studies have shown higher prevalence and intensity of *S. vulgaris* infection in donkeys than the other strongyle species (Table 1.2). Almost all studies reported a 100% prevalence of *S. vulgaris* in donkeys (Pandey and Eysker, 1989; Graber, 1970; Pandey, 1980, Vercruysse et al., 1986; Getachew, 1999; Matthee et al., 2000; Matthee et al., 2002). A study conducted in Zimbabwe (Pandey and Eysker, 1989) showed that the intensities of infection of adults in the large intestine and larvae in the cranial mesenteric arteries and its branches were much higher than those recorded for horses from Morocco (Pandey, 1981), Queensland (English, 1979) and Britain (Poynter, 1970; Ogbourne, 1975). Poynter (1970) and Ogbourne (1975) found the maximum number of larvae recovered from arteries of any single horse was 136 and 74 respectively, whereas in donkeys it may be much higher than these (Table 1.3). Moreover, studies by Pandey (1980) and Pandey (1981) in donkeys and horses in the same area of Morocco demonstrated that horses were less heavily

infected than donkeys. Whether this difference between the two host species is due to the higher susceptibility of donkeys to *S. vulgaris*, or to differences in husbandry and management is not clear and needs further investigation.

Table 1.2. Species of large strongyles described infecting donkeys and their prevalence (%).

	Countries							
	B. Faso ¹	Chad ²	Egypt ³	Ethiopia ⁴	Kenya ⁵	Morocco ⁶	S..Africa ⁷	Zimbabwe ^{8*}
Number examined	30	183	4	13	6	168	16	14
Recovery	pwr	pwr	cos	cos/pwr	pwr	pwr	pwr	pwr**
Strongyle species								
<i>S. vulgaris</i>	100	81.0	+	100	100	98.0	100	100
<i>S. edentatus</i>	90.0	8.0	+	53.8	100	26.0	6.3	-
<i>S. equinus</i>	60.0	11.0	-	15.4	67.0	-	18.8	-
<i>S. asini-</i>	-	-	-	-	-	-	+	-
<i>T. brevicauda</i>	-	-	-	46.2	-	-	-	-
<i>T. serratus</i>	47.0	2.0	+	53.8	50.0	-	37.5	79.0
<i>T. tenuicollis</i>	-	15.0	-	30.8	17.0	-	-	-
<i>T. minor</i>	-	37.0	-	-	-	-	-	-
<i>T. hartmannae</i>	-	-	-	-	-	-	68.8	-
<i>T. burchelli</i>	-	-	-	-	-	-	62.5	-
<i>T.nipponicus</i>	37.0	-	-	-	-	-	-	71.0
<i>O .robustus</i>	-	-	-	20.1	-	-	-	-
<i>C. acuticaudatum</i>	-	-	-	-	17.0	-	-	-

** pwr- postmortem worm recovery, cos- coprology/coproculture. + Described

*1.Kaboret et al., 1986; 2.Graber and Gruvel, 1964; 3. El-Abdin et al., 1983; 4. Getachew, 1999, Feseha et al., 1991; 5. Lewa et al., 1998, 6. Khallaayoune, 1991; 7. Matthee et al., 2000, Matthee et al., 2002; 8. Pandey and Eysker, 1989, 1990, 1991.

Table 1.2. continued...

	Countries							
	Germany ¹	Greece ²	India ³	Italy ⁴	Iraq ⁵	Mexico ⁶	Turkey ⁷	USA ^{8*}
Number examined	106	37	71	36	12	?	92	8
Recovery	cos	cos	cos	pwr	pwr	?	cos/pwr	pwr
Strongyles								
<i>Strongylus</i> spps.	-	+	+	+	+	+	+	+
<i>S. vulgaris</i>	44.3	-	+	+	+	+	+	100(6)
<i>S. edentatus</i>	0.9	-	+	+	-	+	+	50.0(6)
<i>S. equinus</i>	-	-	-	+	-	-	+	-
<i>S. asini</i> -	-	-	-	-	-	-	-	-
<i>Triodontophorus</i> spps.	-	-	+	+	-	-	+	+
<i>T. brevicauda</i>	-	-	-	+	-	-	+	-
<i>T. serratus</i>	-	-	+	+	-	-	+	50.0(4)
<i>T. tenuicollis</i>	-	-	-	-	-	-	-	75.0(4)
<i>T. minor</i>	-	-	-	-	-	-	+	-
<i>T. hartmannae</i>	-	-	-	-	-	-	-	-
<i>T. burchelli</i>	-	-	-	-	-	-	-	-
<i>T. nipponicus</i>	-	-	-	-	-	-	-	-
<i>O. robustus</i>	-	-	-	-	-	-	+	-
<i>C. acuticaudatum</i>	-	-	-	-	-	-	-	40.0(4)

*1.Gothe and Heil, 1984; .2.Sotiraki et al., 1997; 3. Sengupta and Yadav, 1998; 4. Ricci and Abatini, 1992; 5. Daoud and Al-Alousi, 1995; 6 de Aluja et al., 1990, Rodriguez-Maldonado, 1991; 7.Burgu et al., 1995, Arslan and Umur, 1998, 8. Tolliver et al., 1985. ? No data

Table 1.3 The prevalence and intensity of *S. vulgaris* infection in donkeys.

N	Prevalence of		Adult and arterial larval burden			
	Adult	Arterial larvae	Adult mean	Adult Range	Larvae mean	Larvae Range
14	100	86	451	63-1278	69	0-1811 ¹
9	89	100	145	0-454	46	12-109 ²
7	100	100	632	78-2426	31	1-114 ³
168	87	98	208	12-900	5	4-15 ⁴
182	-	62	-	-	-	- ⁵
201	-	91	-	-	33	0-1128 ⁶
8	38	63	-	-	-	- ⁷
8	100 (6)*	75	-	-	-	- ⁸

N- number of donkeys examined. *Number in parentheses shows number of donkeys examined where different from total.

1. Pandey and Eysker, 1989. 2. Matthee et al., 2002. 3. Matthee et al., 2000. 4. Khallaayoune, 1991; 5. Graber, 1970, 6. Pandey, 1980. 7. Karanja, et al., 1994; 8. Tolliver et al. 1985.

Although the pathogenesis of *S. vulgaris* in donkeys has not been thoroughly studied, similar pathogenic effects to those seen in horses have been reported. Postmortem examination of 12 donkeys in Kenya showed that 42% of the donkeys had thrombo-enderteritis of various degrees of severity (Lewa et al., 1998). A study by Khallaayoune (1991) in Morocco also demonstrated 87% of the 168 donkeys necropsied were found to have severe aneurysm lesions associated with thrombus formation. Pandey (1980) examined cranial mesenteric arteries and their branches in 201 donkeys and revealed an overall mean of 91% arterial infections. In this investigation lesions were recorded from less prominent arterial lesions and small thrombi to a hard thrombi of tennis ball size with the arterial wall greatly thickened, corrugated and gritty. Similar arterial lesions have been observed in Ethiopia with high prevalence and intensity of arterial larvae (author’s observation). Such arterial lesions

resulting in the interference with blood supply to the major parts of the alimentary tracts (Ogbourne and Duncan, 1985) may be one of the major contributory factors for the cause of weakness, low productivity and the early demise of working donkeys. The danger and severe pathogenic effect of *S. vulgaris* was seen in a 2 year-old male donkey in Florida, in which a fifth stage larva was found that had migrated through the spinal cord and resulted in progressive paraparesis and then tetraplegia. Severe granulomatous myelitis with hemorrhage and necroses were seen, which were quite similar to lesions seen in equine myeloencephalitis (Mayhew et al., 1984). A similar condition has been recorded in ponies in the cerebrum and spinal cord (Little et al., 1974).

Experiments conducted by Amborski et al. (1974) and Duncan (1975) demonstrated that there is development of age-and acquired-immunity to *S. vulgaris* in horses. The latter author found that horses over 3 years of age were completely resistant to infection to *S. vulgaris*. However, studies in working donkeys showed no such findings (Pandey and Eysker, 1989; Graber, 1970; Pandey, 1980, Vercruysse et al., 1986; Getachew, 1999; Matthee et al., 2000; Matthee et al., 2002). As suggested by Duncan (1975), the phenomena of immunological unresponsiveness and immunosuppression might be operating under field conditions, which allow the development of at least a proportion of ingested infective larvae of *S. vulgaris*. In most countries, equids are working animals, often stressed and undernourished. Moreover, they are outdoors throughout the year, acquiring strongyle infections from very early in life and receiving no treatment and thus may acquire heavy worm burdens.

Before the modern anthelmintics era, surveys usually demonstrated a 78-100% prevalence of *S. vulgaris* in horses (Drudge and Lyons, 1977; Slocombe, 1973; Duncan, 1974; Hass, 1979). However, current infection rates are greatly reduced because of the widespread use of modern anthelmintics (Dipietro et al., 1990; Herd, 1990 a, b, Austin, 1994; Love, 1995).

According to Herd (1990b), parasitologists in North America had difficulty finding *S. vulgaris* for specimens to exhibit to students.

The scenario among working equids, particularly donkeys, in developing countries is quite different. The majority of equine owners, and particularly those of donkeys are very poor and cannot afford treatment against parasites (Svendsen, 1997a,b). So, there is a large gulf in the health level of equids in the developed and developing worlds. Available studies have shown that large strongyles are still highly prevalent in working donkeys except where equine charity organizations are operating, where the prevalence is seen to be decreasing (Tables 1.2 & 1.3).

1.4.1.2. Cyathostomins (small strongyles)

Cyathostomins formerly called trichonema or cyathostomes (Lichtenfels, 1975; Lichtenfels et al., 1998) are the most common other genera of strongyles infecting equids. The adult worms live in the large intestine and have a non-migratory life cycle (Soulsby, 1982; Urquhart et al., 1996). According to Reinemeyer (1986), cyathostomin ova usually comprise over 95% of the strongylid ova in horse faecal samples. However, studies conducted in South Africa (Well et al., 1998) and Ethiopia (Getachew, 1999) showed a higher proportion of large strongyles in donkeys. This can be justified by the fact that the wide spread use of effective parasite control has reduced the prevalence of large strongyles in horses in developed countries (Uhlinger, 1991), whereas donkeys in the resource-poor areas are generally not dewormed, and as a result a higher proportion of large strongyles still prevails.

Infections with these nematodes typically include very large populations and numerous species. A total of 52 species of cyathostomins have been recorded in horses, donkeys and zebras (Lichtenfels et al., 1998). Although such a large number of cyathostomins have been

described in equids, surveys worldwide have reported about 16-24 species in most regions and 4-14 species with a prevalence of 50% or higher (Ogbourne, 1978b; Reinemeyer et al., 1984; Torbert et al., 1986; Carvalho et al., 1998; Lyons et al., 1999; Lichtenfels, et al., 2001). According to Uhlinger (1991) only five species compose 80-90% of the total cyathostomin adult worm burden present.

Due to the complex taxonomy and difficulties in the identification of cyathostomins, very few workers have identified these parasites to the species level in donkeys. The species of cyathostomins described in donkeys are similar to those described in horses (Table 1.4). Of the over 50 species of cyathostomins 10 species have been reported only from donkeys or zebras (Lichtenfels et al., 1998). As shown in Table 1.4 most of the studies describing cyathostomins were from donkeys in Africa. Few attempts have been made elsewhere. The most comprehensive list came from South Africa (Matthee et al., 2000; Matthee et al., 2002), Turkey (Burgu et al., 1995), USA (Tolliver et al. 1985), Ethiopia (Getachew 1999) and Zimbabwe (Eysker and Pandey, 1989), where 23, 23, 23, 17 and 11 species, respectively, were described. It is observed that there is a marked variation in the abundance and species of cyathostomins described in donkeys in different regions. These variations could be due to several factors, e.g., physical environmental condition, geographical distribution, the general health and nutrition of the donkeys and the condition under which the donkeys are kept.

There is sparse knowledge of the biology of individual cyathostomins. As part of their life cycle, cyathostomins undergo a period of arrested development as early third stage larvae (EL3) in the large intestinal mucosa of the horse (Eysker et al., 1984; Eysker et al., 1990; Love, 1992, 1995; Paul, 1998; Proudman and Matthews, 2000), which could be extended for more than 2 years (Reinemeyer, 1986). In temperate regions winter is an unfavorable period for larval development and survival (Ogbourne, 1975) but in many tropical and subtropical

areas it is the dry season (English, 1979a; Eysker, 1987; Mfitilodze and Hutchinson, 1988). Whether such biological dynamics of hypobiosis or arrested development documented in temperate regions in horses exists in donkeys under tropical condition is not well known.

A study conducted in Zimbabwe by Eysker (1987) showed that survival of cyathostomins throughout the unfavorable dry season primarily occurred in the adult stage and not as inhibited early third stage larvae, suggesting that cyathostomins may not undergo inhibited development in donkeys. Another recent study in South Africa (Matthee et al., 2002) also showed that all recovered mucosal larvae from necropsied donkeys were larger developed L4. Although the necropsies took place in the mid summer, the complete absence of early third stage larvae (EL3) in the mucosa may be attributed to the fact that there was no need for hypobiosis during the preceding winter, thus the newly-acquired infective larvae continued development or all the hypobiotic larvae might have undergone development, which is most unlikely. Moreover, according to an observation made by the author (four years of clinical work experience with donkeys), profuse and sudden onset of diarrhea, which is the typical acute clinical syndrome associated with larval cyathostominosis, formerly called cyathostomiasis or cyathostomosis (Lichtenfels et al., 1998), in horses (Paul, 1990, Love, 1992; Love et al., 1992; Love, 1995, Love et al., 1999) is not seen in working donkeys in Ethiopia. In general this may suggest that arrested larval development does not play an important role in the survival of cyathostomins of donkeys although it cannot be ruled out. Rather they survive primarily as long-living adult worms as suggested by Eysker (1987). Detailed and thorough investigation, however, is needed before one comes to the conclusion that cyathostomins in donkeys do not undergo arrested larval development.

Table 1.4. Species of cyathostomins described in donkeys and their prevalence (%).

	Countries							
	B. Faso ¹	Chad ²	Ethiopia ³	Kenya ⁴	S. Africa ⁵	Turkey ⁶	USA ⁷	Zimbabwe ⁸
Number examined	30	183	7	6	16	10	4	14
Cyathostomins								
<i>Cyathostomum alveatum</i>	-	-	-	-	56.3	+	25.0	93.0
<i>C. catinatum</i>	7.0	-	100	50.0	31.3	+	75.0	-
<i>C. coronatum</i>	-	-	71.4	33.0	50.0	+	50.0	86.0
<i>C. labiatum</i>	-	-	100	-	87.5	+	100	-
<i>C. labratum</i>	-	-	85.7	-	31.3	+	100	-
<i>C. montgomeryi</i>	-	-	14.0	-	100	+	-	100
<i>C. pateratum</i>	-	-	-	-	12.5	+	25.0	-
<i>C. poculatum</i>	-	-	-	-	-	-	25.0	-
<i>C. tetracantum</i>	50.0	1.0	100	-	68.8	+	50.0	86.0
<i>Cyathostomum</i> sp.	-	-	-	-	18.8	-	-	-
<i>Cylicocyclus adersi</i>	-	-	-	-	-	-	-	71.0
<i>C. asini n.sp.</i>	-	-	-	-	87.5	-	-	-
<i>Cylicocyclus</i> sp. b	-	-	-	-	6.3	-	-	-
<i>C. auriculatus</i>	57.0	49.0	100	17.0	75.0	+	50.0	93.0
<i>C. brevicapsulatus</i>	-	-	85.7	-	-	-	-	-
<i>C. elongatus</i>	27.0	-	100	-	62.5	+	25.0	79.0
<i>C. insigne</i>	3.0	-	-	-	50.0	+	50.0	-
<i>C. leptostomum</i>	-	-	-	-	18.8	+	100	-
<i>C. nasatus</i>	-	-	100	-	31.3	+	100	100

Table 1.4. continued.....

	Countries							
	B. Faso ¹	Chad ²	Ethiopia ³	Kenya ⁴	S. Africa ⁵	Turkey ⁶	USA ⁷	Zimbabwe ⁸
Number examined	30	183	7	6	16	10	4	14
Cyathostomins								
<i>C. radiatus</i>	-	-	-	83	56.3	+	75	-
<i>C. ultrajectinus</i>	-	-	-	17	-	-	25	-
<i>Cylicostephanus asymmetricus</i>	-	-	-	-	93.8	-	-	-
<i>C. bidentatus</i>	-	-	100	-	-	-	-	100
<i>C. calicatus</i>	-	11	42.9	-	50	+	50	-
<i>C. goldi</i>	13	-	-	-	37.5	+	75	-
<i>C. hybridus</i>	-	-	-	-	-	+	-	-
<i>C. longibursatus</i>	-	32	100	-	50	+	100	-
<i>C. minutus</i>	-	-	85.7	-	87.5	+	100	100
<i>Cylicodontophorus bicoronatus</i>	-	-	100	-	-	+	75	7
<i>Poteriostomum ratzii</i>	-	-	100	-	-	+	25	-
<i>P. imparidentatum</i>	-	-	-	-	-	+	25	-
<i>Parapoteirostomum schuermanni</i>	-	-	-	-	18.8	-	-	-
<i>Gyalocephalus cappitatus</i>	-	-	100	-	-	+	75	-

+ Described

1. Vercruysse et al., 1986; 2. Graber, 1970; 3. Getachew, 1999; 4. Lewa et al., 1998; 5. Matthee et al., 2000, Matthee et al., 2002; 6. Burgu et al., 1995; 7. Tolliver et al., 1985;

8. Eysker and Pandey, 1989 .

Previously, the pathogenic importance of cyathostomins has been overlooked and veterinarians have tended to regard them as having low or limited pathogenicity. Until recently, the more obvious lesions caused by *Strongylus vulgaris*, as well as the frequent finding of very large burdens of cyathostomins in apparently healthy horses (Love et al. 1998) resulted in a perception that cyathostomins were not pathogenic. After a marked decrease in the prevalence of large strongyles, particularly *S. vulgaris* because of the widespread use of modern anthelmintics (Austin, 1994), there have been an increasing number of reports worldwide of larval cyathostominosis in horses. The pathogenic roles of cyathostomins are no longer questioned and they are now frequently referred to as the principal pathogens of horses (Herd, 1990a; Love et al., 1992; Mair, 1993, 1994; Kelly and Fogarty, 1993; Love, 1995, Paul, 1998; Love et al., 1999). In larval cyathostominosis there is up to 50% mortality, even following intensive treatment (Proudman and Matthews, 2000). Moreover, cyathostomins have surpassed *S. vulgaris* as a major cause of verminous colic and are recognised as one of the major causes of colic in most regions of equine industry (Uhlinger, 1990).

Cyathostomins as a group have features that are relevant to clinical disease: the predilection for the large intestine; the propensity for extremely prolonged, arrested larval development in intestinal mucosa, and a markedly reduced susceptibility to the action of anthelmintics during such time (Love, 1995). Larval cyathostominosis is associated with diarrhoea, severe enteritis that occurs following synchronized reactivation of arrested larvae in the gut wall, weight loss, subcutaneous oedema and/or pyrexia (Herd, 1990a; Reilly et al., 1993; Mair, 1993, 1994; Murphy et al., 1997; Love et al., 1999; Proudman and Matthews, 2000).

1.4.2. Gasterophilids.

Gastrophilus larvae in the digestive tracts are the only arthropods reported frequently from donkeys from African countries (Table 1.5), and rare in other countries except from USA (Tolliver et al., 1985) and Germany (Beelitz et al., 1996). Although eight species are known to occur in equids (Zumt, 1965), only *Gastrophilus intestinalis* and *Gastrophilus nasalis* are frequently reported. *G. pecorum* (Graber & Gruvel, 1964; Kaboret et al., 1986; Kilani et al., 1987; Matthee et al., 2000); *G. ternicinatus* (Kaboret et al 1986); *G. inermis* (Kilani, 1986) and *G. haemorroidalis* (Khallaayoune, 1991) are very rarely reported.

Although these arthropods are considered to be non-pathogenic and well tolerated by their hosts (Soulsby, 1982), they have been incriminated in inducing gastric erosion, ulcers, abscesses, ruptures and peritonitis (Rooney, 1964; Waddell, 1972; Pandey et al., 1980; Dart et al., 1987) in horses. According to a study by Cogley and Cogley (1999) and Sequeira et al. (2001) the common macroscopic lesions associated with *G. intestinalis* and *G. nasalis* were the raising of gastric tissues into well-circumscribed ulcerated mounds surrounded by distinct rims and erosions.

Table 1.5. Species of *Gasterophilus* larvae described in donkeys and their prevalence (%).

	Countries									
	B. Faso ¹		Chad ²		Tunisia ³		Egypt ⁴		Ethiopia ⁵	
	Morocco ⁷		S. Africa ⁸		Zimbabwe ⁹					
Number examined	30		67	91	118	7	14	366	16	14
Recovery	pm		pm	pm	pm	pm	pm	pm	pm	pm*
<i>Gastrophilus intestinalis</i>	100		75	90	98	100	33(6)	98	75	100
<i>G. nasalis</i>	87		54	70	87	100	100(8)	96	-	7
<i>G. pecorum</i>	87		9	48	-	-	-	-	25	-
<i>G. haemorrhoidalis</i>	7		-	-	-	-	-	70	-	-
<i>G. ternicinctus</i>	27		-	-	-	-	-	-	-	-
<i>G. inermis</i>	-		-	57	54	-	-	-	-	-

*pm- postmortem

1.Kaboret et al., 1986; 2.Graber and Gruvel, 1964; 3.Kilani et al., 1986; 4. Hilali et al., 1987; 5. Getachew, 1999; 6. Lewa et al., 1998, Karanja et al., 1994; 7. Pandey et al., 1992b, Khallaayoune, 1991; 8. Mathee et al., 2000, Mathee et al., 2002; 9. Pandey and Eysker, 1988, 1990.

1.4.3. Equine ascariasis

Parascaris equorum is among the largest nematodes of the family ascarididae infecting equine species (Urquhart et al., 1996). Both larvae and adult stages are of veterinary importance. The adults live in the small intestine. The duodenum and proximal ileum are the preferred sites for parasitic growth and development although in heavy infestations, they may be found throughout the length of the small intestine (Clayton, 1986). *P. equorum* has a direct life cycle. Eggs produced by the mature adult females are passed in faeces and under ideal environmental conditions (25 °C to 35°C) reach the infective stage (L2) within 10-14 days (Southwood, et al. 1998). Immature ascarid larvae that are ingested migrate through the liver and the lungs before they return to the small intestine via the tracheo-oesophageal route to complete their development (Lyons et al, 1976; Clayton and Duncan, 1977, 1979a; Srihakim and Swerczek, 1978). Experimental studies have demonstrated the prepatent period of *P. equorum* to be in the range 72 to 110 days (Bell et al., 1973; Lyons et al., 1976; Clayton and Duncan, 1977, 1979a), while in naturally-acquired infections, foals begin to pass eggs at 77-98 days of age (Russell, 1948; Todd and Doherty, 1951). The similarity of the prepatent periods of experimentally and naturally acquired infections suggests that foals become infected with *P. equorum* soon after birth.

P. equorum is an important parasite of younger horses, particularly sucklings and weanlings (Russell, 1948; Poynter, 1970; Austin et al., 1990; Southwood et al., 1998). The infection has a worldwide distribution, and is usually present wherever horses and other equidae are raised (Drudge and Lyons, 1983; Joseph, 1986; Austin et al., 1990). Prevalences of 31-61% in horses younger than one year of age (Lyons et al., 1981; Lyons et al., 1985; Haas, 1979) and 25% in horses older than one year of age (Haas, 1979) have been reported. Unlike the strongylids, which have a free-living preparasitic stage able to migrate away from the dung, the infective ascarid's egg is unable to spread by active means (Clayton, 1986). The main

important factors in the epidemiology of parascariasis are therefore, the high fecundity of the adult female worm and the extreme resistance of the eggs to adverse environment conditions and to disinfectants. This ensures their persistence for several years. In addition eggs are adhesive which facilitates passive spread on to stable surfaces, implements, footwears and pasture (Fairbairn, 1957; Clayton and Duncan, 1979a; Clayton, 1986; Austin et al., 1990; Ihler, 1995; Southwood et al., 1998). It has been reported that a mature female can produce more than 200,000 eggs per day (Bello, 1982; Pillier and Davice, 1996). This shows that a patent infection of equids only once in a period of a few years is sufficient to perpetuate the parasite, and the potential for contamination of soil with *P. equorum* eggs is high (Austin et al, 1990).

Infections are of concern to veterinarians and horse owners because of economic losses caused by reduced weight gain, and occasional deaths caused by intestinal obstruction or rupture (Clayton and Duncan, 1978; Clayton, 1986; Joseph, 1986). A study by Clayton and Duncan (1978) showed that over a period of 3 months the weight gain of ascarid-infected foals was reduced by as much as 50% compared with parasite free control foals. According to Joseph (1986), this parasite caused an annual loss of 2 million dollars in horses and mules in the United States in 1954.

A wide variety of clinical signs have been attributed to parascariasis, including coughing and nasal discharge during the larval migratory phase; depression, lethargy, unthriftiness, pot-bellied appearance, seizures and weight loss associated with large masses of worms in the intestine (Russell, 1948; Wiltshire, 1954; Clayton and Duncan, 1978; Clayton, 1986; Austin et al., 1990; Southwood et al., 1996). In field cases the disease picture is complicated by the presence of multiple pathogenic agents, making it difficult to determine a precise cause-and-effect relationship. Mono-specific infections in young foals of less than 6 months of age,

however, have shown that the migratory phase was characterised by the presence of respiratory signs (Clayton and Duncan, 1977, 1978; Srihakim and Swerczek, 1978). Studies combining experimental *P. equorum* infection with strategic anthelmintic treatments showed that unthriftiness develops as a result of the presence of large numbers of worms in the gut lumen and not as a consequence of hepatic or pulmonary damage caused by larval migration (Clayton et al., 1980).

The use of mono-specific infections in worm-free foals indicates that the pathogenicity of *P. equorum* can be attributed both to the migration of larvae into liver and lungs, and the intestinal stages. The hepatic migration of larvae produces focal petechial haemorrhages and small foci of peripheral necrosis followed by widely scattered white foci and nodular lesions due to the infiltration by eosinophils and lymphocytes (Clayton and Brown 1979; Srihakim and Swerczek, 1978 Clayton, 1986). Thrombosis and the development of lymphoreticular nodules and granuloma were reported in older animals (Clayton and Brown 1979). The pulmonary pathology associated with larval migration includes petechial haemorrhages with localized oedema and atelectasis followed by eosinophilic and lymphocytic infiltration producing multiple yellowish-white nodules, and pneumonia (Nichols et al. 1978; Clayton, 1978; Srihakim and Swerczek 1978; Joseph, 1986).

The intestinal stages of *P. equorum* are the most pathogenic, causing mechanical obstruction leading to colic, intussusceptions, bowel rupture, peritonitis and death (Wiltshire, 1954; Orr, 1972; Clayton and Duncan, 1977; Drudge and Lyons, 1983; Dipietro et al., 1983; Adair, 1990; Mueller and Baxter, 1992; Cohen and Chaffin, 1994; Vatistas et al., 1996). Chronic absorption of whole-worm antigen has been suggested to cause stasis and lead to impaction and intestinal rupture (Bello, 1982). Enteritis and diarrhoea due to irritation of intestinal mucosa by numerous parasites, and abdominal abscesses secondary to penetration of the

bowel have also been reported (Austin, 1990; Dipietro et al., 1983; Joseph, 1986). Moreover, intestinal stages can impair digestion resulting in reduced weight gain and a major infection can interfere with albumin synthesis and amino acid absorption (Clayton, 1986; Clayton and Duncan, 1978; Clayton et al., 1980).

P. equorum is a highly antigenic and immunogenic parasite in younger horses and ponies. Experimental studies have shown that, even in the absence of previous exposure, significant age-dependent immunity develops by six months of age (Bello et al., 1974; Clayton and Duncan, 1979b; Bello, 1985; Dipietro and Todd, 1988). Surveys in various part of the world have shown that the incidence of infection decreases from 6 months of age onwards (Poynter, 1970; Clayton, 1986; Austin et al., 1990; Southwood et al., 1998). It has been suggested that the immune response arrests larval migration in the liver and/or lungs (Clayton, 1986) and probably also plays a significant role in the spontaneous elimination of worms from the digestive tract (Clayton and Duncan, 1977). A study by Bello (1985) has shown that serum precipitin titres against whole-worm antigen increase as foals and yearlings develop active immunity, and adult horses maintain high titres. It was reported that without the benefits of anthelmintics, most horses pass very low egg count or stop passing eggs by one year of age (Bello, 1985; Austin et al., 1990).

1.4.3.1 Ascarid infection in donkeys

Research on ascaridinae from African zebra and zebras living in the Vincennes zoological garden in France, and those from horses confirmed that they belong to the same species, *Parascaris equorum* (Ansel et al., 1974). Ascarid infections have been reported in donkeys from various regions of the world (Table 1.6). Most of the reports were however from small numbers of animals, and the information is rather fragmentary.

Table 1.6. Infection of *Parascaris equorum* in donkeys in various regions.

Countries	N	Method	Number positive	Sources
B. Faso	30	pwr	13(43)	Vercruysse et al., 1986
Chad	183	pwr	132(72)	Graber, 1970
Ethiopia	13	pwr	6(46)	Feseha et al. 1991; Getachew, 1999
Germany	106	cos.	3(2.8)	Gothé & Heil, 1984
India	83	cos.	7(8.4)	Kotwal, et al. 2000
Iraq	12	pwr	+	Daoud & Al-Alousi, 1995
Italy	36	cos.	+	Ricci & Sabatini, 1992
Kenya	6	pwr	1(17)	Lewa et al. 1998
Morocco	168	pwr	62(37)	Khallaayoune, 1991
S. Africa	16	pwr	4(25)	Matthee et al. 2000, 2002
Turkey	10	pwr	2(20)	Burgu et al. 1995
USA	8	pwr	1(13)	Tolliver et al. 1985
Zimbabwe	14	pwr	7(50)	Pandey & Eysker, 1990

N- number of donkeys examined; pwr- post-mortem worm recovery; cos- coprology. Numbers in parentheses indicate percentage. +- Some reported parasite.

A coprological study by Wells et al. (1998) to determine helminth levels of working donkeys under different management systems in South Africa showed that the highest infection prevalence and highest egg counts were found in the 6 months to 8 years age groups, which is contrary to the results in horses (Russell, 1948; Poynter, 1970; Austin et al., 1990; Southwood et al., 1998). According to Soulsby (1982) and Proudman and Matthews (2000), patent infections are occasionally found in mature horses, but they are of little clinical significance and play no part in the transmission of the disease. Tolliver et al. (1985) in his study in Kentucky recorded parascaris only from one out of 8 donkeys necropsied. They speculated that this could have been because the donkeys were too old to have harboured this parasite. On the other hand, Vercruysse et al. (1986) reported a prevalence of 43% (n=30) and egg counts ranging from 100 – 500 epg in adult donkeys in Burkina Faso.

1.4.4. Equine tapeworms

Tapeworms or cestodes belong to the group of parasites called flatworms or platyhelminths. Three cestodes species, in two genera of the family Anoplocephalidae, occur as adults in domestic equids. These are *Anoplocephala perfoliata*, *A. magna* and *Anoplocephaloides mamillana* (formerly *Paranoplocephala mamillana*) (Soulsby, 1968; Lichtenfels, 1975; Proudman et al., 1995). A fourth species, *Moniezia pallida*, has been reported in South Africa and Angola (Soulsby, 1965; Georgi, 1974; Spasskii, 1951).

Apart from their size and shape, various structural differences separate the species of equine cestodes (Lichtenfels, 1975). Their predilection sites within the gastrointestinal tract also vary. *A. magna*, the largest equine tapeworm, is located in the posterior small intestine, particularly in the jejunum, and the smallest equine tapeworm, *A. mamillana*, lives in the anterior small intestine, duodenum, and occasionally the stomach (Soulsby, 1982; Urquhart et al., 1996). Despite the widely accepted belief that the ileocaecal junction is the predilection site for *A. perfoliata* (Soulsby, 1982; French and Chapman, 1992), a study by Williamson et al. (1997) on the distribution of *A. perfoliata* in the intestines of 130 horses showed that *A. perfoliata* was found attached in four regions of the gastrointestinal tract. According to this study, 81% of the worms were found attached to the caecal wall, 17% to the ileocaecal junction, 1.7% to the terminal ileum and 0.2% to the ventral colon. A similar study by Fogarty et al. (1994) found a high percentage of horses (47%, n=363) with worms attached exclusively to the caecal wall, whilst only 9% had worms attached only at the ileocaecal junction. The remaining 44% had worms at both sites. The most favorable environment for the worms however, seems to be near the ileocaecal junction, with 93% of all clusters being within 10 cm of this site (Williamson et al., 1997).

The life cycle of equine cestodes is indirect requiring an acarine intermediate host, in which the immature stage, cysticercoids, develop. These acarines are generally referred to as oribatid mites (Fukui, 1960) and they exist as free-living forms, more commonly on permanent pastures (Jacobs, 1986; Bain and Kelly, 1977). They can be exceptionally numerous, and densities as high as 20,000 mites per square metre have been recorded (Jacobs, 1986). Equids accidentally ingest these mites while grazing and become infected with cysticercoids. Within the intestine the cysticercoids develop into adult tapeworms. Adults release gravid proglottids and eggs within the gravid proglottids or free eggs, pass in faeces and are eaten by free-living oribatid mites. Development of infective cysticercoids in mites takes 2 to 4 months, and maturation of adult tapeworms in the host requires an additional 4 to 10 weeks (Soulsby, 1982; French and Chapman, 1992; Urquhart et al., 1996).

A. magna and *A. mamillana* were the most common tapeworms described in horses at necropsy in the early 1900s (Hall and Hoskins, 1918; Olsen, 1938) and *A. perfoliata* was a rare finding (French and Chapman, 1992). However, recent studies have shown that *A. perfoliata* is the most common of equine tapeworms with prevalences ranging from 18-87% in various geographical regions of the world (Bain and Kelly, 1977; Bell, 1979; English, 1979; Slocombe, 1979; Lyons et al., 1984; Reinemeyer, et al., 1984; Dunsmore and Jue Sue, 1985; Torbert et al., 1986; Imrie and Jacobs, 1987; Owen et al., 1988; Pearson, 1993; Yoshinara et al., 1993; Fogarty et al., 1994; Bucknell et al., 1995; Nilsson et al., 1995; Ihler et al., 1995; Williamson et al., 1997; Lyon et al., 2000).

The increasing prevalence of *A. perfoliata* has been attributed by some authors to changes in climate that may directly influence the propagation of oribatid mites (Geering and Johnson, 1990) and also to the widespread use of ivermectin, (Edwards, 1986; Proudman and Holdstock, 2000), which has no documented cestocidal effect. A study by French and

Chapman (1992) carried out for five years on the intensity and prevalence of equine tapeworm infection in pony herds showed no significant effect for the use of ivermectin. Similarly, Nilsson, et al. (1995) and Torbert, et al. (1986) indicated that the level of infection appeared to be unaffected by the use of anthelmintics against nematodes. Thus the increased uses of ivermectins do not explain the increased prevalence of *A. perfoliata* infection in equids.

Horses of all ages appear to be affected by tapeworm infection: cestodosis. Some authors have stated that the prevalence is highest in young horses (Fukui, 1960; Dunn, 1978; Bello, 1979; Reinemeyer et al., 1984; Urquhart et al., 1996), while others have not reported such association (Bain and Kelly, 1977; Hass, 1979; Lyons et al., 1984; Owen et al., 1988). Moreover, there is no apparent acquired or age resistance to tapeworm infections in horses (Bain and Kelly, 1977; Hass, 1979; Lyons et al., 1984; Tolliver et al., 1987; Owen et al., 1988).

There appears to be seasonal variations in the prevalence of cestode infections in horses with the greatest prevalence being seen during the winter months both in the northern and southern hemispheres (Bain and Kelly, 1977; Hass, 1979; Lyons et al., 1984; Hoglund et al., 1995). This has been attributed to the animals' greater exposure to the intermediate host, the oribatid mites, during these periods (Owen et al., 1988).

Although the life cycles of equine tapeworms have been known for many years (French and Chapman, 1992), knowledge about the parasitic phase of the life cycle and the pathogenesis of the infection is limited. Equine tapeworms were considered for many years to be accidental findings and regarded as relatively harmless. Their significance as pathogens was not clear. Infections usually went undetected because of the difficulties with coprological

diagnosis in live animals (Beroza et al., 1986; Proudman and Edwards, 1992) and because no clearly-defined clinical symptoms were associated with them (Soulsby, 1982; Urquhart et al. 1996). However, reports on equine cestodosis have increased during the past two decades. Numerous clinical cases have been associated with tapeworm infection. In particular *A. perfoliata*, has been associated with significant gastrointestinal disease, often necessitating surgical intervention. The parasite has been incriminated as a cause of ileal, ileocaecal, caecocaecal and caecocolic intussusceptions (Rodgers, 1966; Foerner et al., 1980; Barclay et al., 1982; Beroza, 1983, 1986; Edwards, 1986; Cosgrove et al., 1986; Owen et al., 1989; Williamson, et al., 1997), caecal perforation leading to peritonitis (Barclay et al., 1982; Beroza et al., 1983, 1985), intestinal obstruction, caused either by mass of worms attached to the wall of the caecum or terminal ileum (Slocombe, 1979; Beroza et al., 1983; Carmel, 1988) or by mechanical obstruction in the ileocaecal junction (Bello, 1979; Carmel, 1988); and ileocaecal colic, spasmodic colic and ileal impaction (Proudman and Edward, 1993; Proudman et al., 1998; Proudman and Trees, 1999; Proudman and Holdstock, 2000).

The major features of lesions produced by the attachment of *A. perfoliata* have been well described (Bain and Kelly, 1977; Beroza et al., 1985; Pearson et al., 1993; Fogarty et al., 1994; French and Chapman, 1994; Nilsson et al., 1995; Ihler et al., 1995; Williamson et al., 1997; Rodriguez-Bertos et al., 1999). These include ulceration, diphtheritic membrane production, catarrhal enteritis, thickening of mucosa, submucosa and lamina propria, oedema and excessive granulation tissues. Studies by Pearson et al. (1993), Fogarty et al. (1994) and Williamson et al. (1997) have shown that the severity and depth of lesions, particularly ulceration of the mucosa, increased as the number of tapeworms attached in the immediately surrounding area increased. Ulceration of the ileocaecal junction was often more severe than that of the caecal wall when equivalent number of worms were attached in each of the two regions. There is only one case reported in which *A. magna* was associated with the rupture

of the small intestine. This was in a nine month-old colt, which lead to death (Oliver et al., 1977).

1.4.4.1 Diagnosis of cestode infection in equids

Coprological diagnosis

The discharge of gravid tapeworm segments (proglottids) is sporadic. Their integrity is often lost before being shed with the faeces, and the eggs are not equally distributed in the faecal masses (Nilsson, et al., 1995). Consequently, there is usually a low probability that eggs are identified in infected equids. Thus flotation techniques are insensitive for the definitive diagnosis of tapeworm infection in individual animals (Slocombe, 1979; Beroza et. al., 1986; Meana et al., 1998). More sensitive centrifugation/flotation techniques for the detection of tapeworm eggs from faecal masses were developed (Proudman and Edwards, 1992; Williamson, et al., 1998; Meana et al., 1998).

Although, studies by Proudman and Edwards (1992) and Williamson et al. (1998) have shown no statistically significant correlation between the number of eggs observed and the number of tapeworms present, the likelihood of finding eggs is significantly higher in horses with a high worm burden (Proudman and Edwards, 1992; Nilsson, et al., 1995; Williamson, et al., 1998). The significantly higher probability of the technique in identifying horses most at risk of clinical disease makes it considerably more valuable to the clinician than the overall sensitivity would indicate. The technique could also be used as a tool for the investigation of whether there are seasonal or diurnal variations in egg output, host factors involved with egg output and whether eggs are excreted continuously or intermittently (Proudman and Edwards, 1992). It may also be acceptable for diagnosis and epidemiological studies at the herd level (Nilsson, et al., 1995) where other more sensitive tests are not available.

Immunodiagnosis

Coprological diagnosis of equine tapeworm infection is highly specific as the eggs have a characteristic appearance. But faeces of infected horses contain only small numbers of eggs at any time resulting in poor sensitivity (Proudman and Trees 1999). Therefore the centrifugation/flotation technique may be inadequate to diagnose equine cestodosis (Proudman and Edward, 1992; Ihler et al., 1995; Williamson, et al., 1998). The need for an improved diagnostic method has led to diagnostic tests based upon the host's immune response to infection. Serum antibody responses to scolex somatic antigen, whole worm somatic antigen and excretory/secretory (E/S) antigen have been investigated (Proudman and Trees, 1996a; Hoglund et al., 1995). Western blot analysis of the antibody response to E/S antigen revealed immunodominant antigens at 12 and 13 kDa (Proudman and Trees, 1996a). Furthermore, the antibody response to this antigen doublet was isotype restricted to IgG(T). Based on these findings, an anti-12/13kDa IgG(T) enzyme-linked immunosorbent assay (ELISA) was developed for the serodiagnosis of equine cestodosis (Proudman and Trees, 1996b).

Validation of the assay with sera from horses of known parasite status revealed an overall sensitivity of 68% (n= 38), a slight improvement over the coprological method (Proudman and Trees, 1996a). However, and more importantly, significant correlation ($r=0.63$) was obtained between ELISA optical density values and infection intensity in individual animals (Proudman and Trees, 1996b). The observed correlation with infection intensity is of particular importance in relation to clinical disease and to parasite epidemiology, as intestinal helminth disease is related to infection intensity and not to the sole presence of parasites (Anderson and May, 1991).

1.4.4.2 Cestodes infection in donkeys

The species of cestodes infecting donkeys are similar to those described in horses (Table 1.7). All three species have been reported only from Morocco (Khallaayoune, 1991) and *A. magna* from Burkina Faso, Chad and Morocco (Graber, 1970; Vercruysse et al., 1986; Khallaayoune, 1991). The most prevalent species was *A. perfoliata*, as it is in horses (French and Chapman, 1992; Owen et al., 1986). Most studies, however, reported the presence of these parasites while conducting postmortem examinations or through the standard flotation technique, and were not specifically targeted at the detection of cestodes. The exception to this is a pilot study made by Proudman and Ellis (1992), which revealed 27% (n=29) and 40% (n=15) by coprological and serological diagnosis, respectively.

Table 1.7. Species of tapeworm described and their prevalence (%) in donkeys.

Countries	N	Method	Cestode species (%)		
			<i>A. perfoliata</i>	<i>A. magna</i>	<i>A. mamillana.</i>
B. Faso ^{1*}	30	pwr**	-	8(27)	-
Chad ²	183	pwr	-	11(6)	-
Ethiopia ³	7	pwr	1(14)	-	-
Germany ⁴	37	cos	+	-	-
Kenya ⁵	6	pwr	2(33)	-	-
Morocco ⁶	168	pwr	4(2)	2(1)	2(1)
S. Africa ⁷	16	pwr	4(25)	-	-
Turkey ⁸	110	pwr	11(10)	-	-
UK ⁹	29	cos	8(27)	-	-

N- number of donkeys examined. **pwr postmortem worm recovery; cos- coprology. Numbers in parentheses indicate percentage.

1*. Graber, 1970, 2. Vercruysse et al., 1986, 3. Getachew, 1999, 4. Beelitz et al., 1996, 5. Lewa et al. 1998, 6. Khallaayoune, 1991, 7. Matthee et al., 2000, Matthee et al. 2002, 8. Burgu et al. 1995, Gonenc, 1999, 9. Proudman and Ellis, 1995.

According to Trawford (1997), less than 1% of the donkeys admitted to the Donkey Sanctuary, England, had evidence of tapeworm infection. This is attributed to the poor habitat for the oribatid mites on the sanctuary farms. Most of the reports from postmortem findings were from a small number of animals, which may not show the true prevalence of the cestode infection in donkeys. In addition, the low sensitivity of the flotation techniques used may also affect the uniformity of this information.

1.4.5. *Fasciola*

Flukes of the family Fasciolidae, Dicrocoeliidae, Paramphistomatidae and Schistosomatidae are the major trematodes of veterinary importance. The family fasciolidae, consisting of three important genera: *Fasciola*, *Fascioloides* and *Fasciolopsis* (Soulsby, 1982; Urquhart et al., 1996) is by far the most important. Among these, members of the genus *Fasciola*, commonly known as liver flukes, are responsible for widespread morbidity and mortality in domestic ruminants (Boray, 1982; Torgerson and Claxton, 1999) and for human fasciolosis (WHO, 1995; Mas-Coma et al., 1999). The two most important species of this genus are *F. hepatica*, found in temperate areas and in cooler high altitude areas in the tropics and subtropics, and *F. gigantica*, which predominates in tropical areas (Urquhart, et al., 1996; Torgerson and Claxton, 1999; Spithill et al., 1999).

Adult liver flukes are found in bile ducts and immature parasites in the liver parenchyma. Occasionally aberrant flukes become encapsulated in other organs such as the lung, pancreas and lymph nodes (Urquhart et al., 1996; Andrews, 1999). They have an indirect life cycle. Snails of the genus *Lymnaea*, which has a wide geographical distribution throughout the world, are the intermediate hosts (Soulsby, 1982; Urquhart et al., 1996, Andrews, 1999). In contrast to nematodes where egg can develop into only a single adult, one trematode's egg may eventually develop into hundreds of adults due to the phenomena of paedogenesis in the

molluscan intermediate host. The prepatent period varies between the two *Fasciola* species. It is 10-12 weeks for *F. hepatica* and 13-16 weeks for *F. gigantica* in bovine and ovine species (Urquhart et al., 1996; Andrews, 1999; Spithill et al., 1999).

Liver flukes may parasitise a large number of definitive host species among livestock, human and wild animals (Boray, 1982; Torgerson and Claxton, 1999; Mas-Coma et al., 1999). There is a considerable variation between definitive host species in susceptibility to infection and in immune response against liver fluke (Mulcahy et al., 1999). Cattle have proved to develop acquired immunological resistance against *F. hepatica* (Kendall, 1967; Mulcahy et al., 1999). Sheep, on the other hand develop little or no acquired protective immunity (Haroun and Hillyer, 1986) although there are breeds of sheep, like the Indonesian Thin Tailed (ITT) and Merino, which are found to be resistant to *F. hepatica* and *F. gigantica* (Roberts and Suhardono, 1996; Roberts et al. 1996; Roberts et al., 1997). Equids are generally considered more resistant than domestic ruminants (Boray, 1969; Nansen et al., 1975). However, some studies have shown a high prevalence of fasciolosis, particularly in donkeys (Kearney 1974; Fahmy and El-Attar, 1990; Trawford and Tremlett, 1996; Haridy, et al., 2002).

The pathogenesis and clinical signs vary according to the phase of parasitic development in the liver and the species of host involved (Urquhart et al., 1996). The pathogenesis is in two phases: the first phase occurs during the migration of the immature flukes in the liver parenchyma and is associated with liver damage and haemorrhage. Sheep suffer the most from this acute form (Urquhart et al., 1996; Beham and Sangster, 1999). The second occurs when the parasites are in the bile ducts, and results from the haematophagic activities of adult flukes and from damage to the mucosa by their cuticular spines causing cholangitis and hepatic fibrosis (Urquhart et al., 1996). The main clinical features are anaemia, weight loss

and hypoproteinaemia (Soulsby, 1982; Urquhart et al., 1996; Beham and Sangster, 1999). In light infections, the clinical effects may not be readily discernible, but the parasites can have a significant effect on production due to an impairment of appetite and to their effect on post-absorptive metabolism of protein, carbohydrate and minerals, which is a typical feature of chronic fasciolosis, the most common form of the disease in cattle and sheep (Urquhart et al., 1996; Beham and Sangster, 1999).

Fasciolosis has long been recognised as an important veterinary problem, causing morbidity and mortality of livestock, particularly cattle and sheep. It is a significant constraint on productivity of ruminants with important economic consequences (Fabiyyi, 1987; Rim et al., 1994; Hillyer and Apt, 1997; Spithill et al., 1999; Torgerson and Claxton, 1999).

The public health importance of human fasciolosis has also increased in recent years and is recognized as an important re-emerging zoonotic disease (Chen and Mott, 1990; Mas-Coma and Bargues, 1997). Previously it was viewed as a secondary disease and generally it was believed that man was not a suitable host. Seventeen million humans are estimated as being infected with liver fluke worldwide and over 18 million are at risk of infection (Hopkins, 1992; WHO, 1995; Mas-Coma et al., 1999; Haseeb et al., 2002). In northern Bolivia in South America, a prevalence of 72-100% has been reported (Mas-Coma et al., 1999; Esteban et al., 1999; Esteban et al. 1997; Fuentes et al., 1999). The World Health Organization (WHO, 1995) has stressed the serious health problem caused by fasciolosis in several countries due to the pronounced pathogenicity in humans (Chen and Mott, 1990; Mas-Coma et al., 1999). There is a high variability in human prevalence in different regions, which may be partly explained by the behavioural and dietary habits of the people. Consumption of contaminated water and aquatic plants such as watercress and mint, and living close to livestock (Mas-

Coma, 1998; Cats et al., 2000; Doherty et al., 1995; Taira et al., 1997) are considered as some of the main risk factors.

1.4.5.1. Fasciolosis in Ethiopia

Many studies have shown that fasciolosis is one of the common parasitic diseases in Ethiopia. Although the most important fluke, with a distribution over three-quarter of the nation, is *F. hepatica*, *F. gigantica* is also found in Ethiopia, mainly in the western humid zone that encompasses approximately one quarter of the nation (Yilma and Malone, 1998). *F. hepatica* exists at altitudes above 2000 m, *F. gigantica* at altitudes below 1500 m and both at intermediate altitudes (Anon, 1972; Yilma and Malone, 1998). With the exception of a few foci in the north, east and south arid escarpments, fasciolosis is widespread in Ethiopia encompassing the major productive highland plateaus, areas inhabited by more than 85% of the country's human and livestock populations, including donkeys and horses (EMA, 1988). Together with malnutrition, trypanosomosis and nematodosis, fasciolosis is considered as one of the most significant constraints on livestock production in the country (Njau et al., 1988; ILCA, 1991; Ngategize et al., 1993; Yilma and Malone, 1998). Despite the widespread distribution of fasciolosis in the country, interest is focused on ovine and bovine species (Anon, 1972; Scot and Goll, 1977; Goll and Scott, 1978; Gemechu and Mamo, 1979; Lemma, 1985; Njau et al., 1988; Njau et al., 1989; Njau and Scholtens, 1991; Ngategize et al., 1993; Yilma and Malone, 1998). Equine fasciolosis has not been investigated in Ethiopia. However, there are some reports of the existence of the infection in donkeys (Feseha et al. 1991; Yilma et al., 1991; Getachew, 1999).

1.4.5.2. Equine trematodes

Helminths of the family fasciolidae are considered under unusual, accidental or occasional parasites of domestic equids (Lichtenfels, 1975). Only four species in two genera

(*Gastrodiscus*: *G. aegyptiacus*, *G. secundus*, *G. equi*, and *Psuedodiscus*: *P. collinsii*) have been recorded as primary trematode parasites of equids (Lichtenfels, 1975). Some consider fasciolosis as an unusual condition in equids whilst others consider them to be more resistant hosts than domestic ruminants (Boray, 1969; Nansen et al. 1975). In general they are believed to be of minor epidemiological importance in maintaining the life cycle of *Fasciola* spp in the field. Similarly, Morel (1959) reported frequent infection by *F. gigantica* in West African domestic ruminants but considered infections in human and solipeds rare. However, many reports and some studies have shown that fluke infections in horses and donkeys may not be as uncommon as previously suggested. This is particularly true in *Fasciola*-endemic areas and in areas where environmental conditions are favourable to the development of *Fasciola* and their intermediate host, *Lymnaea*.

Infection with liver flukes in **horses** (Moisant et al., 1972; Kearney, 1974; Owen, 1977; Alcaïno et al., 1983; Montes et al., 1984; Krawiecki, 1986; Rubilar et al., 1988; Alves et al., 1988; Soule et al., 1989; Dorchies et al., 1990; Fahmy and El-Attar, 1990; Celano et al., 1991; Toll Vera et al., 1995; Perler et al., 1997; Morales et al., 2000; Haridy et al., 2002) and **donkeys** (Collins, 1961; Pankhurst, 1963; Hatch, 1966; Graber, 1970; Kearney, 1974; Alcaïno et al., 1983; Pandey, 1983; Soule et al., 1989; Fahmy and El-Attar, 1990; Burgu et al., 1995; Beelitz et al., 1996; Hasslinger and El-Seify, 1996; Trawford and Tremlett, 1996; Ashmay and Diab, 1998; Getachew, 1999; Haridy et al., 2002; Haseeb et al., 2002) have been reported from many countries. Higher infection prevalences of flukes were reported in donkeys compared to horses.

Owen (1977) reported 38 cases of *Fasciola* infection in horses sharing pasture with dairy cows. According to his observations, some of the horses showed signs of poor appetite, lowered performance or loss of condition. Blood analysis showed slight anaemia. However,

he emphasised that very few horses showed the classic clinical signs of fluke infection seen in cattle and/or sheep (Urquhart et al., 1996). He inferred the infection on the basis of lowered performances and the use of fluke-infested pasture. This was later confirmed by laboratory faecal examination and post-mortem fluke recovery. Although the total number of horses and donkeys sampled was not mentioned, Kearney (1974) reported an infection prevalence of 77% and 91% in horses and donkeys, respectively, with high fluke burdens.

Many clinical cases and deaths from fluke infection have been reported both in horses (Moisant et al., 1972; Montes et al., 1984; Krawiecki, 1986; Dorchies et al., 1990; Celano et al., 1991; Toll Vera et al., 1995; Perler et al., 1997) and donkeys (Collins, 1961; Pankhurst, 1963; Green et al., 1968; Fahmy and El-Attar, 1990). Moisant et al (1972) reported an outbreak of fasciolosis in a French racing stable with an infection prevalence of 12% (n=96), in which one horse died, and proved to have been anaemic. He diagnosed the infection with indirect immunofluorescence and found 11 positive cases from 96 horses examined. According to his observation, horses were listless, had symptoms of colic and subicteric mucus membranes. Krawiecki (1986) and Perler et al (1997) found similar clinical signs in horses infected with liver fluke, in which they observed poor appetite, poor condition and a pronounced loss of weight and anaemia. Dorchies et al. (1990) conducted a controlled experiment, in which they inoculated 11 ponies with 800 *F. hepatica* metacercariae at 7-9 months of age. Two of the ponies died after 6-8 weeks of infection.

Biochemical analysis in experimentally infected horses with metacercariae of *F. hepatica* revealed a significantly increased plasma glutamate dehydrogenase and gamma-glutamyl transferase 3-5 months after infection (Soule et al., 1989). Montes et al. (1984) reported similar results indicating mild to moderate damage to the liver.

Similar clinical signs to those seen in horses were reported in donkeys infected with liver fluke. Pankhurst (1963) reported an outbreak of fasciolosis in a group of 20 donkeys used for “Donkey Derby” racing, which were in a very poor condition. Green et al (1968) noted the very poor condition of donkeys imported from Ireland, some of which could not thrive in spite of good feeding and care, and a number of deaths occurred. Faecal samples taken from 24 donkeys showed 12 positive cases for fluke eggs. Moreover, post-mortem examination revealed that some of the donkeys were heavily infected with *F. hepatica*. Of 60 post-mortem examinations performed on Irish donkeys transferred to the Donkey Sanctuary in UK, *F. hepatica* was recovered in 17% of the livers examined (Trawford and Tremlett, 1996). A similar finding was reported by Hatch (1966) in which he found 62.8% infected out of 43 donkeys examined.

The only post-mortem finding reported in horses infected with *F. hepatica* was that of Celano et al. (1991), in which he found gross abnormal dilation of the bile ducts, but no histopathologic findings were reported. Post-mortem examination of donkeys with *F. gigantica* infections by Fahmy and El-Attar (1990) revealed grey patches of 1-3 mm in diameter in the hepatic parenchyma, thickening of the bile duct branches and hyperplasia of the epithelial lining. Moreover, the histopathological findings revealed an increased amount of fibrous connective tissue together with infiltration of inflammatory cells in the portal area. Collins (1961) carried out a complete gross and histopathological investigation of livers of donkeys, which died of *F. hepatica* infection. His post-mortem findings demonstrated greyish white thread like scars over the entire surface of the liver extending into the hepatic parenchyma with thickening of the hepatic bile ducts. Histopathological findings included large amounts of connective tissue, which was collagen in nature, with denser and more mature bundles in areas of the larger interlobular bile ducts and the liver parenchyma

together with more diffuse inflammatory cells, primarily lymphocytes, plasma cells and polymorphonuclear leukocytes.

Fasciolosis in horses and donkeys has been treated successfully with carbontetrachloride (Pankhurst, 1963), hilomid (Green et al., 1968), rafoxanide (Moisant et al., 1972), oxyclosanide (Owen, 1977) and Closantel (Fischer, 1982; Krawiecki, 1986; Dorchies et al., 1990). The lately-developed fasciolicide, triclabendazole (Fasinex or Soferen; Ciba-Geigy) has excellent efficacy against all stages of liver flukes in cattle and sheep (Boray et al., 1983; Fairweather and Boray, 1999). It has also been found to be highly effective against liver flukes in horses (Rubilar et al., 1988; Perler et al., 1997) and donkeys (Trawford and Tremlett, 1996). Treated horses and donkeys no longer shed fluke ova and show improvement in body condition and performance.

1.5 Mathematical modelling

Disease is a result of interactions between components of the agent-host-environment complex (Hurd and Kaneene, 1993). The discipline of epidemiology has developed as a result of efforts to unravel the mysteries of this complex. The use of mathematical modelling as a descriptive and interpretive tool is common in scientific studies. Model construction, whether mathematical, verbal or diagrammatical is, in principle, the conceptual reduction of a complex biological or population-based process into a simple, idealized, and easily understandable sequence of events (Anderson and Nokes, 1991). In epidemiology, models are constructed to attempt to predict patterns of disease occurrence and what happens if various alternative control strategies are adopted. Roberts and Heesterbeek (1994) cited a number of reasons why one may wish to model the dynamics of infectious diseases. Among the most important are that “models provide insight into the mechanisms underlying

observed patterns of disease dynamics and enable the modeller to conduct thought experiments concerning the efficiency of plausible disease control strategies”.

Mathematical modelling of disease dynamics dates back to the 18th century when Daniel Bernoulli used a mathematical method to evaluate the effectiveness of the techniques of variolation/vaccination against smallpox (Bernoulli, 1766). Hamer (1906) and Ross (1908, 1911) were the first to formulate specific theories about the transmission of infectious diseases in simple but precise mathematical statements and to investigate the properties of these models resulting in solutions for the problems of regular recurrence of measles epidemics and the relationship between the number of mosquitoes and the incidence of malaria. Their work in conjunction with the studies of Ross and Hudson (1917), Soper (1929), and Kermack and McKendrick (1927) provided a firm theoretical framework for the investigation of observed epidemiological patterns. Since then progress has been made to the point where mathematical modelling is both realistic and useful, and its growth in the literature has been very rapid in human medicine (Macdonald, 1957; Garret-Jones, 1964; Dietz et al., 1974; Bailey 1975; Becker, 1979). More emphasis has been given to the application of control theory to epidemic models (Wickwire, 1977), the study of spatial spread of diseases (Mollison et al., 1977; Cliff et al., 1983; Kallen et al., 1985), the investigation of the mechanisms underlying recurrent epidemic behaviour (Hethcote et al., 1981; Aron and Schwartz, 1984), and the extension of threshold theory to encompass more complex deterministic and stochastic models (Anderson and May, 1978, 1979; May and Anderson, 1979; Ball, 1983).

If theoretical work is to play a role in the solution of practical problems in disease control and in the interpretation of observed trends, a much greater emphasis must be placed on data-oriented epidemiological studies. Models can be used effectively if supported with reliable

field and experimentally derived data relating to a disease's natural history and its biological dynamics. When used in association with diagnostic and experimental techniques, models can be useful guides to choosing the most efficient disease control techniques as well as resulting in increased understanding of disease dynamics. Modelling now has a broad remit including the conceptual representation of any real event in mathematical terms.

1.5.1 Types of mathematical models

Mathematical models are of a number of different types. They are built for several purposes; different kinds of models solve different kinds of problems. To know what type of model is appropriate to model a system, a number of factors have to be taken into account. Models can be based upon a discrete or continuous time scale; they can either consider individuals as discrete or a continuum; they can be either deterministic or stochastic in nature; Models can also provide results from analytical solutions or from simulation. Models can attempt to mimic our understanding of the real world closely, by being highly complex; or be more general, but give a good overview of the system under consideration (Innocent, 1998).

Depending on how they model the effects of chance models can either be stochastic or deterministic (King and Solkoline 1988). Deterministic models are models in which the values of input parameters can be fixed, and the results obtained do not take account of uncertainty, i.e., random variation. They produce a single output value for any given starting value and set of parameters every time they are run, and one can consistently determine the state of the model for a given set of initial values and parameters (Sherriff, 1996; Innocent, 1998).

Stochastic models on the other hand are models that describe processes or events, which are subject to random variation and chance, so that the outcomes occur with probability

(Thrushfield, 1995; Innocent, 1998). Such models work on the assumption that the future behaviour of the system is not predictable from the present or previous state, but is based on a set of probabilistic rules. Each run of the stochastic simulation will produce a different output value, even when identical initial conditions and parameters are used. This results in a set of output values, possibly theoretically infinitely large, from which meaningful statistics can be derived (Innocent, 1998).

A model is either discrete or continuous in relation to its mathematical treatment of time (Hurd and Kaneene, 1993). Discrete-time models divide time into units of equal duration and employ the algebra of finite difference equations or update rules (Gurney and Nisbet, 1998). On the other hand, continuous-time models treat time as a continuous variable and use differential equations. Continuous-time models or models based on differential equations are generally formulated in terms of the rate of change of either of the parasites or the number of hosts, or the subset of these populations, with respect to time (Sherriff, 1996).

Differential equation models describe a system by defining how measurements of the system vary over time and these measurements can be used to define the state of the system at any instant in time. These models may be used either by solving the equations explicitly, or by determining certain special properties of the system under study. It is possible to determine if any static points are present, and if these points are stable or not. Such static points represent equilibrium solutions to the set of differential equations and if stable, represent the situation in which a model would eventually come to rest (Innocent, 1998).

For the computational treatment of individuals, a model can be classified as a discrete-entity or continuous-entity (Hurd and Kaneene, 1993). Discrete-entity models track one individual at a time through the simulation model so that the behaviour of the system is the sum of the

behaviour of each individual. Continuous-entity models treat the number of individuals in any stage as a real number; such models can be computed in either continuous or discrete time (Ackerman, et al., 1984).

Solutions to models may be either analytic or simulation based. Analytical models depend on mathematical manipulation alone to explore the relationship between variables i.e. they seek a closed-form of solution to the state of the system (Bailey, 1982). Simulation models depend on numerical substitution (according to model-defined rules) to find the expected outcome of the mathematical formulation (Ackerman et al., 1984). The goal of simulation models is prediction of the performance of diseases in relation to conditions, which change either deterministically or stochastically. The life cycles of infectious agents, vectors and host can be integrated with environmental factors that vary from site to site and season to season to model the dynamics of a disease over time. Solving such a complex system as a finite number of equations analytically may be impossible, but simulation of the system using a computer model is relatively simple (Sherriff, 1996).

A first approach to modelling a system is to produce a visual representation of the system. One useful method of visualising a modelling problem is to produce a network diagram (Innocent, 1998). A network diagram consists of blocks with lines between them. Blocks can be considered to represent actions to be taken or state of nature and the lines illustrate the direction of flow between blocks. Thus in disease modelling a network may be used to demonstrate the flow of infection from infectious to susceptible individuals to determine the likely outcome of infection within group. Such diagrams are useful, not only because they represent a concise method of defining a model of our understanding of the system under investigation, but also because they lend themselves to a number of analytical techniques (Innocent, 1998). The network formulation is particularly attractive when time delays are a

feature of the life cycle (such as parasite's) being modelled and when the output response of a biological system is to be measured for a given input. The inability of many models to cope with changing inputs during the period of operation of the model can be circumvented using a network representation of a parasite's life cycle (Thrushfield, 1995).

From model's application perspective, a model can either be functional (descriptive or *a posteriori*) or structural (*a priori* or dynamic). Structural models try to portray the underlying mechanism of the disease transmission process for the purpose of making *a priori* predictions or exploring implications of assumptions. Most simulation models are of this type. Functional models, on the other hand, begin from the standpoint of modelling a process but their goal is to quantify observed phenomena (or to gain estimate of risk factors) with statistical analysis (Hurd and Kaneene, 1993).

1.5.2 Mathematical modelling in veterinary medicine.

Considerable progress has been made towards establishing mathematical descriptions of the epidemiology of diseases of human (Anderson and May, 1991; Isham and Medley, 1996), but less attention has been paid to modelling diseases of farm animals. Despite the existence of mathematical models for human disease since the 18th century (Bernoulli, 1766), it has only been in the last three to four decades that significant attention has been paid to the application and use of mathematical modelling in the study of animal health problems.

Models have been developed for the choice of disease control strategies, such as brucellosis (Hugh-Jones et al, 1976; Dietrich, et al., 1980), bovine tuberculosis (Trewella and Anderson, 1983; Barlow, 1994; Barlow et al., 1998), FMD (Gloster et al., 1981; Kao, 2002; Smith, 2001), paratuberculosis (Beyerbach et al., 2001) and Rabies (Macdonald and Baco, 1980). In the study of genetic resistance of animals to diseases (Barger, 1989), antigenic drift

(Hugh-Jones, 1986b), resistance to anthelmintics and acaricides (Gettinby, 1989; Smith, 1990; Barnes, et al., 1995) and vaccination strategies (Keeling et al. 2003), mathematical models have been found to be invaluable epidemiological tools. Models that assess the cost effective disease control strategies (Beal and McCallon, 1983; Dykhuizen, 1993; Sanson and Thornton, 1997), models of herd health, reproduction and production (Sorensen and Enevoldsen, 1992; Innocent, 1998; Gasqui et al., 2000; Blanc et al. 2001; Graat et al., 2001; White et al., 2002) have also been developed.

More recently, stochastic models as a tool for planning animal health surveys and interpreting screening-test results (Audige et al., 2001), for quantifying the consequences of selecting animals for resistance to microparasitic infectious diseases (MacKenzie and Bishop, 2001) and models for molecular strategies for overcoming antibiotic resistance (Tan et al., 2000) have been produced.

Multiple logistic regression models have become commonplace in clinical research over the last two decades due to their power and broad applicability to clinical problems in veterinary medicine (Hosmer and Lemeshow, 1989; Reeves et al., 1989; Reeves et al., 1990; Reeves and Smith, 1995; Reid et al., 1995; de Jong, 1995; Proudman et al., 2002). Logistic models have two main clinical applications: firstly, they can identify risk factors for a disease or clinical syndrome and secondly, they can be used to estimate the probability of clinical events, such as death or a specific disease (Reeves and Smith, 1995; Reid et al., 1995).

1.5.2.1 Mathematical modelling in veterinary parasitology.

The emphasis of most of the research in veterinary parasitology has been on the development of methods for field studies, parasite type, distribution, abundance, prevalence, on ascertaining the clinical significance of infection, on the development of control methods and

anthelmintic resistances (Soulsby, 1986; Armour, 1980). Until fairly recently, little attention has been paid to the population biology and transmission dynamics of parasites of veterinary importance, and their significance for analysis, interpretation and the design of control policies using mathematical models.

Mathematical models have played an important role in our understanding of parasitic diseases in both human and veterinary medicine, and in the development of efficient disease control strategies (Anderson and May, 1986; Boag and Thomas, 1975; Smith and Grenfell, 1993; Grenfell et al. 1987; Grenfell et al. 1995; Roberts, 1999). Models described by Gettinby et al. (1979), Gettinby and Paton (1981), Paton et al. (1984), Paton (1987), Smith and Galligan (1988) and Smith (1989) were detailed mimics of the life cycle of helminth parasites of bovine and ovine species and they are among the first specific nematode models of veterinary importance to help predict the risk of infection. Earlier models (Ollerenshaw and Rowlands, 1959; Ollerenshaw and Smith, 1966; Ollerenshaw et al., 1978; Thomas and Starr (1978), which involved a simple empirical model linking climate data to some indices of parasite abundance, developed a system for forecasting the intensity of gastrointestinal parasites in ruminants.

Mathematical modelling of the population biology of *Ostertagia ostertagi* is one of the well-studied epidemiological models of veterinary parasitology (Gettinby et al., 1979; Grenfell and Smith 1983; Paton, 1983; Paton et al., 1984, Paton, 1987; Grenfell et al., 1987; Smith and Guerrero, 1993). The models have shown that the natural control and regulation of parasite numbers is mediated by the effect of climate on the development and survival of the free-living and infective stages; changes in the rate of establishment of the infective larvae, and density-dependent variation in parasite survival and fecundity. The models are excellent mimics of the epidemiology of bovine ostertagiosis and can be used, as a simple screening

procedure, to help determine which of many possible anthelmintic control strategies should be selected for more detailed examination in the field. Moreover, they provide a theoretical framework within which ideas concerning the epidemiology of parasitic gastroenteritis can be assessed and refined.

1.5.2.2 Mathematical modelling in equine health problems

Despite the widespread use of mathematical models in the investigation of bovine and ovine diseases and, particularly diseases of man, their application in equine problems is not well developed. Of the over 200 epidemiological models reviewed by Hurd and Kaneene (1993) built for simulation of livestock population and/or diseases, none of the models were specifically intended for equids. However, mathematical models have been developed for a few infectious equine diseases recently. Factors affecting the epidemics of African horse sickness, the likelihood of an epidemic before and after the introduction of virus and the effectiveness of vaccination strategies were simulated (Lord et al., 1997, 1998). Mellor et al. (1998) and Baylis et al. (1999) used mathematical modelling to study the distribution and abundance of *Culicoides imicola*, a vector for African horse sickness, using climatic data and satellite imagery to predict the distribution of outbreaks of African horse sickness in Morocco. Mathematical models have also been developed to study vaccination control strategy, spatial spread and strain variation of equine influenza virus (Menzies and Reid, 2002; Glass et al. 2002) and to assess the risk of importing equine infectious anaemia infected horses (Carpenter et al., 1998).

Increasingly, models are being linked together to produce large-scale systems models, such as EQWISE (Equine Welfare Information System/Expert), which facilitate access to different types of disease model and expert systems for equine health and welfare (Reid et al., 1996). In this programme two models are described; the first, an heuristic-based decision

support system for the diagnosis of coughing in the horse, structured to emphasise the visibility of the underlying inferential mechanism, as well as to provide hypertext links to information and illustrations associated with possible disease diagnosis; in the second, the EQWISE shell is used to provide access to a logistic regression model of the risk factors associated with the need for surgery in cases of equine colic.

Multiple logistic regression models, to identify risk factors associated with musculoskeletal injuries in racehorses (Martin, et al., 1996; Bailey, et al., 1997; Bailey et al., 1998), equine monocytic ehrlichiosis (Kiper et al., 1992), to develop a predictive model for equine encephalomyelitis using weather variables (Francy and Wagner, 1992) and to determine the prognosis of equine colic patients (Reeves et al., 1989; Reeves et al., 1990; Proudman et al., 2002) have been developed recently.

1.5.2.3. Mathematical modelling in equine gastrointestinal parasites

There have been no attempts to model the population biology and dynamics of gastrointestinal parasites of equids. Multiple logistic regression model has been used by Reid et al. (1995) to highlight the value and importance of epidemiological modelling in modern clinical equine cyathostomosis. In this study the model was used to assess epidemiological risk factors associated with the diagnosis of cyathostomosis in horses.

Mathematical modelling of the processes that regulate and control parasite abundance can play an important role in the understanding of the pathogenesis of the disease (Reid et al. 1995). Modelling techniques are very useful in understanding how the timing and frequency of anthelmintic treatment should be regulated by the population biology of the aetiological agents (Smith and Guerrero, 1993). There are two principal ways in which models can yield information on parasitic disease control: they may be used to obtain information directly by

testing the relative efficacy of a range of alternative control strategies or information on control may be obtained indirectly by simulating the host-parasite system in order to achieve as complete as possible an understanding of the epidemiology of the parasite (Paton, 1983).

Very simple generic models such as produced by Smith (1990) are useful to answer fundamental questions about the population dynamics of gastrointestinal parasites of equids, particularly, the strongyle species, for which no modelling attempts have been made so far.

1.6. Population dynamics of equine strongyles

The belief that all species of parasites have their own characteristic biological properties and the epidemiology of each species is different, inevitably leads to the view that the biological properties in which they differ are of a critical epidemiological significance. For example, the course of infestation on pasture could be deduced from characters such as critical temperature and moisture for development, longevity of the larvae, time of maximum infective larvae on pasture, resistance to desiccation and extreme of temperatures (Michel, 1982). Obviously, profound differences might be expected in different climatic regions. It is also noteworthy that there are often greater differences between populations of the same species in different environments than between different species in the same environment (Michel, 1982). A better understanding of the characteristics of the bionomics of the free-living stages and the population dynamics of strongyles is required for developing mathematical models of the parasitic population on pasture and/or in the host. This is pivotal if the aim is to forecast periods with a higher risk of infection for the herd and develop alternative strategies of control using improved managerial practices or more efficient times for deworming. The following sections review the biological dynamics of equine strongyles in order to make reasonable estimates of the relevant parameters used to construct the model.

1.6.1 Seasonal pattern of egg hatching and larval development of equine strongyles

Field studies on the development of equine strongyles have been carried out mainly in temperate and to a lesser extent in subtropical areas. There is very little information on the dynamics of strongyles under tropical conditions. Investigations carried out on nematode larval ecology in parts of sub-Saharan Africa (Chiejina and Fake, 1984; Onyali et al. 1990; Ndamukong and Ngone, 1996; Waruiru, et al. 1998, 2002) have shown that the rate of development and the longevity of eggs and larvae of trichostrongylids of domestic ruminants vary with temperature and rainfall in different geo-ecologic regions (Table 1.8).

Baker et al. (1939) and Ogbourne (1972) found that temperature influences strongyle larval development more than any other factor in cold temperate areas. Laboratory studies conducted in UK (Rupasinghe and Ogbourne, 1978) have shown that the minimum hatching temperature for eggs of equine strongyles was 8°C; between 6°C and 8°C eggs develop but do not hatch, while below 6 °C eggs do not develop. According to Ogbourne (1972), winter temperatures are not low enough to kill many eggs, but larvae which hatch do not reach the infective stage in the UK. However, egg development, hatching and larval development to the infective stage occur at an increasing rate between 10°C-35°C.

Laboratory experiments (Ogbourne, 1972; Mfitlodze and Hutchinson, 1987) and field studies (Baker, 1939; Mfitlodze and Hutchinson 1988; English, 1979b) have shown that temperature and moisture were the most important factors for the development of equine strongyles. However, Mfitlodze and Hutchinson (1988) found that, once moisture is above the threshold value temperature was the main factor determining the proportion of eggs, which give rise to infective stage larvae. Laboratory studies (Mfitlodze and Hutchinson, 1987) in tropical Australia revealed that strongyle egg development was rapid between 25-33°C, with complete hatching within 24 hours, infective larvae started to appear after 48

hours and all developing larvae reached the infective stage by 3-4 days. This laboratory study also showed that the lower and upper temperature limits for development of horse strongyle eggs to infective stages were 10°C and 35°C, respectively, but the optimum temperature and relative humidity at which large numbers of larvae reached the infective stage was 28-33°C and 74-75%, respectively.

Table 1.8 Nematode egg development to infective larvae under tropical and subtropical conditions.

Species	Region	Season	Egg to L3(wks)	Sources
Strongyles	St-Aus*	Cool months	4-5	English, 1979a
Strongyles	St-Aus	Hot summer	1	English, 1979a
Strongyles	Tr-Aus **	Cool & dry	2	Mfitlodze & Hutchinson 1988
Strongyles	Tr-Aus	Hot & dry	1	Mfitlodze & Hutchinson 1988
Trichostrongylids	Kenya	cool & wet	2-6	Waruiru, et al., 1998
<i>H. contortus</i>	Nigeria	Warm & wet	1	Onyali et al., 1990
<i>H. contortus</i>	Nigeria	Hot & dry	-	Onyali et al., 1990

* St-Aus- Sub-tropical Australia ** Tr-Aus- Tropical Australia

The generally warm climate of tropical environments provide adequate conditions for the development of helminth larvae to the infective stage throughout the year but many studies have shown more nematode eggs including that of equine strongyles complete their development one week following contamination and the maximum larval count occurred within 1-6 weeks depending on the prevailing temperature during the wet season, whereas high temperature and low humidity during the dry season bring about rapid faecal desiccation resulting in high mortality among pre-infective larvae (Reinecke, 1960; Mfitlodze and Hutchinson, 1988; 1989; Onyali et al. 1990; Banks et al., 1990; Tembely,

1998; Waruiru et al. 1998, 2002). Mfitilodze and Hutchinson (1988) observed high negative correlations between larval yield and faecal and air temperatures under tropical condition.

In the subtropics the development of strongyle larvae to the infective stage also occurs throughout the year (English (1979a, b) but seasonal variations are not highly pronounced as they are in the tropics. During the cool months of the subtropics, eggs hatch within 1-2 weeks; during the warm summer hatching is complete in the first week, and all eggs are hatched within 48 hours during the hottest period of the summer. All larvae reach the infective stage within one week, while it takes 2-3 weeks in spring and autumn, and 5 weeks in winter before all larvae were infective (Table 1.8). A laboratory study by Mfitilodze and Hutchinson (1987) showed that at the optimum temperature of 28-33°C and relative humidity of 74-75% larval yield of strongyles ranged between 70-100% after 7 days of incubation.

The cooler months of the subtropics (English, 1979a) and tropics (Mfitilodze and Hutchinson, 1988) are highly conducive both for the development of the free-living stages and for their survival. This results in a high yield of infective larvae at these times (Table 1.9).

Table 1.9 Larval yields of strongyles in different seasons of tropical and subtropical conditions 7 days after faecal deposition on pasture.

Region	Season	Larva yield (%)	Sources
St. Aus*	Hot & wet	1-10	English, 1979a
St. Aus	Cool & wet	80	English, 1979a
Tr. Aus**	Cool & wet	29-32(31)†	Mfitilodze and Hutchinson, 1988
Tr. Aus	Cool & dry	31-35(32)	Mfitilodze and Hutchinson, 1988
Tr. Aus	Hot & wet	18-29(24)	Mfitilodze and Hutchinson, 1988
Tr. Aus	Hot & dry	7-22(16)	Mfitilodze and Hutchinson, 1988

†Numbers in parentheses indicate average yields. *sub-tropical Australia, ** Tr-Aus- Tropical Australia.

Winter temperatures in the tropics and subtropics are rarely below those which prevent development of eggs and larvae, in contrast to cold temperate regions (Ogbourne, 1972); when they do so it is only for short periods (at night) and the day temperature usually compensates for this (Mfitilodze and Hutchinson, 1987). It is important to note that within certain temperature limits a small amount of thermal energy is required for larvae to develop to the infective stage, and it does not matter whether this energy is obtained continuously or at intervals (Hsu and Levine, 1977). There is always adequate thermal energy for larval development throughout the year in the tropics, and any delays in winter are unlikely to be epidemiologically significant (Mfitilodze and Hutchinson, 1987).

Laboratory studies conducted both in the tropics and in temperate regions have shown that there were no major differences between the different species of horse strongyles in their basic temperature requirement for development (Mfitilodze and Hutchinson, 1987; Rupasinghe and Ogbourne, 1978). Moreover, no differences in the proportions of infective larvae of various species of strongyles at the various temperatures, and no substantial differences in the rate of developments were observed (Mfitilodze and Hutchinson, 1987).

1.6.2 Longevity or survival of infective larvae of strongyles on pasture

Infective strongyle larvae are well known to survive in a desiccated state under laboratory conditions; 15-25% of infective larvae survived for 1 year in faeces with 8-21% moisture content (Pukhove, 1941). Laboratory experiments conducted by Mfitilodze and Hutchinson (1987) showed that 42% and 10% infective larvae were still alive in dry faeces after 2 and 3 months, respectively, while no infective larvae were recovered from moist faeces kept at 37°C after 2 months. The likely explanation for this could be that under moist conditions, higher temperatures cause the non-feeding infective larvae to utilise their limited energy reserves faster, leading to reduced survival period, while under dry conditions larvae are not

active and hence conserve energy (Mfitilodze and Hutchinson, 1987). The ability of desiccated infective larvae to withstand cryopreservation at a very low temperature has also been documented (Bemark, 1978).

Many field studies under tropical and subtropical conditions have shown that the survival of infective larvae of nematode parasites on pasture varies according to seasons (Table 1.10). A study conducted by English (1979a) showed that conditions, which are optimal for larval development, are not necessarily optimal for survival. During the hot and dry period, larval development is very rapid but survival is very limited. This could be due to a direct temperature effect, in which the non-feeding, infective larvae use up their limited food reserves more rapidly at higher temperatures. This is more likely in the hot wet summer of the subtropics (English, 1979a). However, the higher temperatures and low humidity of the dry season of tropical weather may result in a faster rate of faecal desiccation than the rate of development, with critical moisture levels being reached before many of the larvae have reached the infective stage. Although there are considerable seasonal variations in the survival rate of strongyle larvae in faeces, there were no indications that different equine strongyle species survive differently (Hutchinson et al., 1989; English, 1979).

Table 1.10 Survival of nematode infective larvae on pasture under tropical and subtropical conditions

Species	Region	Season	Max. survival time (wks)	Sources
Strongyles	St-Aus*	Hot & wet	4	English, 1979a
Strongyle	St-Aus	Hot & wet	2-3	English, 1979b
Strongyle	St-Aus	Cool & dry	3-8	English, 1979b
Strongyles	Tr-Aus**	Hot & wet	12	Hutchinson et al. 1989
Strongyles	Tr.Aus	Cool & wet	12-16	Hutchinson et al. 1989
Strongyles	Tr-Aus	Cool & dry	4-8	Hutchinson et al. 1989
Strongyles	Tr-Aus	Hot & dry	0	Hutchinson et al. 1989
Trichostrongylids	Tr-Aus	Cool & wet	12-14	Fabiyi et al. 1988
Trichostrongylids	Ethiopia	Cool & wet	2-6	Timbely, 1998
Trichostrongylids	Kenya	Warm & wet	12-16	Waruiru et al. 1998
<i>H. contortus</i>	Nigeria	Cool & wet	10	Onyali et al. 1990
<i>H. contortus</i>	Nigeria	Hot & dry	0	Onyali et al. 1990
<i>H. contortus</i>	Fiji	Warm & wet	9-13	Banks et al. 1990
<i>H. contortus</i>	Fiji	Cool & wet	13-17	Banks et al. 1990

* St-Aus - subtropical Australia. ** Tr-Aus - Tropical Australia.

1.6.3 Larval translations and pasture larval recovery

The size of the infective larval population of helminth parasites on pasture are the result of the number of eggs spread in faeces by the animals, the development rate, survival and translation onto pasture (Rossanigo and Gruner, 1994). The course of herbage infestations is determined by the release of infective larvae from their reservoir faecal masses on the one hand and by the growth of the herbage on the other hand. Many studies have shown that larval translation to pasture and pasture larval recovery vary greatly and is generally limited to wet seasons, and the migration of infective larvae from faecal masses to herbage is likely to occur in waves coincident with falls of rain (Ogbourne, 1973; English, 1979; Craig et al., 19 83; Hutchinson et al., 1989). The highest numbers of infective larvae were recovered during and immediately following the wet warm season from herbage samples collected 7 days after faecal deposition under tropical and subtropical conditions (Hutchinson et al. 1989; English, 1979b), whereas in cooler periods pasture larvae will not reach a peak until

sometime from 4-12 weeks and is not necessarily accompanied by marked reductions in the number of larvae in faeces (Hutchinson, et al., 1989; Mfitilodze and Hutchinson, 1988; English, 1979a). This indicates that migration of larvae in cool tropical and subtropical conditions results in the gradual accumulation of surviving larvae on pasture. Even under optimum weather conditions the number of strongyle infective larvae recovered varies (Table 1.11).

Table 1.11. Pasture strongyle larval recovery under optimum weather conditions

Larvae per kg dry herbage*	Sources
10,000-60,000	Hutchinson et al., 1989
>10,000	Craig et al., 1983
>140,000	Slocombe et al., 1987

*Number of infective strongyle larvae recovered from herbage samples collected 7 days after faecal deposition.

Seasonal fluctuations in the availability of infective larvae on pasture are not as pronounced under subtropical condition (English, 1979b) as they are in tropics (Hutchinson et al. 1989; Onyali, et al. 1990) and temperate regions (Michel, 1969; Ogbourne, 1972; Duncan, 1973, 1974; Ogbourne, 1973; Eysker et al., 1986). These studies have shown that the period of availability of larvae on pasture is influenced to a greater extent by rainfall both in tropics and subtropics, and to a lesser extent by temperature than in temperate regions. However, Hutchinson et al. (1989) found no direct correlation between the numbers of larvae recovered from herbage and the amount of rainfall. According to this study, the amount of rainfall is important only in as much as it provides the minimum amount of moisture required for larval translation. Above the threshold, extra rain may in fact destroy the faecal pats or flood the area reducing larval availability. Studies conducted both under tropical and subtropical conditions have shown that little or no larval translation onto pasture was observed during

most of the dry hot season of the tropics and subtropics because of lack of rainfall. The high environmental temperature, particularly under the dry tropics, in fact leads to rapid faecal and larval desiccation and death of infective larvae (Hutchinson et al. 1989; Chiejina and Fake, 1984; Onyali et al. 1990; Ndamukong and Ngone, 1996; Waruiru, et al. 1998, 2002).

The great seasonal variation in the availability of infective larvae on pasture in the tropics emphasises the marked seasonal nature of rainfalls in these regions. Therefore, it seems more likely that animals in the dry tropical environment have a high probability of acquiring infection from pasture during and immediately following the wet season and the rest of the year poses little danger with regard to the acquisition of infective larvae from pasture. It should be noted, however, that faecal pats might act as a refuge or reservoir in the late dry season (Chiejina and Fake, 1984), particularly if accompanied by unseasonal rain, and can pose a danger.

1.6.4 Larval distribution and ingestion rate of infective strongyle larvae

The development to an infective stage is only partially indicative of infective potential. As many as 80% of eggs produced may reach the infective stage under optimum weather conditions (Mfitilodze and Hutchinson, 1988; Callinan, 1979) but all do not disperse successfully on to pasture (Callinan, 1979eq44). Horizontal dispersal is largely dependent on moisture, but vertical dispersal is less so (English, 1979b; Stromberg, 1997). There are reports that trichostrongylids of domestic ruminants migrate into the soil regardless of incident rainfall (Gronvold and Hogh-Schmidt, 1989). Larval distribution on pasture is not random, but in clumps following the distribution of faecal masses (Crofton, 1954). The concentrations of infective larvae are close to the faecal masses and there appears to be little migration beyond 30 cm from the faeces, with the majority of larvae (greater than 89%) being found within 15 cm (Baker et al., 1939; Michel, 1969; English, 1979b; Herd and

Willardson 1985; Herd, 1986; Hutchinson et al., 1989). However, there might be danger of spread over a wider area by temporary localised floodings, hooves, etc..

The epidemiological importance of such high concentrations of infective larvae around the faecal masses is related to the grazing behaviour of animals. Horses and donkeys grazing within this radius of faecal masses are at high risk of ingesting large numbers of infective larvae. However, horses (Taylor, 1954) and donkeys (authors observation) acquired an abhorrence of faeces as part of their adaptation to life in a parasitised environment. This may not be always true as study made by Herd and Willardson (1985) and Getachew (personal observation) revealed that such protection is transient and of little significance once conditions favours larval migration and dispersal. This is particularly true in most tropical countries where there is scarcity of pasture, and as the grazing season progresses, animals are forced to graze the roughs, the portion on which there is a high concentration of infective larvae, and which normally the animals reject.

The vertical distribution of larvae on pasture is not also uniform, with a greater concentration of larvae a short distance above the ground level (Mossand Vlassoff, 1993; English, 1979b). So, length of the grass is another factor to be considered. Horses and donkeys usually graze close to the ground and also cover a large area in order to obtain sufficient feed (Baker, et al. 1939) but are less likely to do so when the grass is long, hence decreasing the chance for infective larvae to be ingested. This also may not hold true in most tropical countries, where there is a scarcity of pasture.

Infestation of larvae on herbage is measured as numbers of larvae per unit weight of herbage dry matter (Kao, et al., 2000). Therefore, larval intake can be estimated from herbage intake and the density of larvae on pasture. Considering larvae to be evenly distributed over pasture.

larval ingestion rate is simply daily grazing rate for an individual animal divided by the available herbage (Kao et al. (2000). Although there are studies conducted in determining pasture larval distribution (Baker et al., 1939; Michel, 1969; English, 1979b; Herd and Willardson 1985; Herd, 1986; Hutchinson et al., 1989), no study has been made to determine the ingestion rate of infective strongyle larvae either by horses or donkeys.

1.6.5 Establishment of infective larvae in the host

There is evidence that many of the infective strongyle larvae ingested by horses (Britton, 1938; Baker et al. 1939; Taylor, 1933) and the infective trichostrongylids larvae ingested by domestic ruminants (Gibson and Parfitt, 1972; Harrison et al. 1999; Niezen et al. 1998) never reach maturity in the gut. There is a strong suggestion that age resistance and increased susceptibility due to poor nutritional status may be factors, which limit or increase the percentage of ingested larvae that reach maturity. Larval establishment is typically high in young and parasite naïve animals and low in adults (Adams 1982; Love and Duncan, 1992). It is also probable that weakened infective larvae, which have nearly depleted their available food reserve, are incapable of exsheathment and penetration of the gut wall, and hence are passed out in the faeces.

Rate of exposure, degree of previous exposure, resistance of worms to anthelmintics and age of the host were found to be the main determining factors for the establishment of trichostrongylid infective larvae in sheep (Barger, 1989; Dobson et al. 1990 a, b; Kao et al. 2000). Dobson, et al. (1990a) in estimating the establishment of *T. colubriformis* in helminthologically naïve merino sheep given infective larvae at different rates found approximately 65% of the infective larvae given in the first week became established as adults, then the establishment fell to below 5% after 7, 10, and 14 weeks of continuous intake for the high, medium and low infection rates, respectively. This suggests that a

threshold of worm exposure was required before resistance to establishment developed. Similarly, a study on the effect of host age on the establishment of infective larvae showed that the rate of development of resistance to new infection was faster in older sheep and establishment declined to approximately 2% over 5 and 9 weeks for the 36 and 12 week-old sheep, respectively (Dobson et al., 1990b).

A study conducted by Love and Duncan (1992) showed that previous exposure of ponies to parasites resulted in reduced susceptibility to infection reflected in acquiring a lower worm burden. Moreover, yearling ponies had a higher total worm burden than the adult group. However, no quantitative work has been carried out to determine the establishment rate of strongyle infective larvae and factors that may influence it in equidae.

1.6.6 The pre-patent period (PPP) of cyathostomins

The pre-patent period is the period of time from ingestion of infective larvae to the appearance of eggs in faeces or to the development of patent infection (Urquhart et al. 1996). Although the pre-patent periods of individual species of cyathostomins have not been separately determined, experimental and field studies have shown that parasitic development of cyathostomins can vary from 5 to 18 weeks but the majority have pre-patent periods of 5-9 weeks (Table 1.12). On the other hand, a prolonged pre-patent period of more than 2 years, which might indicate arrested development, has been observed after repeated treatment of housed horses (Gibson, 1953; Smith, 1976).

Since most animals are repeatedly exposed to cyathostomin infection throughout their lives, it is difficult to investigate whether the development of small strongyle infection is affected by the age of the host. An experimental study by Smith (1976) has shown longer pre-patent periods when the ponies were infected with large numbers of cyathostomin larvae at 7-8

years of age than when they had been given a similar infection 3 years earlier. However, a study conducted by Love and Duncan (1992) revealed no differences in the apparent pre-patent periods either between adult ponies (age 2.5-14 years) or between adults and yearlings (age 16-18 months). This may suggest that age and pervious exposure to cyathostomins does not markedly affect the time for naturally acquired infections to develop to patency in horses.

Table 1.12 Pre-patent periods (ppp) of cyathostomins of horses and ponies

Infection	Age	Exposure	ppp(wks)	Sources
Experimental	Foals	Naive	5-8	Round, 1969
Experimental	Foals	Naive	5-8	Russell, 1948
Experimental	4-5 yrs	Exposed	12-15	Smith, 1976
Experimental	9-10	Exposed	17-18	Smith, 1978
Natural	3-6m	Naïve	7-9 (8)*	Love &Duncan, 1992
Natural	4-5m	Exposed	5-8 (8)	Love & Duncan, 1992
Natural	16-18m	Exposed	7-12(9)	Love & Duncan, 1992
Natural	2.5-14yrs	Exposed	8-10 (9)	Love & Duncan, 1992
Experimental	2-6m	Naïve	6-7	Reinemeyer et al. 1988

*Numbers in parentheses indicate average pre-patent period.

1.6.7 Fecundity of equine cyathostomins

Fecundity is the rate of egg production per matured adult female worm per day (Michael et al. 1991; Urquhart et al. 1996). It is usually estimated in terms of faecal worm egg count if worm burden and daily faecal mass are known (Kao et al. 2000). According to the study by Love and Duncan (1992), the general pattern of faecal worm egg output were similar in foals, yearlings and adults with the counts showing a sharp increase at about 100 days after first grazing and then remaining fairly constant until treatment on day 186. But the mean worm egg count in adult ponies was lower than those detected in foals and yearlings, which they attributed to modified cyathostomin development as a consequence of host age and/or previous exposure to parasites. A similar result was obtained by Smith (1978) in which he

recorded significantly lower faecal worm egg counts when he infected ponies for a second time.

A study on infection with *Ostertagia ostertagi* and *T. circumcincta* (Smith, 1988, 1989) showed that fecundity is regulated by density dependent constraints. A similar result was found by Dobson et al. (1990a) in which he measured daily egg production per female *T. colubriformis* for 2-11 weeks post infection and found egg production to be density dependent. Increased fecundity was also shown to be associated with increased worm length of *Trichostrongylus circumcincta* (Stear et al., 1995; Stear and Bishop, 1999).

In contrast to the above findings, a study by Coyne et al. (1991) showed no evidence for density dependent regulation of parasite fecundity in *H. contortus*, *Trichostrongylus* species, *Nematodirus* species and *Oesophagostomum venulosum*. Moreover, Coyne et al. (1991) found that analysis of the linear regression of fecundity on the duration of infection indicated that parasite fecundity remained unchanged throughout the grazing season. This absence of strong density dependent constraints on fecundity may indicate that the natural regulation of these parasites is through density dependent constraints on parasite survival, which could be directed at the newly ingested larvae, or at the adult parasites already in the gastrointestinal tract or both (Smith, 1988; Barger and Le Jambre, 1988).

No study has been made on the fecundity of strongyles of equids and whether it is density dependent or not has not been determined. Baker et al. (1939) estimated that each female strongyle produces between 0.5 and 2 epg per day. Miller (1953) indicated that the lower rate of egg production is characteristic of the cyathostomins and females lay eggs at markedly different rates varying from about 10 or 12 eggs per female per day.

1.6.8 Cyathostomin burden of horses and donkeys

Post-mortem surveys of naturally occurring cyathostomin burdens of adult horses and donkeys have shown great variability (Table 1.13). Seasonal trends in the age structure of cyathostomin burden were recognised by Ogbourne (1976) and Reinemeyer et al. (1986). This seasonal trend may in part explain the wide range of burden, although it seems probable that host, management and parasite factors may also contribute to the wide variation in worm numbers seen in individual animals. Length of time since initial larval challenge, daily larval challenge, and total larval challenge (Anderson, 1986; Gregory and Woolhouse, 1993; Dobson et al. 1990a; Grenfell et al. 1995) can affect worm burden. Love and Duncan (1992) have shown that the total cyathostomin burden was highest in the helminth-naïve foals and lowest in the grazed foals of the same age; similarly the yearling group had higher total worm burdens than the adult group.

Table 1.13. Gastrointestinal cyathostomin burden of horses and donkeys.

Equines	Age group	Worm burden	Sources
Horses	Adults	12000-1239000	Ogbourne, 1976
Horses	Adults	680-663100	Reinemeyer et al. 1984
Horses	Adults	415-190316	Krecek et al. 1989
Horses	Adults	20-165000	Mfitilodze & Hutchinson, 1990
Horses	Adults	510-65250	Love and Duncan, 1992*
Horses	Yearlings	5560-173060	Love and Duncan, 1992*
Horses	Foals	5130-84220	Love and Duncan, 1992*
Donkeys	Adults	25900-46700	Eysker, 1987
Donkeys	Adults	3900-222767	Eysker and Pandey, 1989*
Donkeys	Adults	3580-217643	Eysker and Pandey, 1989
Donkeys	Adults	7509-93734	Malan et al. 1982
Donkeys	Adults	1286-127990	Matthee et al. 2000

*Total worm count including all mature and immature larvae.

1.6.9 Life expectancy or longevity of adult cyathostomins

There are no natural predators of parasites in the alimentary canal and, consequently, death must be due to senescence or to some protective activity on the part of the host (Poynter, 1954). A study by Colglazier (1979) showed that about 34% of the total worm burden of 29 ponies he examined was eliminated spontaneously before treatment suggesting some host protective mechanism. Any larval challenge could provoke an immune response, and thus it is difficult to separate the acquired immunity-mediated death rate from the death rate in the absence of acquired immunity. Britton (1938) and Taylor (1933) found that acquisition of infection from birth to 3 years of age exceeded rate of loss thus increasing the degree of parasitism. Parasite burden then remained fairly constant until the age of 10 when the degree of infection gradually diminished, indicating a gradually acquired resistance to strongyle infection. In such situations, it is very difficult to estimate the longevity or life expectancy of worms.

Studies made in the USA (Reinemeyer et al., 1986) and UK (Ogbourne, 1976) have shown seasonal variation of adult cyathostomins in horses. According to the work of Reinemeyer et al. (1986), each reproductive category (immature, gravid and spent females) was dominant for three or more consecutive months during only one period per year. Gravid females were most common from late spring to late summer, and were long-lived because spent females did not increase in prevalence until autumn and winter. Reinemeyer et al. (1986) suggested that spent females presumably died and were replaced by a succeeding generation of immature worms in the following spring indicating several months longevity of female cyathostomins in horses.

Eysker (1987) and Eysker and Pandey (1989) found that the populations of third and fourth stage larvae were low during the wet as well as during the dry periods in donkeys in

Zimbabwe. They found that more than 80% of the total worm count was adult cyathostomins during the dry season. Eysker (1987) suggested that cyathostomins in donkeys survive the unfavourable dry winter mainly as adult worms and not as inhibited larvae. As the unfavourable dry climatic conditions last for more than six months in Zimbabwe, Eysker and Pandey (1989) suggested that the longevity of cyathostomins in donkeys is probably several months.

CHAPTER TWO

GENERAL MATERIALS AND METHODS

2.1 Study country- Ethiopia

The Federal Democratic Republic of Ethiopia (FDRE) is a landlocked country in the horn of Africa bounded to the north by Eritrea, to the west by Sudan, to the south by Kenya, to the east by Somalia and to the northeast by Djibouti (Fig.2.1). It lies within the tropics between 3°24' and 14°53' North; and 32°42' and 48°12' East with a total area of 1,104,300 square kilometres. It is twice the size of Texas or approximately the size of France, Spain and Portugal combined. This makes Ethiopia the fourth largest nation in Sub-Saharan Africa and the 21st largest nation of the world. With an average population growth rate of 3% at present, the population of Ethiopia had reached 74 million as estimated by FAO (2004).

The main geo-physical feature of the country is the diversity in altitude and the accompanying climatic and ecological variations. Taking the two extreme altitudes, temperature ranges from mean annual of 34.5°C in the Danakil depression, which is 125 metres below sea level (mbsl) and below zero up to -5 °C in the upper reach of Mt Ras Dashen, which is 4620 metres above sea level (masl). Between these extremes are vast areas of plateaus and marginal slopes where mean annual temperatures are between 10-28 °C. The traditional climatic zones and their physical characteristics include *Bereha* (hot arid, 500-1500 masl and rainfall below 900 mm), *Kola* (warm semi-arid, 500-1500 masl and rainfall 900-1400 mm), *Woyna Dega* (warm to cool sub-humid, 1500-2300 masl and rainfall above 1000 mm), *Dega* (cool humid, 2300-3200 masl and rainfall above 1000 mm) and *Wurch* (cold and moist to alpine, above 3200 masl and rainfall above 1400 mm) (MOA, 2000).

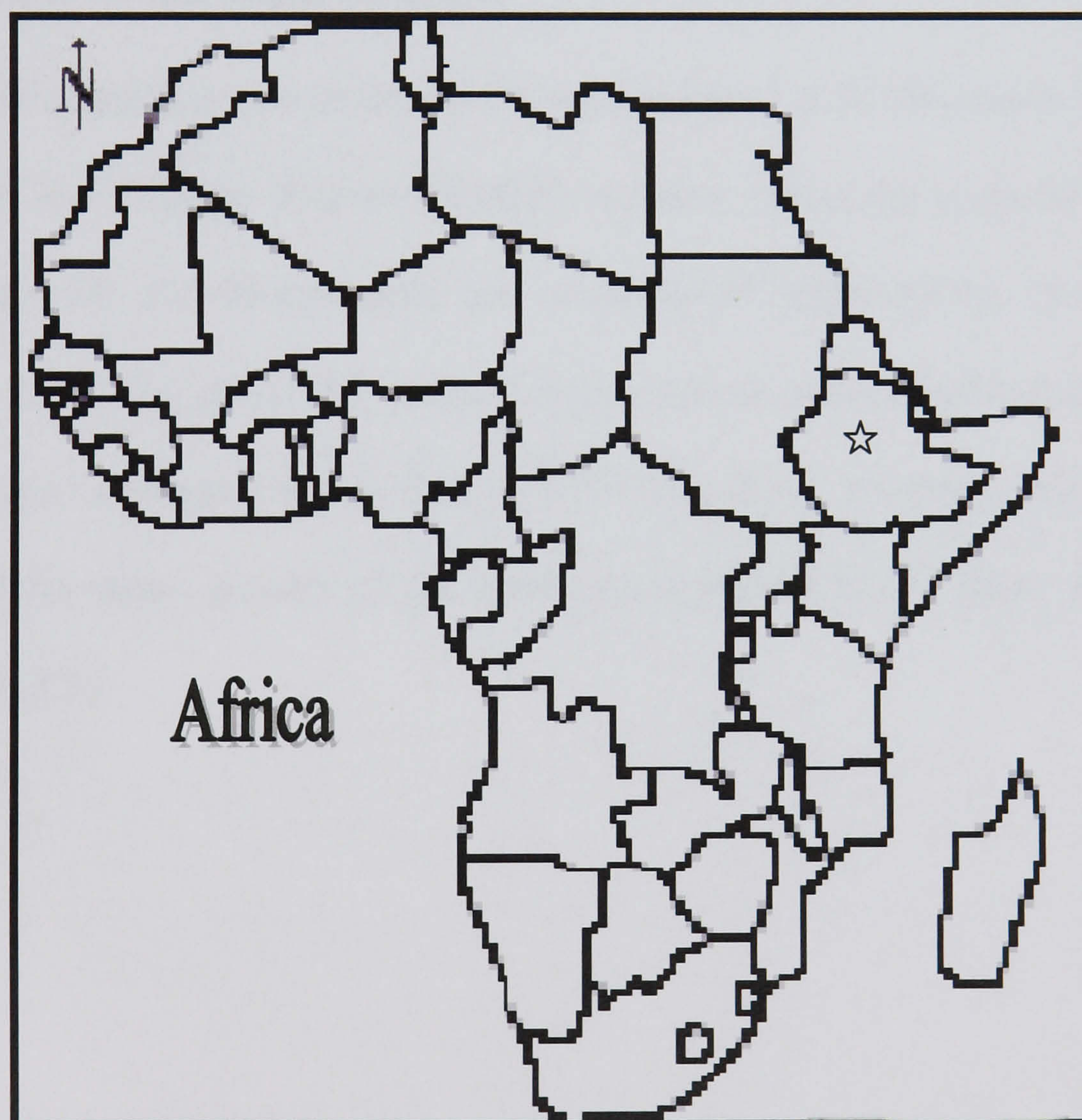


Fig. 2.1 Country location map of Ethiopia (MOA, 2000)

Such diversity in altitude and the accompanying climate and ecological variations should be taken in to account during a study made as these affect the collected data on parasitic infection of domestic animals itself, and therefore its interpretation.

Agriculture has always been, and remains, the cornerstone of the Ethiopian economy. Small-scale farming is the backbone of the sector. Subsistence sector technology is largely traditional and rainfed, with very limited areas of irrigation. Livestock production is an integral part of the country's agricultural system. The various ecological zones allow the production of several species of livestock, which represent a major national resource. Ethiopia has the largest livestock population in Africa: 30 million head of cattle, 23 million sheep, 18 million goats, 8 million equidae (donkeys, horses and mules), 1.5 million camels and 53 million poultry (FAO, 2004).

2.2 Description of the study regions

Studies conducted and reported in this thesis were carried out in Ethiopia in areas where the Donkey Health and Welfare Project (DHWP) operates, under the auspices of the Donkey Sanctuary. They are 20-150 km north and southeast of Addis Ababa, the capital city of Ethiopia (Fig. 2.2). The regions are situated in the central highland plateau having different climatic and agro-ecological zones and with varying land coverage, human and equine population. Eighty three percent of the total equine population of these areas consists of donkeys (Table 2.1).

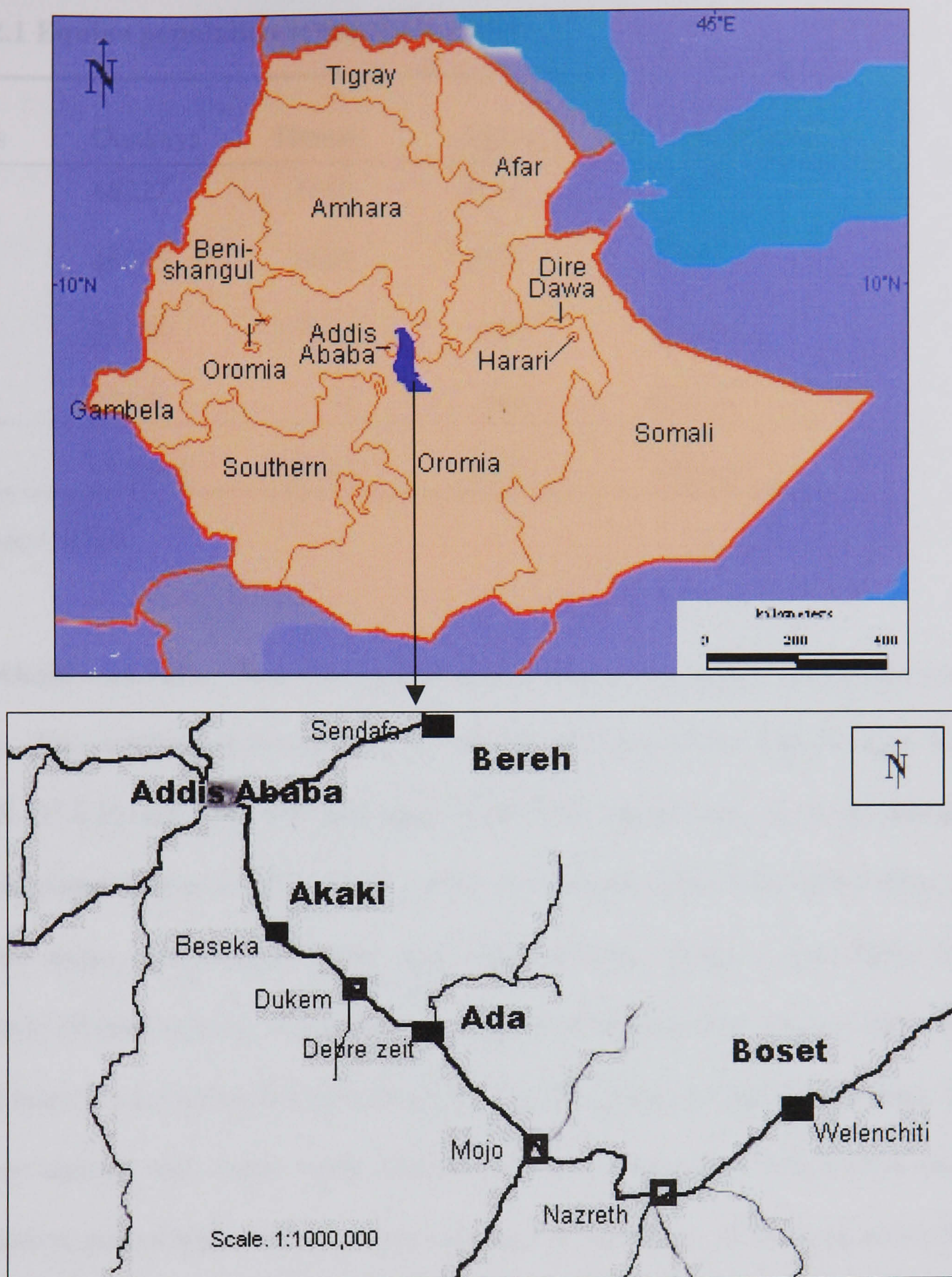


Fig. 2.2 Location map of study sites: Ada, Akaki, Bereh and Boset.

Table 2.1 Equine population of the study areas.

Regions	Donkeys	Horses	Mules	Total equidae
Ada	46222	2116	2025	50363
Akaki	16035	3518	2354	21907
Bereh	24395	13219	390	38004
Boset	37181	0	592	37773
Total	123833	18853	5361	148047

Source: MOA (2004)

2.2.1: Akaki and Ada: These two regions are similar in geography and so are considered together. They are located 20 and 50 km southeast of Addis Ababa and lie at lat. 9.02° 8’N, long. 38.45° 8’E, and 8.48° 8’N and long. 39.38° 8’E, respectively. They are located in the central highland plateau at mid altitude on the escarpment of the Great Rift Valley, although there are some mountainous areas with high altitudes (Fig.2.2 and Table 2.2). The topography of these regions is marked by the presence of a number of crater lakes. However, most of them are not accessible to animals, having only small outlets for watering. There are ponds or lagoons and water wells used as a water source for both human and animal consumption, particularly during the dry seasons, in most places of the rural areas. Some flat bottomlands are swampy and waterlogged. There are also few areas where irrigation is being practiced.

These regions practice an integrated crop-livestock production system: cattle play an important role in supplying draught power, while equidae are the areas beast of burden for transporting agricultural produce, goods and water. The majority of the flat bottomlands are used for crop production and permanent pasture is very scarce. Animals, most of the time, graze on road and hillside, weeds of arable lands and fallows where the availability of grass

is very scarce and almost non-existent during the long dry season (October to June). Crop residues from cereals such as teff (*Eragrostis abyssinicus*) and wheat as well as pulses like peas, beans, vetch and chickpea are the major sources of feed during the long dry season. The major soil types of the areas are Alfisol/mollisol and vertisol of clay to clay-loam in texture (Murphy, 1959; EARO, 2004).

Table 2.2. Climatic data and agro-ecological zone of the study areas.

Regions	Altitude (masl*)	Annual Rainfall (mm)	Mean Annual temp. max (min)	Agro-ecological zone
Ada	1500-2300	800-1200	26.5 (13.0)	mid-lowland (moist, warm/cool)
Akaki	1700-2100	1000-1300	24.7 (14.0)	mid-lowland (moist, warm/cool)
Bereh	2300-3000	1000-1300	22.4 (10.5)	Highland (wet, cool)
Boset	1000-1500	550-1200	31 (16.6)	Lowland (moist to dry/hot)

Source: MOA (2004) * metres above seas level

Table 2.3. Monthly Rainfall (mm) distribution of the study areas (2003/2004)

Months	Ada	Akaki	Bereh	Boset
January	38.3	19.6	75.5	32.5
February	55.4	24.3	0.0	32.1
March	61.6	23.9	29.7	119.6
April	100.3	114.0	126.9	152.6
May	21.1	2.9	1.7	0.0
June	81.4	125.4	120.6	61.2
July	277.9	325.1	304.4	393.2
August	285.1	307.0	373.4	321.7
September	119.4	112.4	122.4	19.5
October	6.0	0.0	0.0	0.0
November	3.6	1.9	0.0	0.0
December	35.4	0.0	19.7	28.3
Annual RF*	1085.5	1056.5	1174.3	1160.7

Source: ENMSA (2004). *RF- Rain fall

2.2.2. Bereh (Sendafa): Bereh is located about 50 km north of Addis Ababa within the northern central highland plateau. It lies at lat. 08° 86’N and long. 39° 021’E). The area is at high altitude and has a cool-wet agro-ecological zone (Table 2.2). The agricultural system of the region is an integrated crop-livestock production system where cattle play an important role as draught animals and equidae are the area’s beast of burden. The region is characterized by a large area of flat bottomland and uphill lands, which are used for crop production, as a permanent pasture and for haymaking. Hay from both sources and crop residues from cereals such as teff, wheat and barley, as well as pulses like peas, beans, chickpea and lentils are used as animal feed during the dry period. Most of the bottomland grazing areas are flooded during the rainy season and are waterlogged. There are also few rivers, which run all year making the surrounding areas productive throughout the year. The major soil types of the region are Vertisol in the bottomland and Nitosol and Alfisol in many of the steeper slopes (EARO, 2004).

Table 2.4 Mean daily maximum and (minimum) temperature of the study areas (°C) for each month during the study period (2003/2004).

Months	Ada	Akaki	Bereh	Boset
January	26.9(11.2)	26.6(11.8)	23.0(10.5)	30.0(15.2)
February	28.9(13.0)	28.3(13.2)	24.0(10.7)	32.5(16.7)
March	28.6(14.0)	27.5(14.2)	22.0(12.0)	32.7(15.2)
April	27.8(15.0)	27.3(15.1)	22.0(12.0)	32.1(18.0)
May	29.3(14.1)	28.9(15.0)	25.0(11.0)	34.4(16.7)
June	27.6(14.1)	27.1(15.0)	24.0(11.0)	33.1(18.8)
July	23.6(14.2)	23.7(14.2)	20.0(10.0)	28.5(17.9)
August	23.0(13.7)	23.6(14.1)	20.0(10.0)	28.9(17.7)
September	25.0(13.7)	24.9(15.0)	22.0(10.0)	30.0(17.5)
October	26.6(11.2)	26.6(14.0)	18.0(9.0)	31.5(15.4)
November	26.3(11.4)	26.6(14.9)	19.0(10.0)	30.7(15.7)
December	24.7(10.1)	25.7(12.8)	18.0(10.0)	28.5(14.1)

Source: ENMSA (2004)

2.2.3. Boset (Welenchiti): Boset is situated in the Great Rift Valley 135 km southeast of Addis Ababa. It lies at lat.08° 43'N and long.039° 41'E (Fig. 2.2). The region has an altitude range of 1000-1500 metres above sea level and a hot dry/moist agro-ecological zone. Agriculture is a mixed crop-livestock production system but livestock herding is the dominant activity. Very few areas bordering the Awash River practice irrigation. Thin grasses and bushes with patches of woodlands cover most areas. There is no permanent pasture and animals graze on the road and hillside, and on weeds of arable lands. Animals spend most of their time within the surrounding bushes and acacia trees, which is not suitable for crop production, and the availability of grass is very scarce and almost non-existent during the long dry season (October to June). Crop residues from cereal such as teff, and maize or sorghum stover are the major feed source for the prolonged dry-period. The areas are not waterlogged but there are small ponds and water wells in the rural areas used as water sources both for humans and animals. The dominant soil types are cambisol/yermisols (Solonchaks) of sandy texture and vertisols very rich in clay (EARO, 2004).

2.3. Study animals

Working donkeys of all age groups and sex were included. Ages of the donkeys were estimated using an age determination chart developed based on dentition while body condition scoring was performed based on the five level condition scoring system developed by the Donkey Sanctuary (Appendices II and III).

2.4 Sample size

Literature reviews showed that some studies had been undertaken to determine the prevalence of strongyles and bot larvae in donkeys. The presence of tapeworm infection, parascariasis and fasciolosis were also reported in donkeys from many countries worldwide (Chapter One). However, no studies had been made specifically to determine their

prevalence and intensity. So their approximate or expected prevalences were not known, particularly under tropical conditions. According to Cannon and Roe (1982), Thrusfield (1995) and Pfeiffer (2002), if the approximate prevalence of a particular disease is unknown, a suitable procedure would be to choose the 50% figure since this results in the largest sample size for all prevalences, or 20% but take additional samples if the prevalence exceeds 20%. We followed the first procedure to calculate the sample sizes as follows:

$$n_r = (N)(n)/(N+n) \quad 2.1$$

Where n_r is the required sample size;

N is the size of the study population;

n is the estimated sample size calculated based on an infinite population with desired fixed confidence limits and absolute precision, and expected prevalence of a disease. The calculation is based on the normal approximation of the binomial distribution. The values of n are given in table form for different desired accuracy, level of confidence and expected prevalence of a particular disease (Cannon and Roe, 1982; Thrusfield, 1995; Pfeiffer, 2002). This value is then corrected for a finite population using equation 2.1, which is equivalent to $1/n_r = 1/n + 1/N$, to estimate the required sample size from such a population (see Cannon and Roe 1982 for detail). Therefore, a sample size of $n=663$ animals would need to be sampled from an infinite number of population with expected 50% prevalence and a desire to estimate a 99% confidence interval with an absolute precision of 5%. Substituting this for n in the above equation and with finite population size of $N=123,833$ donkeys, the required sample size will be: $n_r = (663 \times 123833)/(663 + 123833) = 659$

This sample size is used in the cross-sectional coprological survey of cestodes, ascarids, and trematodes of donkeys, and sero-epidemiological study of equine cestodosis.

2.5 Sampling procedures

2.5.1 Peasant associations (PAs)

From each region four peasant associations (PAs) were randomly selected using a random number generator (Microsoft Excel, 2000) out of eight PAs in each region (Table 2.5).

Table 2.5. Peasant associations randomly selected from each region.

Ada	Akaki	Bereh	Boset
1. Bekejo	Menedelo	Dale	D-wanga
2. Dembi-1	Wad-Warabu	L-Beri	Tedecha tuledimtu
3. Filtino	Lale	Dire	T-abeye
4. D.koticha	S-Awash	Lebu	T-Sangota

2.5.2 Donkeys

Based on the total sample size calculated (section 2.4), the proportion or the number of donkeys to be sampled from each region was calculated as follows:

$$n_d = (N_D * n_r) / N$$
2.2

Where

n_d number of donkeys to be sampled from each region.

N_D : total donkey population of the region.

n_r : total number of donkeys to be sampled.

N : total donkey population. The results are shown in Table 2.6.

A stratified sampling method coupled with simple random sampling was applied to sample donkeys from each region. The number of donkeys to be sampled from each PA in each region is then calculated according to equation 2.3 (rounding up to the next whole number in each case).

$$n_p = n_d / N_p$$
2.3.

Where

n_p : the number of donkeys to be sampled from each PA

n_d : number of donkeys to be sampled from each region (equation 2.2)

N_p : number of PAs randomly selected from each region, which is 4.

The number of donkeys to be sampled from each PA is then divided by 12 to get the number of donkeys to be sampled per month. Due to rounding up to the next whole number and multiplication the total number of donkeys sampled became 684, which is more than the calculated sample size (Table 2.6). To sample the required number of donkeys from each PA in each region, the donkey owners were randomly selected without replacement from the record in the project database using a random number generator (Microsoft Excel, 2000). When the farmer has more than one donkey a blind random sample was applied at the sampling point to select one of them.

Table 2.6. Estimated number of donkeys to be sampled from each study region

Region	Ada	Akaki	Bereh	Boset	Total
Donkey population	46,222	16,035	24,395	37,181	123,833
Number of donkeys to be sampled/region	246	85	130	198	659
Adjusted total * number of donkeys to be sampled/region	246	96	144	198	684

*These are adjusted number of donkeys to be sampled due to rounding up when calculating the number of donkeys sampled per PAs and per month.

2.6. Data management and analysis

All field and laboratory data of the study animals were recorded on a pre-designed data record sheet and transferred to computer files using Microsoft Access and Excel. Graphs and descriptive statistics were produced using data analysis tools in Microsoft Excel 2000 and

Minitab statistical software, version 12.21. Other statistical analyses were performed using R, version 2.0.1 and Minitab statistical software, version 12.21. Details of statistical analysis and modules used for specific studies are described in the material and method section of their respective chapters.

2.7 Faecal sample collection and laboratory processing

Faecal samples were collected directly from the rectum of the donkeys. However, some samples were picked up off the ground only if the donkeys concerned were seen to pass the faeces and could be identified, and the sample could be picked up immediately. The samples were transported to the Donkey Health and Welfare Project Clinic Laboratory and stored at 4°C until examination. From each sample an appropriate amount was taken and weighed on an electronic scale, according to the specific parasite under study, for further processing. Each processed sample was then kept in a refrigerator at about 4 °C until faecal worm egg recovery and counts were performed. These were done within 48 hours.

2.7.1. Cestodes

The faecal samples were analysed by a centrifugation/flotation technique as described by Proudman and Edwards (1992), Nilsson et al. (1995) and Meana et al. (1998).

Technique

1. 30 gm of faeces were broken down and mixed vigorously in 450 ml of tap water.
2. The resulting homogenate was strained through two sieves of apertures: 850 µm (on top) and 300 µm mesh, and allowed to sediment for 24 hrs in a half-litre decanter.
3. After siphoning off the supernatant, the sediments were resuspended in 30 ml tap water. The suspension was then divided into two centrifuge tubes (15 ml each) and spun at 1500 rpm for 10 minutes.

4. The supernatant in each tube was discarded and the sediment was again resuspended in 15 ml tap water, and re-spun at 1500 rpm for 10 minutes.
5. After discarding the supernatant the faecal plugs were resuspended in 15 ml saturated sucrose solution and spun at 1500 rpm for 10 minutes. (sg = 1.28 at 20 °C, prepared by dissolving 450 gm granulated sugar in 350 ml of warm water. Two ml of 37% formaldehyde was added to deter growth of fungi (Carmel, 1988; Foreyt, 2001).
6. The suspension from the top of one of the tubes was transferred by a pipette to the two McMaster chambers (0.3ml), allowed to stand for 5 minutes and the number of eggs were counted under a x 100 magnification to identify and determine epg. All eggs in the two chambers were counted. The number of eggs per gram of faeces (epg) was obtained by multiplying the total eggs in the two chambers by a factor of 3.3 ($\text{epg} = (\text{total eggs counted}/0.3\text{ml})(30 \text{ ml } /30\text{gm})$), where 0.3 ml was the volume of aliquot examined, 30 ml total volume of the faecal suspension and 30 gm the amount of faeces examined.
7. A few drops of sucrose solution were added on the top of the second tube until a convex meniscus was formed and a standard cover slip was placed on top and allowed to stand for an hour.
8. The cover slip to which eggs had attached was placed on a microscope slide, scanned for cestodes eggs and these counted.
9. Standard positive faecal samples with recovery rate of 5-20 cestode eggs were regularly processed in order to check that the analytical procedure showed consistent results. Moreover, the specific gravity of the sucrose solution was adjusted and monitored using hydrometre.

2.7.2. Trematodes (*Fasciola* and *Gastrodiscus*)

The faecal samples were analysed by a sedimentation/flotation technique as described by MAFF (1986), Anderson, et al (1999) and Conceicao et al. (2002).

Technique

1. 10 gm of faeces were broken down in 100 ml tap water and stirred well with a stirring rod until homogenised.
2. The mixture was strained through two sieves of aperture: 850 μm (on top) and 300 μm . As much water as possible was pressed out of the debris through the sieve and the debris left on the screens was discarded. The strained fluid was allowed to stand for 5-10 minutes.
3. The supernatant was discarded and the sediment was re-suspended with 15 ml tap water in 18 ml Clayton Lane tube and centrifuged for 2 minutes at 1500 rpm. This process was repeated.
4. The tube was agitated until the sediment was loosened and formed sludge at the bottom of the tube. The tube was filled with saturated zinc sulphate solution to the same 15 ml level as before. One to two drops of a 1% aqueous solution of methylene blue was added. (Zinc sulphate of 1.20 specific gravity was prepared by dissolving 371 g zinc sulphate in 1000 ml tap water (Foreyt, 2001)).
5. The contents of the tube were thoroughly mixed by inverting the tube five to six times with the end covered and immediately a sufficient amount of the fluid was withdrawn with Pasteur pipette and carefully allowed to run into one of the McMaster chambers. After further mixing, the second sample was withdrawn and run into the other chamber.
6. All eggs of *Fasciola* and *Gastrodiscus* in the two chambers (0.3 ml) were counted immediately. The number of eggs per gram of faeces was obtained by multiplying the

total egg counts in the two chambers by 5 ($\text{epg} = (\text{total eggs counted}/0.3\text{ml})(15\text{ml}/10\text{gm})$), where 0.3 ml is the amount of aliquot examined, 15 ml total volume of the suspension and 10 gm amount of faeces examined.

2.7.3. *Parascaris equorum*

Faecal worm egg counts using the modified McMaster technique were performed (MAFF, 1986).

Technique

1. 10 gm of faeces was broken down in 100 ml tap water and stirred well with a stirring rod until homogenised.
2. The mixture was strained through two sieves of 850 μm (on top) and 300 μm aperture and the filtrate was centrifuged for 2 minutes at 1500 rpm.
3. The supernatant was discarded and the sediment was re-suspended with 15 ml tap water and centrifuged for 2 minutes at 1500 rpm. This process was repeated.
4. The tube was agitated until the sediment was loosened and formed sludge at the bottom and filled with saturated sucrose solution (sg. 1.28) to the same 15 ml level as before.
5. The contents of the tube were thoroughly mixed by inverting the tube five to six times with the end covered, and immediately sufficient amount of the fluid was withdrawn with Pasteur pipette and carefully allowed to run into one of the McMaster chambers of 0.15 ml of volume. After further mixing, the second sample was withdrawn and run into the other chamber.
6. All parascaris eggs in the two chambers (0.3 ml) were counted. The number of eggs per gram of faeces was obtained by multiplying the total eggs in the two chambers by 5 ($\text{epg} = (\text{total eggs counted}/0.3\text{ml})(15\text{ml}/10\text{gm})$), where 0.3 ml is the amount of

aliquot examined, 15 ml total volume of the suspension and 10 gm amount of faeces examined.

2.8. Retrospective data on post mortem worm recovery and identification

A total of 122 donkeys were examined for several species of internal parasites at necropsy at the Donkey Health and Welfare Project (DHWP) clinic from 1995 to 2004. History of parasiticide usage in these animals showed that no anthelmintics had been given within the last 6 months prior to the animals's death. Ten animals had been treated with ivermectin while they were hospitalised at the clinic and were not considered in the present study. Many animals had been drenched with traditional herbal medicines to treat the illness from which they were suffering.

The 112 donkeys were examined for cestodes, ascarids, trematodes, bot larvae and some of them for strongyles, spirurids and *O. equi*. The number of donkeys examined, their age and sexes, the species of parasites searched for, and months of necropsy were all recorded. Information on clinical history and death showed that the donkeys either died of various clinical ailments or were euthanased because of poor prognosis with no chance of recovery. A complete examination of the contents and mucosal lining of each portion of the gastrointestinal tracts was conducted to recover any mature adult worms. The stomach, small intestine, caecum, ventral and dorsal colon and small colon/rectum were examined. Organs of the abdominal and thoracic cavity, particularly, liver for liver flukes and lungs for lungworms were also examined. The standard procedures used to recover the strongyles and methods of their identification are outlined in the previous study (Getachew, 1999). The present study focused on cestodes, trematodes, ascarids and bot larvae, and the standard procedures followed during the post-mortem examination to recover these parasites are outlined below.

2.8.1. Gasterophilus larvae

The stomach and the first 50 cm of duodenum were removed from the rest of gastrointestinal tracts. It was opened with a single incision extending from the duodenum through the pylorus along the greater curvature of the stomach to the oesophagus. All the stomach contents were removed and the detached larvae were collected. Larvae recovered from contents and those found attached to their respective sites were collected and counted separately and preserved in 10% formalin for later species identification. Larvae found in the posterior part of the gastrointestinal tract were also collected. Gross pathological lesions caused by these larvae at the sites of their attachment were also recorded.

2.8.1.1. *Gastrophilus* larval identification

The recovered larvae were differentiated into species and 2nd and 3rd stages according to the keys proposed by Zumpt (1965). These were mainly based on the following morphological features:

1. Body measurements (length and width).
2. Shape of body segments.
3. Number of rows of spines on each body segment.
4. Shape of the spines (blunt or pointed).
5. The absence or presence of spines in some or all body segments whether ventrally, dorsally or both.
6. Absence or presence of pseudocephalon with groups of denticles
7. Shape of mouth-hooks and their curvature.

2.8.2. *Parascaris equorum*, *Anoplocephala magna* and *Anoplocephaloides mamillana*

The small intestines were searched for these large roundworm and tapeworms, respectively. After removal of the intestine, the contents and two water rinses were emptied into a plastic

container. Intestinal contents and rinses were washed through a 10-mesh sieve (2 mm aperture) and the residues were examined for *Parascaris*, tapeworms and other small nematodes. The small intestine was then opened with scissors and any parasites found were removed. The specimens were preserved in 10% formalin.

2.8.3. *Anoplocephala perfoliata* and *Gastrodiscus aegyptiacus*

The whole caecum, large colon, and ileum were searched for any tapeworms and *Gastrodiscus* attached. After removal of the intestines the contents and two water rinses were emptied into a large plastic container. Intestinal contents and rinses were washed through a 10-mesh sieve and the residue thoroughly examined for the presence of *A. perfoliata* and *Gastrodiscus*. The intestines were then opened with scissors and the mucosa of each part of caecum, colon and ileum were examined and any attached parasites were removed. All recovered parasites were counted and preserved in 10% formalin. Any gross pathological lesion at the site of parasites attachment were also recorded

2.8.4. Trematodes (*Fasciola* spp)

The liver of necropsied donkeys was removed. The main bile ducts were searched for adult flukes. Any gross pathological lesions of the bile ducts were recorded. The whole liver was then examined by cutting into 1 cm slices after soaking in warm water for about an hour. Each sliced strip was thoroughly squeezed from end to end. The contents of the basin were sieved and put into a Petrie dish. Adults, cut pieces and immature flukes were collected with fine brushes and forceps. Counts of the heads of cut flukes were made and added to the appropriate count of adult flukes. The flukes were preserved in 10% formalin for later identification (see Appendix VI).

2.9 Enzyme-linked immunosorbent assay (ELISA) for the detection of antibody to *A. perfoliata* in donkey sera.

2.9.1 Antigen

A 12/13kDa IgG(T) antigen, which is an excretory/secretory antigen of *A. perfoliata* supplied by Diagnosteq, diagnostic laboratory, University of Liverpool, Faculty of Veterinary Medicine, was used.

2.9.2 Serum collection and ELISA

Blood samples were collected and allowed to clot overnight at room temperature. Serum was prepared by centrifugation at 800 g for 5 minutes and stored at –20 °C until required (Proudman and Tree, 1996; Hoglund et al., 1995). Sera from 797 donkeys of all ages and sexes were collected.

The following protocols for anti-12/13kDa IgG(T) tapeworm ELISA were used (Proudman and Tree, 1996):

Protocol

1. Prepare affinity purified 12/13kDa antigen to a concentration of 5 µg/ml in carbonate coating buffer (pH 9.6).
2. Coat appropriate number of wells within Immulon 4 plates (Dynatech Laboratories, Billingham, West Sussex) with 100 µl per well and incubate at room temperature for an hour.
3. Wash with 2% Skimmed milk powder (SMP) wash solution, 100 µl per well three times.
4. Block each well with 100 µl 2% SMP block solution and incubate at room temperature for an hour. Then wash three times.

5. Dilute sera 1/100 in 2% SMP dilution solution, add 100 µl/well and incubate for an hour at room temperature. Wash three times.
6. Add 100 µl/well of goat anti-equine IgG(T) (ICN Biomedicals Inc., USA) diluted 1/400 in 2% SMP dilution solution. Incubate for an hour at room temperature and wash three times.
7. Add 100 µl/well of peroxidase conjugated anti-goat Mab (SIGMA, USA), diluted 1/1000 in 2% SMP dilution solution. Incubate for an hour at room temperature. Wash three times. Perform a final wash with phosphate buffer solution (PBS) only.
8. Prepare substrate by dissolving a 10 µg ABTS (azino-bits (3-ethylbenzthiazoline-6-sulphuric acid) tablet in 25 ml citrate buffer (pH 4.2). Just before use add 20 µl of 30% hydrogen peroxide. Incubate 100 µl per well for 10-20 minute.
9. Read the optical density (OD) at 405 nm (TiterTek Multiskan PLUS MK II, International marketing flow laboratories, Switzerland).

2.9.3. Reagent recipes used for the tapeworm ELISA (Diagnosteq, equine division, University of Liverpool):

1. Carbonate coating buffer (pH 9.6). Store at 4 °C. (1 month maximum)
 - a. 0.795g Na₂CO₃
 - b. 1.465g NaHCO₃
 - c. 0.1g NaH₃
 - d. Quantity sufficient to 500 ml with purified water
2. Citrate buffer (pH 4.2). Store at 4 °C. (1 week maximum)
 - a. 0.98g citric acid
 - b. 1.44 g trisodium citrate.2H₂O
 - c. Quantity sufficient. to 100 ml with purified water

3. ABTS substrate solution. (Make up fresh each assay).
 - a. 10 µg tablet ABTS
 - b. 25 ml citrate buffer
 - c. 20 µl 30% H₂O₂
4. Wash solution (2% SMP, 0.01 Tween-20). (Make up fresh each assay).
 - a. 2 g Skimmed milk powder (SMP)
 - b. 100 ml PBS
 - c. 10 µl Tween-20
5. Blocking/dilution solution (2% SMP). (Make up fresh each assay).
 - a. 2 g SMP per 100 ml PBS

2.10. Determining efficacy of praziquantel against cestode and assessing serum antibody level of *A. perfoliata* after treatment.

- Forty four donkeys positive for cestode both coprologically and serologically were randomly selected.
- The donkeys were randomly allocated to treatment and control groups, each comprising 22 animals.
- The treatment group was treated with the paste form of praziquantel (Equitape, Fort Dodge, Bayer) and ivermectin (Ivomec injectable, Merial) at a recommended dose rate of 1mg/kg and 0.2 mg/kg body weight, respectively, orally. The control group received only ivermectin per os at recommended dose rate of 0.2 mg/kg. Then both groups received ivermectin at 8 weeks interval.
- Faecal and blood samples were taken immediately before treatment from all treatment and control donkeys.

- Then faecal samples were collected every two weeks for the first 16 weeks after treatment.
- The second and third sera were collected 8 and 16 weeks post-treatment, respectively, from all groups of donkeys.
- Faecal and sera samples were collected and analysed using the methods and techniques outlined in sections 2.7.1 and 2.9.2, of this chapter.
- Details of statistical analyses are given in the respective chapter (Chapter Six).
- The study was conducted from February to July 2004.

CHAPTER THREE

CROSS-SECTIONAL COPROLOGICAL SURVEY OF CESTODE, TREMATODE AND ASCARID INFECTION IN DONKEYS, ETHIOPIA.

3.1. Introduction

In contrast to the size of the donkey population in the world and the service they provide to society and the national economy, these animals are largely ignored by society, governments and the scientific community. Available literature (Pandey et al., 1994; Starkey, 1994a; Svendsen, 1994, 1997b) shows that few attempts have been made to study the different aspects of donkeys worldwide.

Some studies have shown that helminths in working donkeys are not only highly prevalent but the intensity rate or worm burden is also very high (Pandey and Eysker, 1988, 1990; Eysker and Pandey, 1989; Eysker, 1987; Pandey, 1980a,c 1981; Getachew, 1999; Matthee et al., 2000; Matthee et al., 2002). Thorough studies carried out to date to identify the gastrointestinal parasites of donkeys include those conducted by Matthee et al. (2000) and Matthee et al. (2002) in South Africa, Getachew (1999) in Ethiopia, Eysker and Pandey (1989) in Zimbabwe and Burgu et al. (1995) in Turkey. Most of these studies were focused on strongyles and few attempts have been made to study non-strongyle helminths of donkeys, particularly cestodes, trematodes and ascarids. Some coprological studies in various regions of the world, however, have shown that donkeys may be infected with these parasites (Collins, 1961; Pankhurst, 1963; Hatch, 1966; Green, et al., 1968; Kearney, 1974; Gothe and Heil, 1984; Vercruysse et al., 1986; Feseha et al. 1991; Yilma et al., 1991; Proudman and Ellis, 1995; Trawford and Tremlett, 1996; Trawford, 1997; Ashmay and Diab, 1998; Wells et al., 1998; Getachew, 1999; Gonenc, 1999; Kotwal, et al. 2000; Haridy et al., 2002). Most of these reports are from a small number of animals and the information is rather disparate and fragmentary. Moreover, no studies have been made specifically to

determine the epidemiology, population biology and pathogenecity in donkeys of these genera. Therefore a cross-sectional coprological survey was designed to study the epidemiology of cestodes, *Fasciola*, ascarids and *Gastrodiscus* infection in the donkey population of Ethiopia. This is part of the on-going study to identify the species of helminth parasites: their prevalence and relative abundance: their pathogenic effect and economic significance in working donkeys in Ethiopia. Although this is the primary objective, the study also recorded the presence of other helminth eggs in faeces of donkeys. The current chapter, therefore, presents results obtained from this study.

3.2. Materials and methods

Faecal samples were collected on a monthly basis from June 2003 to May 2004. Eight hundred and three donkeys were rectally sampled. There were 89 samples from donkeys under 2.5 years old, 128 samples between 2.5 and 4.5 years old, 140 samples between 4.5 and 8 years old and 446 samples from 8 years old and above. Four hundred and two horses were also randomly sampled from working horses coming to the veterinary clinic from the surrounding areas, where the project operates, for coprological analysis and recovery of ascarid eggs. The age of the horses was determined according to Evans (2001) while that of the donkeys as is described in appendix II. Details of sampling procedures are outlined in Chapter Two.

Although several diagnostic methods are available, and faecal egg counts are known to be influenced by many factors, the coprological approach to determine the presence and/or number of helminth eggs, is the most widely used diagnostic method in veterinary parasitology (Ward et al., 1997). In epidemiological studies and helminth control programmes, the examination of faecal samples plays a crucial role in deciding whether to

use anthelmintics, and also assists in the identification of anthelmintic resistance and determining the degree of pasture contamination with eggs.

Ova counting procedures, which assess the number of helminth eggs per gram (epg) of faeces, are all based on dilution counting procedures in which an aliquot of the faecal suspension from a known volume of sample is examined microscopically (Nicholls and Obendorf, 1994). The McMaster and its modification methods form the basis of the most universally used technique (Whitlock, 1948). In the present study a dilution factor of 1:10, i.e., 1 gram of faeces to 10 ml of tap water was used to detect trematodes and ascarid eggs (MAFF 1984; Anderson, et al, 1999; Conceicao et al., 2002). A total of 10 g faeces was examined. For the cestode egg detection a total of 30 g of faeces with a dilution factor of 1:15 was used. The more sensitive centrifugation/flotation technique (Proudman and Edwards, 1992; Nilsson et al., 1995 and Meana et al, 1998) was employed in parallel with McMaster quantification. Eggs of cestodes, trematodes and parascaris recovered from matured adult parasites and faecal samples from donkeys were measured using an ocular micrometer in a calibrated microscope (Todd, 1979). Even though emphasis on the survey was on cestodes, trematodes and parascaris, the detection of strongyle, *Ascaris*, *Oxyuris equi*, and *Trichuris* infections were also recorded. Details of the procedures and techniques used in laboratory faecal analysis and egg measurements are outlined in Chapter Two and Appendix IV.

3.2.2. Data analysis

Descriptive statistics and graphs were produced using data analysis tools in Microsoft Excel 2000 and Minitab statistical software, version 12.21. Because of the many zeros and the nature of the distribution of faecal egg count, which was highly skewed to the right, logarithmic transformation did not produce normality. One alternative to logarithmic

transformations is generalized linear modelling with untransformed data (Wilson and Grenfell, 1997) and this is the method employed here. The faecal egg count data were fitted to the negative binomial distribution with identity link function. Analyses on faecal egg counts were performed using the general linear model (GLM) in the R statistical package software version 2.0.1. The association of region, age, sex and body condition scores with infection prevalence was evaluated by multivariate binary logistic regression. Where appropriate the difference between different levels of explanatory variable found to be significant was determined using pairwise contrast (R library Gremisc). The significance level for all statistical tests was set to $P < 0.05$

3.3. Results

Coprological results showed that the majority of donkeys were found harbouring eggs of more than one parasite species (Table 3.1). Strongyle eggs by far were the most abundant nematode eggs found, with a prevalence of 100% (data not shown). Three to eight species of helminth eggs including strongyles, *Oxyuris equi*, *Ascaris* and *Trichuris* spp were recorded per donkey.

3.3.1 Cestodes

Both modified McMaster and centrifugation/flotation techniques were employed in the detection of cestode eggs. The McMaster technique detected only 13.8% while the centrifugation/flotation 24.4%. Descriptive data and statistical analysis on faecal egg counts however, were restricted to the McMaster technique, as that of flotation technique was semi-quantitative. However, the latter was used in determining infection prevalences. An overall prevalence of 27.9% of cestode infection was detected using both McMaster and flotation techniques. No egg of *Anoplocephaloides mamillana* was detected. The maximum prevalence of cestode (46.9%) and the highest overall mean faecal egg count (11 ± 4.8 s.e.)

were recorded in the highland region of Bereh, while the minimum prevalence (20.7%) and lowest overall mean faecal egg count (1.5 ± 0.69 s.e.) were detected in the mid-lowland region of Akaki (Table 3.2).

Table 3.1. The prevalence of non-strongyle polyparasitism determined by coprological examination in donkeys, Ethiopia (n=803).

Helminths	Number positive	%
1. Cestode, <i>P. equorum</i> , <i>Fasciola</i> spp, <i>Gastrodiscus</i>	17	2.1
2. Cestode, <i>P. equorum</i> , <i>Fasciola</i> spp	54	6.7
3. Cestode, <i>P. equorum</i> , <i>Gastrodiscus</i>	34	4.2
4. Cestode, <i>Fasciola</i> spp, <i>Gastrodiscus</i>	33	4.1
5. Cestode, <i>P. equorum</i>	104	13.0
6. Cestode, <i>Fasciola</i> spp	120	14.9
7. Cestode, <i>Gastrodiscus</i>	63	7.9
8. <i>P. equorum</i> , <i>Fasciola</i> spp, <i>Gastrodiscus</i>	84	10.4
9. <i>P. equorum</i> , <i>Fasciola</i> spp	183	22.8
10. <i>P. equorum</i> , <i>Gastrodiscus</i>	162	20.2
11. <i>Fasciola</i> spp, <i>Gastrodiscus</i>	140	14.4
Total	994	

Table 3.2. The prevalence (%) and the overall mean faecal egg counts of equine tapeworm in four study regions, Ethiopia.

Regions	Number of donkeys examined	Prevalence	Mean \pm se [†]
Ada	194	24.7 ^{a*}	5.6 \pm 2.3 ^{a*}
Akaki	164	20.7 ^b	1.5 \pm 0.68 ^b
Bereh	175	46.9 ^{abc}	10.9 \pm 4.8 ^b
Boset	270	22.2 ^c	3.5 \pm 1.2 ^c
Total	803	27.9	-

*Significant differences at P<0.05 level are indicated by the same superscript down the column. Example, the infection prevalence in Ada is significantly different from Bereh, both with the same superscript ‘a’, so also that of Akaki from Bereh, both with superscript ‘b’, but Ada is not significantly different from Akaki, having different superscript ‘a’ and ‘b’. [†]Fitted mean value for the negative binomial distribution linked with identity function.

Amongst coprologically cestode positive donkeys the egg count ranged from 3 to 373 eggs per gram (epg), averaging from 17.6 \pm 25 sd to 53.3 \pm 80.2 sd depending on the region of study. Multiple binary logistic regression analysis considering region, age group, sex and body condition score showed region as a significant risk factor (P<0.0001). Moreover, pair wise analysis between regions revealed significant differences (P<0.0001) in infection prevalences between Bereh and the other regions (Table 3.2). Bereh had odds ratio (OR) of 2.65 (P< 0.0001, 95% CI = 1.69-4.15) and was the region with highest risk for cestodosis. Age, sex and body condition had no significant effect on the infection prevalence. A generalised linear model analysis of faecal egg count data fitted using a negative binomial distribution linked with identity function showed no statistically significant differences (P>0.05) in the mean faecal egg count among regions, except between Akaki and Bereh (P=0.03) nor among the different age groups, sex and body condition scores (Tables, 3.6-3.8).

Eggs of tapeworms were detected throughout the year. However, the data indicated a possible increase in prevalence and faecal egg count during and following the rainy seasons irrespective of regions of study (Figs. 3.1 and 3.2). The rainy seasons are March to April, and June to September (Fig. 3.3). Significantly lower ($P<0.05$) prevalences were recorded in April in all regions except in Ada, and in the month of November in Ada and Boset.

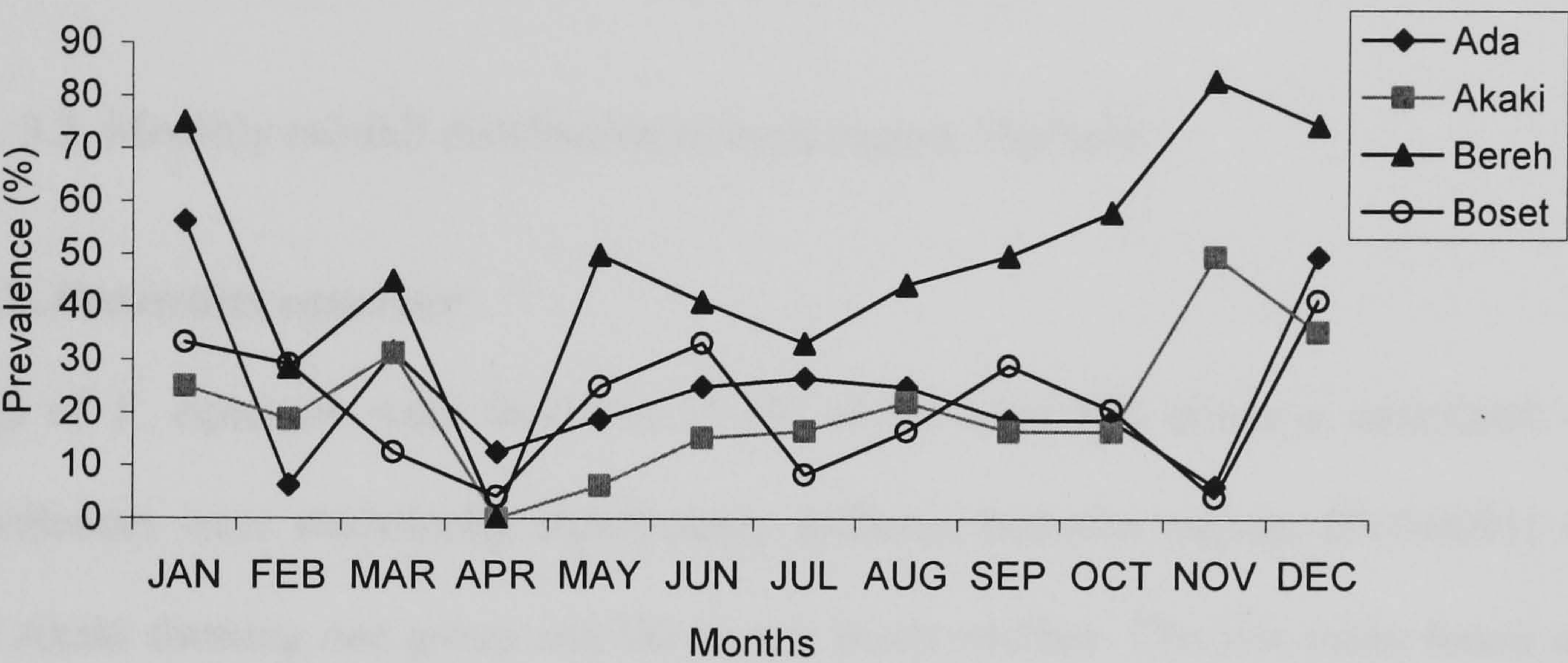


Fig 3.1 Seasonal prevalence of cestode infection of donkeys in the study regions, Ethiopia.

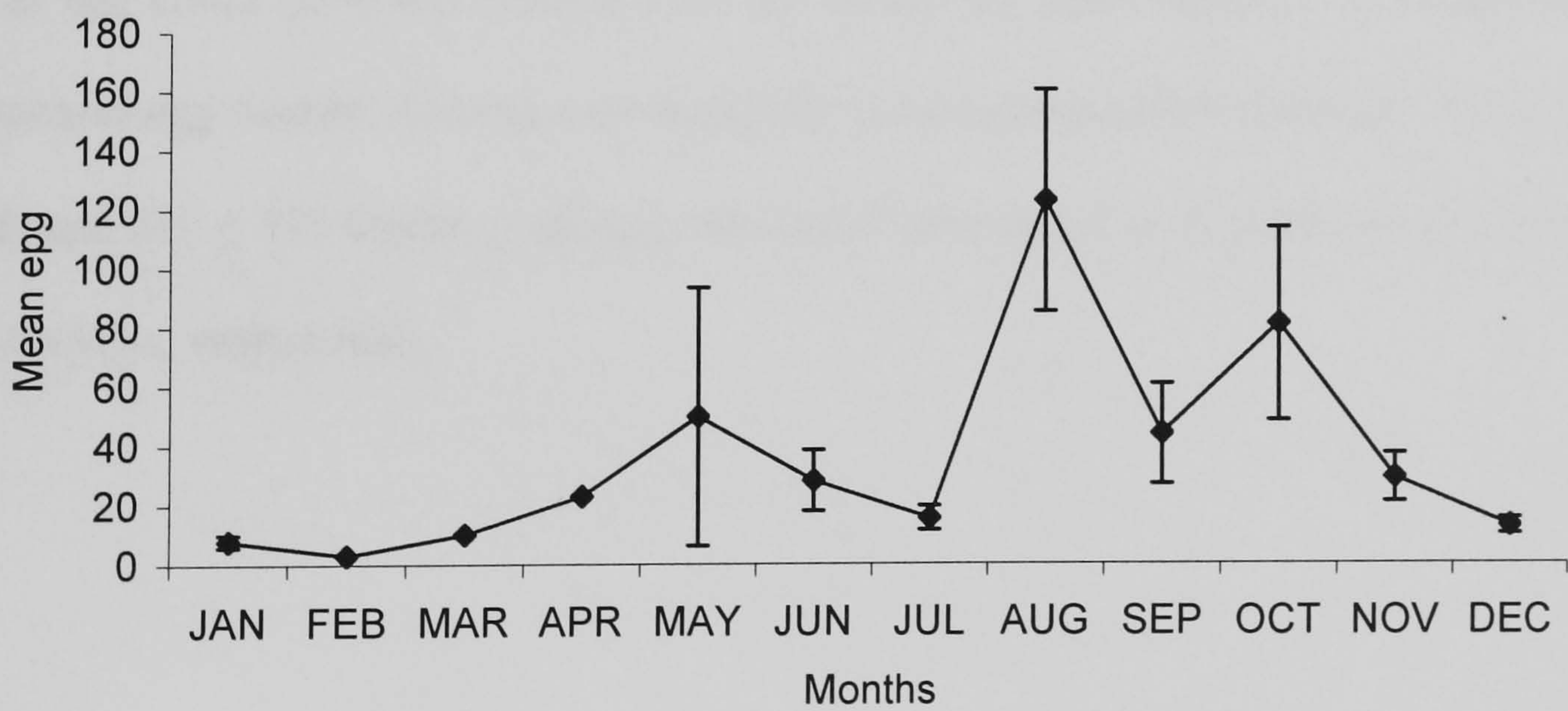


Fig.3.2 Seasonal distribution of the overall mean faecal egg counts of tapeworm of donkeys, Ethiopia. Error bars indicate mean \pm se. The mean is a fitted value for the negative binomial distribution linked with identity function.

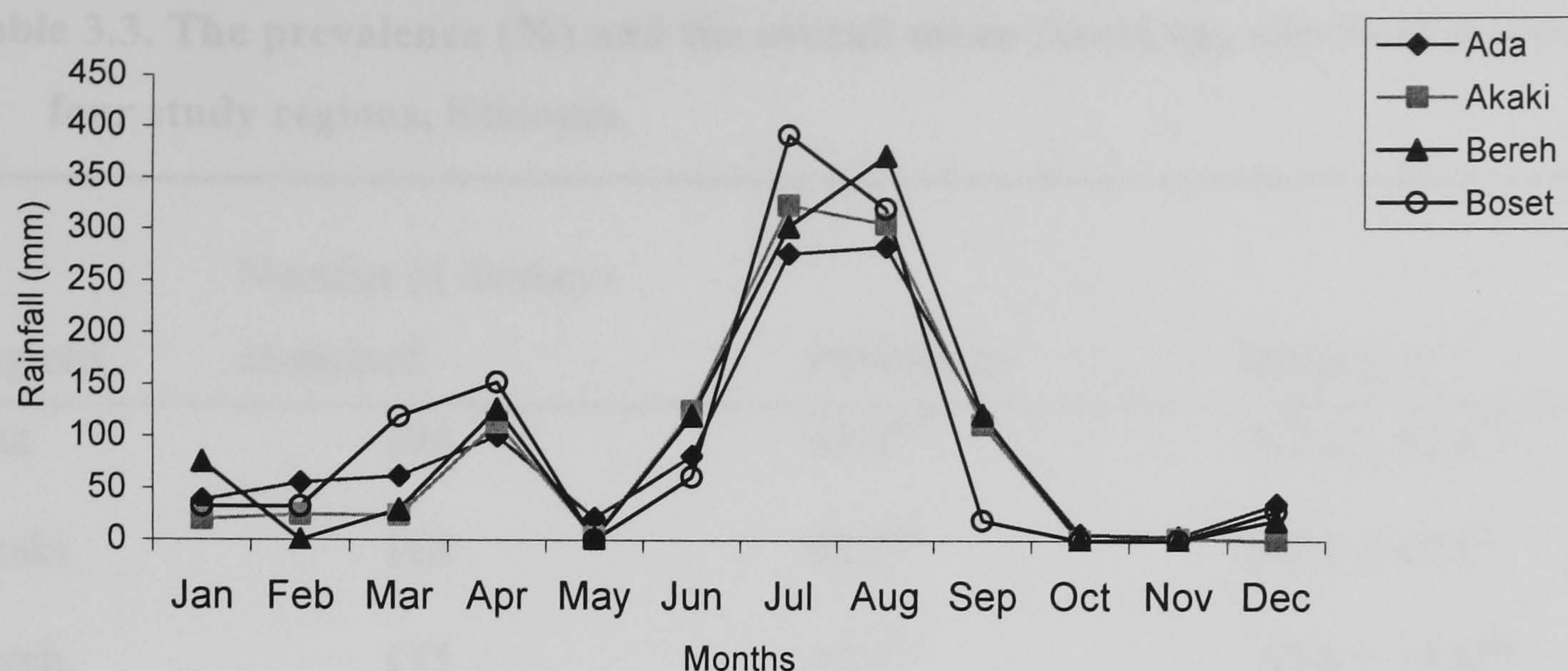


Fig. 3.3. Monthly rainfall distribution of each region, Ethiopia.

3.3.2. *Parascaris equorum*

Eggs of *P. equorum* were found in 51.1% of the total 803 donkeys examined. Infection prevalences were statistically significantly different between regions ($P < 0.0001$) with Ada and Akaki forming one group and Bereh and Boset another. The low mean faecal egg count observed in Bereh was significantly different from the rest of the regions ($P < 0.0001$) (Table 3.3). Age, sex and body condition had no significant effect on infection prevalence and faecal egg count ($P > 0.05$) (Tables 3.6-3.8). However, older donkeys had high mean faecal parascarid egg counts. Among coprologically parascarid positive donkeys, 251 ± 300 (mean \pm sd) and 331 ± 415 (mean \pm sd) epg were recovered in 4.5 to 8 years old and above 8 years old donkeys, respectively.

Table 3.3. The prevalence (%) and the overall mean faecal egg counts of *P. equorum* in four study regions, Ethiopia.

Regions	Number of donkeys examined	Prevalence	Mean \pm se [†]
Ada	194	61.3 ^{ac*}	207.3 \pm 43.4 ^{a*}
Akaki	164	62.2 ^{bd}	207.1 \pm 47.1 ^b
Bereh.	175	37.1 ^{bc}	62.6 \pm 13.8 ^{abc}
Boset	270	45.9 ^{ad}	146.7 \pm 26.0 ^c
Total	803	51.1	-

*Significant differences at P<0.05 level are indicated by the same superscript down the column.

[†]Fitted mean value for the negative binomial distribution linked with identity function.

Faecal samples examined from 402 working horses for the presence of ascarid eggs also revealed a prevalence of 16.2%. An infection prevalence of 12.2% was obtained in horses of 8 years old and above. Among horses positive for ascarid eggs the mean faecal egg count of 799 \pm 1552 (mean \pm sd) was found in horses of 4.5 years old an above. The minimum and maximum epg found in this age group were 50 and 7800, respectively.

There were similarities in seasonal distribution of *P. equorum* infection and faecal egg output among the study regions. Eggs were recovered throughout the year although the infection prevalence and faecal egg counts were relatively higher during the wet season (Figs 3.4 and 3.5).

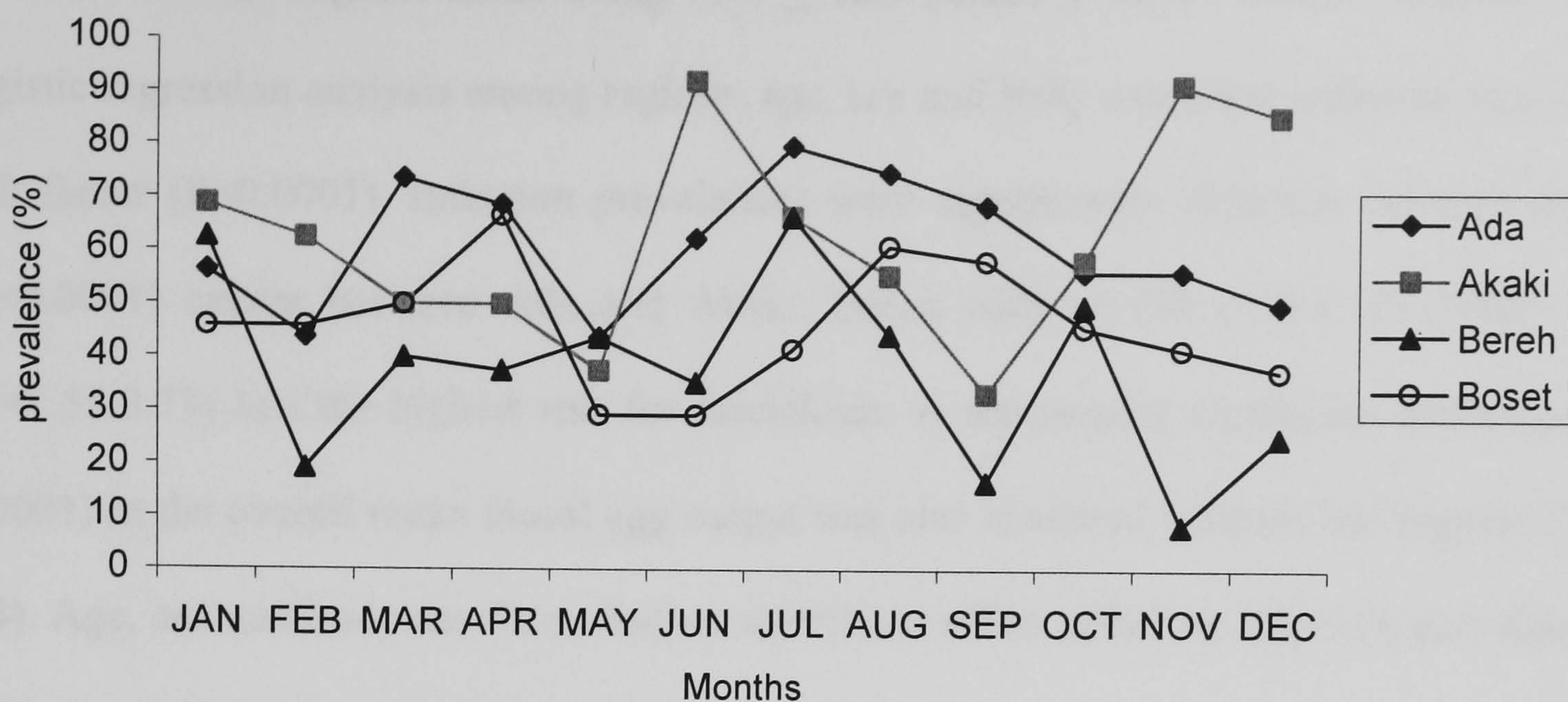


Fig 3.4 Seasonal prevalence of *P. equorum* of donkeys in the study regions, Ethiopia.

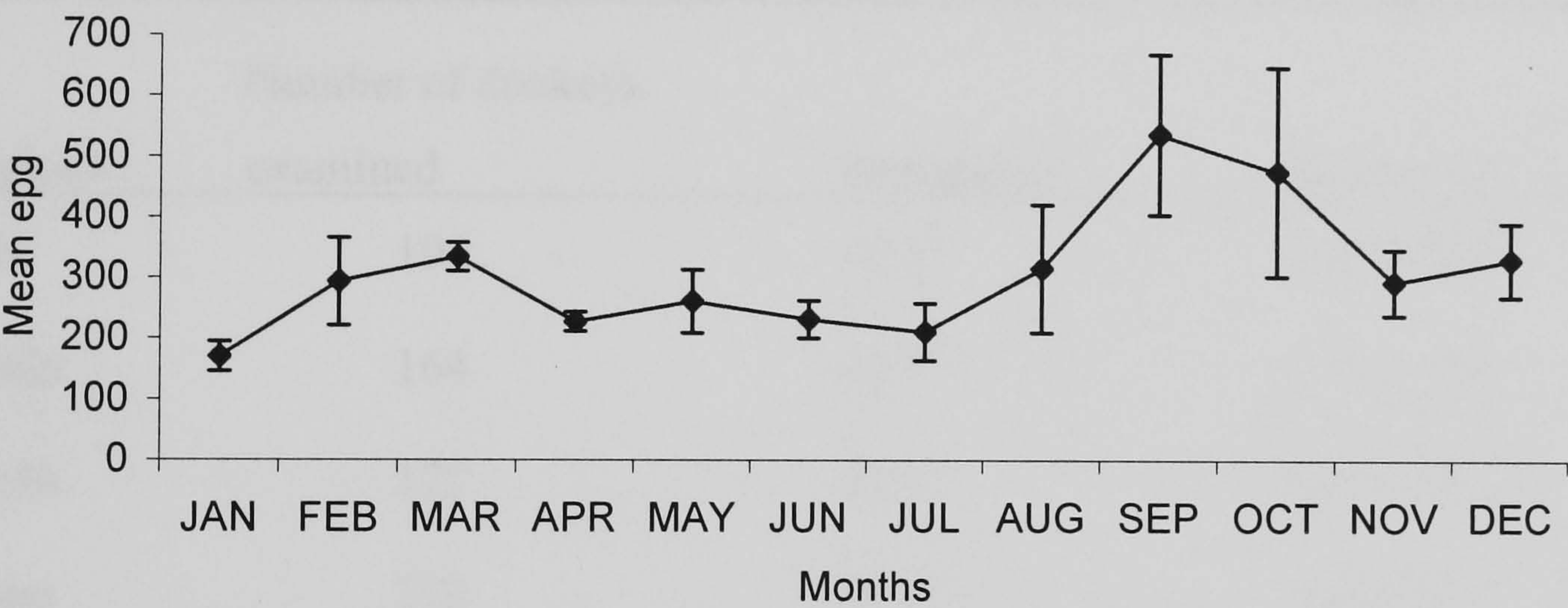


Fig.3.5 Seasonal distribution of the overall mean faecal egg counts of *P. equorum* of donkeys, Ethiopia. Error bars indicate mean \pm se. The mean is a fitted value for the negative binomial distribution linked with identity function.

3.3.3. Trematodes

Fluke infection was found widely distributed among the donkey population in Ethiopia. An overall prevalence of 44.7% with the highest prevalence of 72.6% being in the highland region of Bereh was recorded (Table 3.4). Among donkeys positive for *Fasciola* eggs the egg output ranged from 10 to 960 epg with the lowest mean being 34.4 ± 28.9 (mean \pm sd)

in Boset and the highest mean being 77.4 ± 72.9 (mean \pm sd) in Bereh. Multiple binary logistic regression analysis among regions, age, sex and body condition indicated region as a risk factor ($P<0.0001$). Infection prevalences were significantly different between regions ($P<0.0001$) except between Ada and Akaki. Bereh with an OR of 2.4 ($P<0.0001$, 95% CI=1.55-3.73) had the highest risk for fasciolosis. A statistically significant difference ($P<0.0001$) in the overall mean faecal egg output was also observed between the regions (Table 3.4). Age, sex and body condition had no significant effect either on infection prevalence or mean faecal egg counts (Tables 3.6-3.8).

Table 3.4. The prevalence (%) and the overall mean faecal egg counts of *Fasciola* spp in four study regions, Ethiopia.

Regions	Number of donkeys examined	Prevalence	Mean + se [†]
Ada	194	52.6 ^{a*}	32.0 \pm 6.1 ^{a*}
Akaki	164	43.9 ^{bc}	17.3 \pm 3.6 ^a
Bereh	175	72.6 ^{ab}	56.1 \pm 11.3 ^a
Boset	270	21.5 ^{ac}	8.0 \pm 1.2 ^a
Total	803	44.7	-

* Significant differences at $P<0.05$ level are indicated by the same superscript down the column.

[†]Fitted mean value for the negative binomial distribution linked with identity function.

Eggs of *Fasciola* were recovered throughout the year in all regions except in July at Boset. The general trend however, indicated increased prevalence following the rainy seasons (Fig. 3.6). The overall monthly distribution of faecal egg counts was similar among the different regions. Relatively more eggs were recovered following the short rainy season (March and April) and during the long rainy season (June-September) than in dry season (Fig. 3.7).

Table 3.5. The prevalence (%) and the faecal egg count (epg) of *Fasciola* spp. in the study regions, Ethiopia

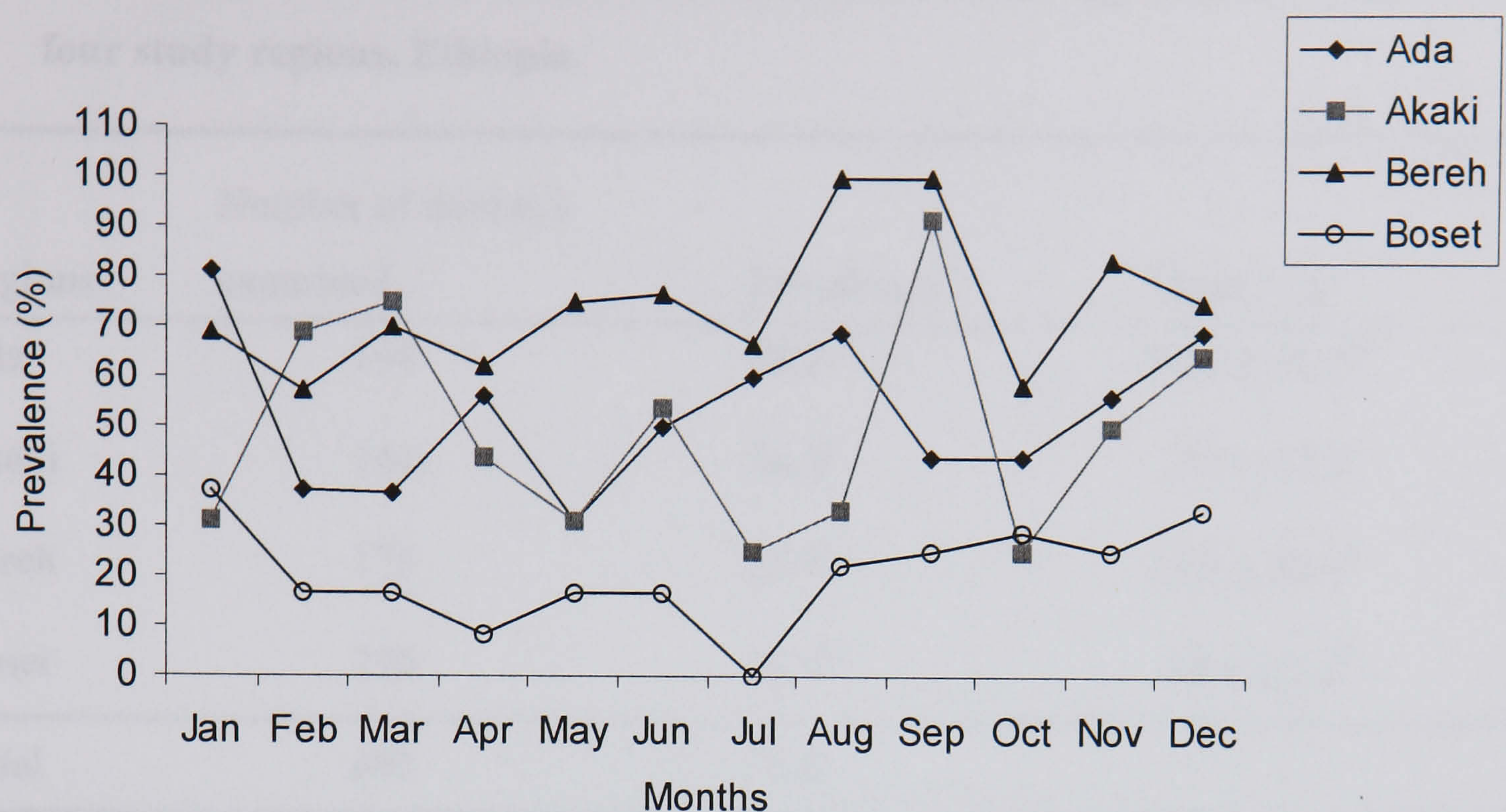


Fig 3.6 Seasonal prevalence of *Fasciola* spp of donkeys in the study regions, Ethiopia

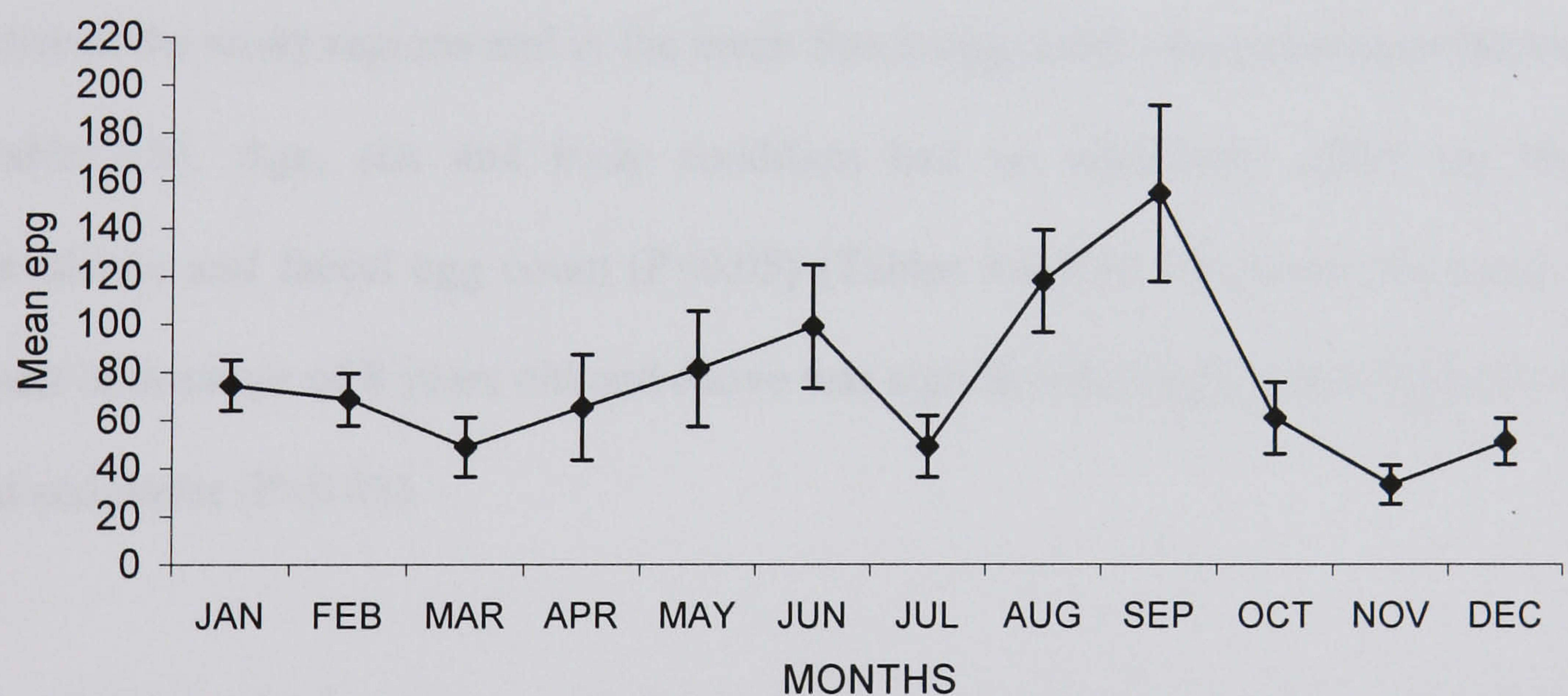


Fig. 3.7 Seasonal distribution of the overall mean faecal egg counts of *Fasciola* spp of donkeys, Ethiopia. Error bars indicate mean \pm se. The mean is a fitted value for the negative binomial distribution linked with identity function.

Results for *Gastrodiscus* faecal egg recovery revealed an overall prevalence of 35%. The egg counts ranged between 10 and 2235 epg in coprologically positive donkeys. The maximum prevalence (58.2%) and highest overall mean faecal egg count (74.9 ± 18.3 se) were recorded at Ada (Table 3.5).

Table 3.5. The prevalence (%) and the overall mean faecal egg counts of *Gastrodiscus* in four study regions, Ethiopia.

Regions	Number of donkeys examined	Prevalence	Mean \pm se [†]
Ada	194	58.2 ^{a*}	74.9 \pm 18.3 ^{ab*}
Akaki	164	44.5 ^a	27.3 \pm 7.3 ^a
Bereh	175	27.4 ^a	13.8 \pm 33.6 ^a
Boset	270	17.4 ^a	10.4 \pm 2.2 ^b
Total	803	35.0	-

* Significant differences at P<0.05 level are indicated by the same superscript down the column.

[†]Fitted mean value for the negative binomial distribution linked with identity function.

Statistical analysis revealed significant differences in infection prevalence (P<0.0001) between the study regions and in the mean faecal egg count except between Bereh and Boset (Table 3.5). Age, sex and body condition had no significant effect on the infection prevalence and faecal egg count (P<0.05) (Tables 3.6-3.8). However, the mean faecal egg count of donkeys of 8 years old and above was significantly higher than donkeys of 2.5 years old and under (P=0.03).

Bimodal infection prevalence of *Gastrodiscus* was observed. Peak prevalences were seen following the short and long rainy seasons (Fig. 3.8). The monthly faecal egg output however, was similar to that of *Fasciola* (Fig. 3.9).

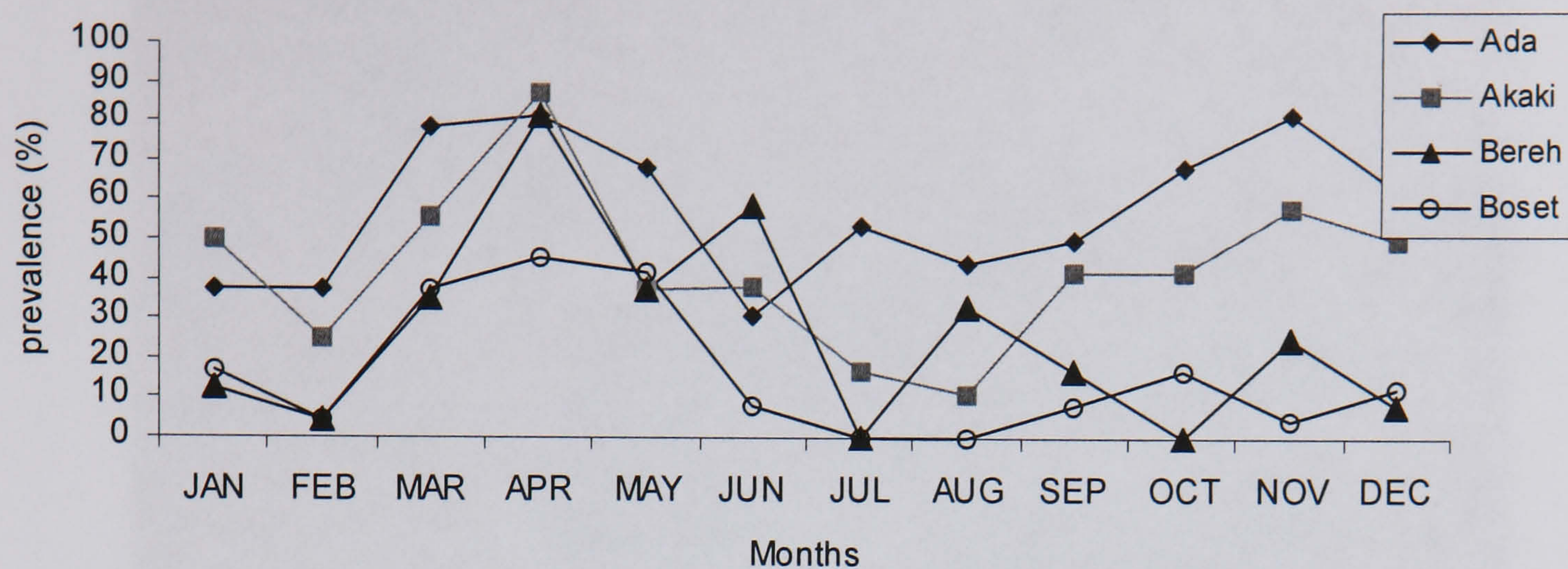


Fig 3.8 Seasonal prevalence of *Gastrodiscus* of donkeys in the study regions, Ethiopia.

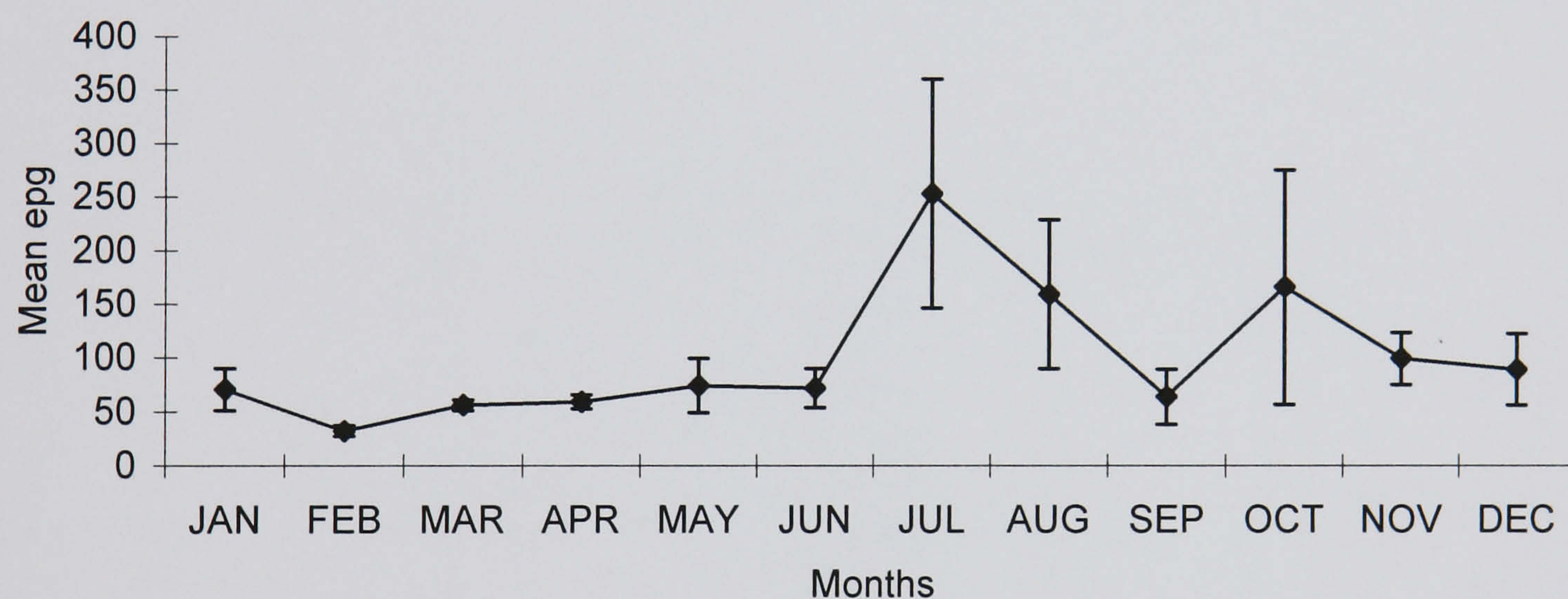


Fig 3.9. Seasonal distribution of the overall mean faecal egg counts of *Gastrodiscus* of donkeys, Ethiopia. Error bars indicate mean \pm se. The mean is a fitted value for the negative binomial distribution linked with identity function.

Eggs of *Trichuris* spp, *Ascaris* spp and *O. equi* were also recovered with prevalences of 27.4%, 29.6% and 12.6%, respectively (Fig 3.10).



Fig 3.10. Eggs of ascarids (white arrow) and *Trichuris* (thin black arrow) recovered from donkey faeces: The thick black arrow shows *P. equorum* egg.

Table 3.6. The overall mean faecal egg counts (epg) calculated fitted to the negative binomial distribution, and the prevalences of the four helminth parasites in four age (years) categories of donkeys, Ethiopia.

Helminths	Faecal egg counts (mean epg \pm se)*				Prevalence (%) (n= 803)			
	≤ 2.5	2.5-4.5	4.5-8	≥ 8	≤ 2.5	2.5-4.5	4.5-8	≥ 8
Cestode	11.52 \pm 7.13	3.79 \pm 1.96	5.01 \pm 2.48	4.45 \pm 1.24	4.5	5.1	4.5	13.8
<i>P. equorum</i>	130.79 \pm 40.87	164.81 \pm 42.94	125.64 \pm 31	166.88 \pm 23.29	5.2	9.1	8.7	28.0
<i>Fasciola</i>	31.97 \pm 9.48	22.27 \pm 5.51	42.49 \pm 10.04	20.65 \pm 2.74	5.5	6.9	9.1	23.3
<i>Gastrodiscus</i>	14.55 \pm 5.51 ^{a**}	31.0 \pm 9.75	35.97 \pm 10.85	31.2 \pm 5.27 ^a	3.6	6.0	6.4	19.1

* Fitted mean value for the negative binomial distribution linked with identity function.

**Significant differences at P<0.05 level are indicated by same superscripts across rows.

Table 3.7 The overall mean faecal egg count (epg) calculated fitted to the negative binomial distribution, and the prevalence of four helminth parasites in each sex groups of donkeys, Ethiopia.

Helminths	Mean faecal egg count (epg \pm se*)		Prevalence (%)	
	Male	Female	Male	Female
Cestode	4.77 \pm 1.60	5.66 \pm 1.46	12.8	15.1
<i>P. equorum</i>	170.06 \pm 20.24	142.81 \pm 26.07	24.2	26.9
<i>Fasciola</i>	24.85 \pm 3.65	26.93 \pm 3.65	20.6	24.2
<i>Gastrodiscus</i>	22.53 \pm 6.28	36.64 \pm 4.18	15.8	19.2

* Fitted mean value for the negative binomial distribution linked with identity function.

No statistically significant differences in infection prevalence or mean faecal egg outputs were observed between male and female for all helminths studied.

Table 3.8 The overall faecal egg counts (epg) calculated fitted to the negative binomial distribution, and the prevalence of the four helminth parasites in the three body condition score (BCS) categories of donkeys, Ethiopia.

Helminths	Faecal egg counts (mean epg ± se*)			Prevalence (%), (n=803)		
	BCS1	BCS2	BCS3	BCS1	BCS2	BCS3
Cestodes	2.32 ± 1.35	5.50 ± 1.38	6.30 ± 2.99	3.9	19.1	5.0
<i>P. equorum</i>	117.96 ± 34.27	154.73 ± 19.50	182.78 ± 43.55	5.6	34.9	10.6
<i>Fasciola</i>	22.96 ± 6.37	28.86 ± 3.47	17.65 ± 4.02	6.0	31.0	7.7
<i>Gastrodiscus</i>	35.15 ± 12.37	32.36 ± 9.49	18.82 ± 5.44	4.7	24.0	6.2

* Fitted mean value for the negative binomial distribution linked with identity function.

No statistically significant differences in infection prevalence or mean faecal egg outputs were observed between the different body condition score for all helminths studied.

3.4. Discussion

3.4.1. Cestodes

The high prevalence (27.9%) of cestode infection obtained shows the wide distribution of this parasite among the donkey population of Ethiopia. Although practically it is difficult to differentiate eggs of *A. magna* from that of *A. perfoliata*, particularly if they present with abnormal shapes under the microscope, the majority of the observed eggs had many morphological similarities with *A. perfoliata*. Moreover, egg measurements conducted revealed that they are within the range of that of *A. perfoliata* suggesting it was the dominant species (Appendix IV). The prevalence of *A. perfoliata* obtained from donkeys on post-mortem examination (Chapter Four) and serological results (Chapter Five) supports this suggestion.

A pilot coprological study by Proudman and Ellis (1992) revealed a similar prevalence of 27% (n=29). Infection prevalences of 25% (n=160) and 33% (n=6) have also been reported from studies made on post-mortem examinations of donkeys in South Africa (Matthee et al., 2000, 2002) and Kenya (Lewa et al. 1998), respectively. On the other hand, post-mortem examinations made by Khallaayoune (1991) in Morocco and Burgu et al. (1995) in Turkey revealed relatively low prevalences of 2% (n=168) and 10% (n=110), respectively. Although the number of donkeys examined was not mentioned, less than 1% infection prevalence of tapeworm was reported at The Donkey Sanctuary, England, which was attributed to the poor habitat for the oribatid mites on the sanctuary farms (Trawford, 1997). However, the small number of donkeys examined as compared to the present study in some and the different methodology used in others does not allow us to make an epidemiologically sound comparison. Moreover, no associations were reported with age or any other factors that may influence the infection prevalence or intensity of infection.

The high prevalence of tapeworm in the present study may indicate the wide distribution of oribatid mites, which are the intermediate hosts for cestodes and in which the immature stage, cysticercoids, develop. It may also indicate the favourable environmental conditions for the survival and development of both the mites and of cestode eggs. As there is no treatment for cestodes of donkeys in Ethiopia, the significantly high infection prevalence and faecal egg output recorded in the highland region of Bereh, might be taken as an indication that environmental conditions in this area are more favourable than those found in the other study areas. Bereh is characterized by low-lying, wet and more permanent pasturelands as compared to the other regions. Such highland regions are known to support numerous mites and provide a high potential for transmission (Dipietro, et al., 1990). Studies have shown that oribatid mites exist as free-living forms and can be numerous, as high as 20,000 mites per square metre on undisturbed permanent pastures (Jacobs, 1986; Bain and Kelly, 1977). In the Bereh area the pasture management system is also quite different from the other areas. Most highland regions are characterised by a wide area of permanent pasture specifically kept for animals and for haymaking. According to Ihler et al. (1995), permanent pastures are land that has been grazed by animals for at least the last 5 years in succession without being disturbed. According to the information obtained from the regional ministry of agriculture (personal communication) these regions have been used as permanent pasture for animals to graze and for hay making for more than 50 years without being disturbed, and are still in use. Grazing pastures are available throughout the year resulting in longer grazing periods, which could result in the ingestion of more mites. These could be the main reasons why the donkeys in this region are at a greater risk of infection.

On the other hand, the other three regions (Ada, Akaki and Boset) have more or less similar pasture and pasture management system. In most of these areas the land is continuously being converted to crop lands and grazing lands are very scarce. The tradition of leaving

cultivated lands fallow for a year or two to allow them to recover their fertility is widely practiced. Such land often used as occasional pastureland. Such land management may disturb the ecology of the mites and affect their ability to develop and survive. Moreover, grazing on all pasturelands is seasonal. Grasses are very scarce and virtually unavailable during the long dry season. During this time of the year animals depend on the available crop residues and straw. Therefore, the grazing period is restricted to the wet season (June-September) and the donkeys are therefore less exposed to infection. The lack of availability of vegetation during the dry period may also influence the density of the mites reducing the risk of infection in the next wet season. These could be important factors for the relatively low prevalence of cestode infection in these regions. The influence of other factors, particularly those that affect the survival rate of the mites and cestode eggs, such as different soil types, acidic conditions in the microenvironment and availability of watering points such as ponds, streams, or rivers may also be important.

In the present study donkeys of all ages were found excreting tapeworm eggs. Although, there was considerable individual variation within all age groups, there was no statistically significant difference ($P < 0.05$) in mean faecal egg output between the different age groups. This may show the donkey's lack of immunity against cestode infection. However, care should be taken in interpreting faecal egg output, as cestode eggs are not easily detected in faecal flotation techniques and egg counts may not represent the level of infection intensity. Thorough abattoir studies rather than coprology have to be conducted to know the level of infection in the different age groups. Similar results to ours however, were reported in horses in which both coprological and abattoir studies showed that age did not significantly influence the prevalence of *A. perfoliata* infection (Lyons, et al., 1984; Owen, et al., 1988; Fogarty, et al., 1994; Ihler et al., 1995). Moreover, these studies also revealed similar magnitudes of infection level of *A. perfoliata* irrespective of age and sex, indicating neither

acquired protective immunity nor age resistance to *A. perfoliata* seem to be of any importance. Hass (1979), however, reported that cestodes are more common in adult horses than in foals.

The McMaster technique has a very low sensitivity to detect cestode eggs compared to centrifugation/flotation technique. Studies made have shown that although there is no correlation between the number of detectable *A. perfoliata* and the number of eggs recovered by centrifugation/flotation techniques in individual horses, the egg recovery rate and the number of detectable eggs were significantly higher for high worm burdens (>20 worms) than for low worm burdens (<20 worms) (Proudman and Edwards 1992; Nilsson, et al., 1995; Williamson et al., 1998). Proudman and Edwards (1992) demonstrated an increase in sensitivity of the centrifugation/flotation technique at higher intensities of infection; an increase from 61% overall to 92% when horses with fewer than 20 tapeworms were eliminated from analysis. Similar results were reported by Fogarty, et al. (1994), Nilsson, et al. (1995) and Williamson et al. (1998) in which they demonstrated the existence of association between increased intensity of infection and a high probability of detecting eggs. This indicates that although the overall sensitivity of the method was relatively low, its reliability at high intensities of infection, which predisposes individuals to clinical disease, was significantly higher. The significantly higher probability of a test in identifying the animals most at risk of clinical disease makes it considerably more valuable to the clinician than overall sensitivity would indicate. Therefore, the high prevalence and faecal egg counts obtained in the present study, particularly in the highland region of Bereh, could indicate that donkeys in Ethiopia are at high risk of clinical disease.

The lack of information regarding the intensity of infection and the relationship with egg recovery in the donkey population however makes comparison of our result difficult.

Although the correlation between intensity of infection and a high probability of detecting eggs demonstrated in the horse might also hold true in donkeys, the association between intensity of infection and the probability of egg recovery rate and/or egg count, and clinical disease in the donkey population is unknown. On the other hand, while a high egg count may be reliable indication that an animal is at possible risk of clinical disease, a small number of eggs counted does not preclude the possibility that an animal is harbouring a large number of cestodes. Surveys of adequate sample size need to be conducted using direct worm recovery in necropsy materials or post-mortem to know the true prevalence of cestodosis in the donkey population of Ethiopia. The problem with an abattoir study in Ethiopia is that donkeys are not slaughtered either for human consumption or to be processed for animal feed on religious grounds. Any future study would depend on recovering worms from donkeys that have died or euthanased at different veterinary clinics in the country.

There appears to be some seasonal variation in the prevalence of cestode infection although the eggs were detected throughout the year. More donkeys were found to be positive for cestode eggs starting at the end of the long rainy season and during the dry season. This could be a consequence of the animals' greater exposure to the infection during the wet season and the length of the life cycle, which takes two to four months for the cysticercoids to develop in the oribatid mites and a further 6 to 10 weeks for the development in the host (Soulsby, 1982). The sharp decline in prevalence in the month of April in some regions and in November in others, and subsequent rise in the following months may indicate the presence of more than one generation of cestodes in a year or turnover of the worms. Similar seasonal variation in the prevalence of cestode infection in horses, with the greatest prevalence during the winter months has been reported (Lyons et al., 1984; Hoglund et al., 1995). The presence of cestode eggs throughout the year may indicate the slow turn over of adult tapeworms and/or absence of treatment against cestode infection in the donkey

population in Ethiopia. This might explain the presence of infection throughout the year in regions where there is no permanent pasture and grazing is very scarce during the dry season and hence the chance of continuous reinfection would be rare. Continuous reinfection and/or the above justification would both be possible explanations in regions where there is permanent pasture and the animals graze throughout the year.

3.4.2. *Parascaris equorum*

The present study showed high prevalence of infection and faecal egg output of *P. equorum* in donkeys, irrespective of their age. Although there was no statistically significant difference between the different age groups of donkeys, the finding of high parascaris egg output in adult donkeys may indicate a potential danger for the donkey population as a whole. Few studies have investigated the prevalence of ascarid infection in donkeys. Coprological studies in Germany (Gothe and Heil, 1984) and India (Kotwal et al., 2000) revealed low prevalences of 2.8% (n=106) and 8.4% (n=83), respectively. However, these studies did not show the effect of age either on the prevalence or on faecal egg output and are difficult to compare with our results. A relatively high prevalence of parascariasis (23.5%, n = 131) in adult donkeys was reported by Vercruysse et al. (1986) from Burkina Faso with egg counts ranging from 100 to 500 epg. Similar results to our study were also reported by Wells et al. (1998) with prevalence of 33.9% (n=93) and the highest parascaris egg counts in the 6 month to 8 years age group of donkeys.

The small numbers of horses in the different age groups did not allow us to determine the effect of age on the infection prevalence and faecal egg output. As we did not have the treatment history of the horses, the overall low prevalence might be attributed to the effect of treatment, particularly in horses less than 4.5 years old. Cart owners buy young horses and most of them deworm their horses as soon as they bought. However, among the 65 (16.2%)

coprologically positive horses, 90% were above 4.5 years old and 11% of them were shedding *parascaris* eggs at rates greater than 200 epg. The prevalence of infection is reportedly 31% to 61% in horses younger than 1 year and 25% in horses older than 1 year in temperate regions passing small number of eggs (Hass, 1979). According to Clayton and Duncan (1979b) and Soulsby (1982), it is usually horse foals of three to 9 months of age that suffer from this parasite and even though patent infections are occasionally found in mature horses, they are of little or no clinical consequence. It would therefore appear that, unlike horses under temperate conditions, it is not uncommon to find ascarid infection in adult donkeys and at least in working horses under tropical conditions.

P. equorum is a highly antigenic and immunogenic parasite of young horses and ponies. Experimental studies have shown that, even in the absence of previous exposure, significant age-dependent immunity develops by six months of age (Clayton and Duncan, 1979b; Bello, 1985; Jacobs, 1986; Dipietro and Todd, 1988) and the incidence of infection declines from 6 months of age onwards (Poynter, 1970; Clayton, 1986; Austin et al., 1990; Southwood et al., 1998). It was also reported that even without the benefit of anthelmintics, most horses pass very few eggs or stop passing eggs altogether by one year of age (Bello, 1985; Austin, et al., 1990). Uhlinger (1993) indicated that adult horses occasionally have a small number of eggs in their faeces but the presence of a high egg count may suggest immuno-incompetence. This could be one explanation in working donkeys and horses in Ethiopia as these animals are highly stressed, undernourished and suffering from various ailments (Svendsen, 1997b; Starkey, 1994a; Getachew, 1999). On the other hand, it has been suggested that the few adult horses that harbour this parasite are usually asymptomatic and could be sources of contamination for young stock (Uhlinger, 1993). Hence, the high patent infection found both in adult working donkeys and in horses might put the young animals at risk even if we assume adults are not suffering much from infection. The findings of a number of ascarid

worms in the intestine of adult donkeys (Chapter 4) may lead us to believe that adult donkeys may not develop protective immunity or that they might have become immunocompromised.

Young horses with eggs in their faeces are candidates for treatment as they are considered to be a potential source of contamination for themselves and for other foals for several years. The goal of anthelmintic treatment for ascarid control should be preventing the development of patent infections that will seed the environment. This could be accomplished by suppressive treatment of not only the foals but also adult equids that share the living space and pasture with the young stock. There is no pasture management practice in Ethiopia and all age groups of equids spend their entire life together under an extensive management system. This might have contributed to the widespread infection among equids. Many practitioners and some parasitologists use 100-200 epg as an indication for anthelmintic treatment both in foals and adults with foals. In the present study the majority of adult donkeys and horses were found shedding eggs at more than 300 and 1000 epg, respectively. This is higher than the suggested cut-off number for treatment in adult horses (Uhlinger, 1993). Therefore, the treatment of foals and adults irrespective of the number of eggs present in their faeces is recommended in equids under Ethiopian tropical conditions. Most parasitologists also recommend this, as faecal egg detection techniques may underestimate the number of eggs present in faeces (Uhlinger, 1993). However, thorough experimental studies would need to be undertaken to understand the immune status of working donkeys and horses against *parascaris* infection under tropical conditions in general.

Farmers in Ethiopia use kraals (fenced bare paddocks constructed from locally available bushes and wood branches) to keep their animals during the night to protect them from predators. These kraals are almost permanent, are renovated every year and have frequently

been used for several years. As ascarid eggs are very resistant and able to remain viable for several years, kraals could be one of the potential sources of infection. The infection is further assisted by the donkeys' habit of eating soil when the ground of the kraal becomes dry during the dry season. This may be caused by some mineral deficiencies. A study by Ihler (1995) showed that even the removal of a paddock from use for 18 months did not result in a significant decrease in the number of parascaris infective eggs.

The observed variation in infection prevalence between the different regions could be attributed to the differences in environmental conditions, such as soil types or temperature, which may play a significant role in the development of ascarid eggs. For example, most of the Boset area has a solonchaks soil type, which is a gravel or gravel-sand mixture. In such soil texture the ascarid eggs may have been transported more rapidly down through the soil profile by rainwater, because of the good drainage, reducing the availability of eggs on the surface. This has been demonstrated with *Ascaris lumbricoides* eggs (Storay and Phillips, 1985) and *P. equorum* eggs (Ihler, 1995) through similar soil types. A study made by Ihler (1995) on different bare paddocks as to the distribution of ascarid eggs revealed a high proportion of embryonated eggs of *P. equorum* in the upper soil profile (0-5 cm deep) of clay soil types but a significantly higher proportion in the lower profile (10-15 cm deep) of the gravel and gravel-sand mixture soil types. Clay soil was the dominant soil type in Ada and Akaki where the high infection prevalences were recorded. The significantly low prevalence obtained in Bereh may indicate that the highland environment is not as suitable as the other regions for the survival and development of ascarid eggs. However, to obtain more precise information as to the effect of soil types and texture on the distribution, development and survival of ascarid eggs thorough experimental studies should be undertaken.

The high fecundity of female ascarids and the extreme resistance of their eggs to adverse environmental conditions ensure their persistence for several years. The adhesive property of the eggs facilitates passive spread through stables, paddocks and pastures (Austin et al., 1990; Uhlinger, 1993; Ihler, 1995; Southwood et al., 1998). Therefore the environment may remain a source of infection for a long period. Thus the biology of this parasite makes it unlikely that a change of season would greatly affect the prevalence of ascarid infection in equids. Our result is in agreement with this in that high prevalences were obtained both during the wet and dry seasons of the years although the relative rise in prevalence and faecal egg output during the wet seasons and subsequent months may indicate the high probability of reinfection during this time of the year.

3.4.3 *Fasciola*

The findings of an overall prevalence of 44.7% and the highest prevalence of 72.6% obtained in Bereh region indicate that fasciolosis is widespread in the donkey population of Ethiopia. Such high infection prevalence may not be surprising owing to the endemic nature of the disease in the region with high infection prevalence in domestic ruminants (Lemma, et al., 1985; Scotts and Goll, 1977; Yilma et al., 1998), and the total absence of treatment against trematode infection in equids in Ethiopia. Our results showed a fairly high prevalence compared to some studies made in donkeys. Haridy et al (2002) reported prevalences of 4.6–9.1% from different regions of Egypt with an overall prevalence of 3.03% (n=658), while Ashmawy and Diab (1998) reported relatively high prevalences ranging from 10 to 33.8% from eight governorates in Lower Egypt with an overall prevalence of 18.6% (n=1000). Coprological studies made by Green et al (1968) and Trawford and Tremlett (1996) showed a prevalence of 50% (n=24) and 56.7% (n=30), respectively. Although the total numbers of donkeys sampled were not mentioned, a survey made in West Ireland showed 91% infection prevalence (Kearney, 1974).

The presence of seasonal streams and brooks whose banks are generally irregular in shape, and covered with tangled bushes and undergrowth that provide an hospitable environment for snails and the infective stages of *Fasciola* characterise most of the bottom valley land of Bereh. In general the low-lying regions in the high plateaus of Ethiopia have poor drainage and the soil seems to be acidic (Lemma, et al., 1985). It is most likely that this particular trait of the Ethiopian highland favours the development of fasciolosis in the donkey population. Media with slightly acidic pH and an average temperature range of 10-21°C are considered optimum for the development of the snail Lymnaeidae and liver fluke (Ollerenshaw, 1971; Ollerenshaw, 1974). Above 30 °C snail eggs will not develop (Angus, 1978). In the present study weather conditions favour the development of both infective stage of flukes and snails, except during the long dry period in Ada, Akaki and Boset, particularly in Boset, where the day temperature is usually above 30 °C during this period.

Although the lowest infection prevalence was recorded in Boset, the 21.5% infection prevalence obtained seems to be quite high because studies made in bovine and ovine species reported that fasciolosis was rare in the Rift Valley regions of Ethiopia (Lemma et al, 1985; Graber, 1975). Because of the sandy nature of most of the places there are no waterlogged areas, and streams or brooks are very rare. The presence of ponds scattered in rural regions and the practice of irrigation in some areas near and around the Awash River could be the reason for the observed infection prevalence in this particular region. Another possible explanation is that lowlanders usually buy their donkeys from the highlanders where fasciolosis is endemic and they could have already been infected before they were purchased. On the other hand, the presence of permanent and seasonal shallow water bodies together with seasonal streams and brooks in some places could be the major reason for the prevalences of fasciolosis in Ada and Akaki. Because of the lack of permanent pasture and the poor vegetation cover of seasonal pastures animals are forced to graze these limited

areas. According to our observation, these areas are more crowded with animals in Ada, Akaki and Boset than in Bereh because of the limited number and size of watering areas. Local overcrowding has particular importance in the epidemiology of fluke infection. Firstly, in the heavy contamination of snail habitat with ova. Secondly, in the ingestion of metacercariae, an important dynamic cause for infection transmission. Local crowding of animals along the banks of shallow water bodies, ponds and irrigation canals during the dry season when nutritional conditions are generally compromised provides an important dynamic cause for infection transmission. This seems to best explain the increased prevalence of fasciolosis in these regions rather than the widely spread waterlogged regions at Bereh, which at first sight might appear more suitable for spread of fasciolosis.

Lymnaea truncatula and *L. natalenses*, intermediate hosts for *F. hepatica* and *F. gigantica*, respectively, are present in Ethiopia (Goll and Scott, 1978). According to Goll and Scott (1978), the period of peak snail population was mid August and the peak of infection of snails by *F. hepatica* meracidium was found at the end of September. The present finding indicates that fasciolosis is present throughout the year in the donkey population in all areas investigated except in Boset in which no eggs were detected in July. When the result is viewed in light of the climatological characteristics prevailing in the different regions, fasciolosis in the donkey population seems to be mostly encountered after the heavy long rainy season. Studies made by Scott and Goll (1977) and Yilma et al. (1998) showed that adult fluke numbers were significantly higher in ovine and bovine species during the months of the dry season following the wet seasons rather than at other times of the year. The relatively high infection prevalence and mean faecal egg count seen during the months of July to October may indicate fluke infection acquired during the short rainy season. Erich (1983) and Lemma et al. (1985) reported that aestivating snails are able to survive from the long rainy season to become active during the small rainy season, and might cause

fasciolosis. Although eggs have been detected throughout the year, the lower faecal egg count obtained during the dry season, during the time of the year when high fluke infection has been reported in ovine and bovine species (Scott and Goll, 1977; Yilma et al., 1998) may indicate the density-dependent effect on worm fecundity or different infection level in donkeys. However, any interpretation of fluke infection only based on coprology in the donkey population needs to be cautious as there is no treatment against flukes in donkeys and there are suggestions that egg shedding by fluke occurs throughout the year as the life span of *Fasciola* is considered to be long (Ross et al., 1967; Rangel-Reuiz, et al., 1999). In addition, the immune status of the donkeys against fluke infection is not known.

3.4.4 *Gastrodiscus*

The epidemiology of this parasite is not well known in equids in Ethiopia. This is the first extensive coprological study made to determine its prevalence in the donkey population. The finding of an overall prevalence of 35% and the high prevalence recorded in Ada (58.2%) shows its wide distribution in the donkey population of Ethiopia. Similar results were reported from South Africa with prevalences ranging from 14% to 63% depending on the region from where faecal samples were examined (Wells et al., 1998). The statistically significant difference obtained in the mean faecal egg output between younger and older donkeys is in agreement with the results reported by Wells et. al. (1998).

The snail intermediate hosts so far recognized are *Cleopatra* spps in Egypt and the *Bulinus* spps in tropical Africa (Angus, 1978). Although the exact snail species involved with *Gastrodiscus* infection in equids in Ethiopia are not known, the *Bulinus* species are known to exist in the study areas (Brown, 1967; Bizuneh et al., 1995).

The variation in prevalence and faecal egg output among the different study regions might be due to differences in the distribution of the snail host. However, the general pattern of seasonal prevalence is similar in all regions and seems to follow the rainfall distribution. The prevalence is bimodal and the high peaks after the short and long rainy seasons indicate that the infection might have taken place during the rainy seasons with adults reaching maturity during the subsequent dry season.

3.4.5 *Ascaris* and *Trichuris*

This report of *Ascarid* eggs other than *P. equorum*, and *Trichuris* eggs in equids with such high prevalence is the first of its kind. They are regarded as accidental findings in equids (Soulsby, 1982). Ascarids are thought to be highly host specific and *P. equorum* is considered to be the only ascarid in equids (Soulsby, 1982). The physical characteristics, shape and size of the recovered *Ascaris* and *Trichuris* eggs are quite similar to either *Ascaris suum* or *Ascarid lumbricoides*, and *Trichuris trichiura*, respectively. Whether donkeys are the natural hosts for these parasites or whether infection is accidental is not known. However, the absence of any adult ascarid other than *P. equorum* so far recovered and identified (Chapter 4) from donkeys in the present study might lead us to suggest that accidental ingestion of eggs from the environment has caused the present findings.

Ascaris lumbricoides and *Trichuris trichiura* infection are the two most common human helminthic diseases in almost all parts of Ethiopia, particularly in children. Prevalences as high as 40.7-83% for *A. lumbricoides* and 13–80% for *T. trichiura* have been reported from the central highland and lowland regions, which includes our present study sites (Mamo et al., 1989; Nahmias, et al., 1991; Dagnew et al., 1995; Jemaneh, 1998; Erko and Medhin, 2003). In rural Ethiopia the majority of people use open bushes for defecation without burying. There is no habit or tradition of constructing pit latrines. Donkeys have a habit of

eating dried human faeces and deeply graze the areas where faeces are deposited. This could be related to certain mineral deficiencies in their diet, as donkeys are not supplemented with extra feed or mineral licks. Horses have no such habit and no single egg of these kinds was recovered from the 402 horses examined. Therefore, the high prevalence of these parasites in the human population coupled with the human behaviour of defecating in an open field and the donkeys' habit of eating dried human faeces might explain the high prevalence of these two parasites eggs in donkeys' faeces as passive ingestion without the eggs developing. However, thorough experimental investigation would need to be undertaken to know whether donkeys are a natural host of these parasites.

3.4.6 *Oxyuris equi*

The overall 12.6% prevalence of *O. equi* infection was an unexpected result. Because of the egg-laying behaviour of the adult female around the peri-anal region, acetate tape preparations are primarily used to diagnose oxyuris infection, and recovery of eggs through faecal flotation is rare (Soulsby, 1982). A similar result was reported by Well et al. (1998) with an infection prevalence of 17.7% (n=64) in donkeys through direct faecal examination. The possible capture of eggs from the peri-anal region during faecal sampling could be one explanation for such a high recovery rate. The change in behaviour of female worms in laying eggs could be another possibility. Well et al. (1998) reported *O. equi* almost exclusively in female donkeys, which they thought might be because of the close association between Jennies in a herd and their foals. But in our case there was no significant difference between male and female donkeys. The mutual grooming behaviour of donkeys could play an important part in the transmission of *O. equi* between members of a herd.

Generally the present coprological survey has shown that donkeys in Ethiopia are harbouring quite a substantial number of helminth species apart from the strongyles, which has been

repeatedly reported with 100% prevalences in Ethiopia (Feseha, 1998; Getachew, 1999) and in other countries (Pandey et al., 1994; Svendsen, 1994, 1997a; Feseha, 1998). The non-strongyle gastrointestinal helminths, particularly cestodes, trematodes and ascarids, however, have been rarely reported. The present study revealed a high infection prevalence of these helminths and has demonstrated polyparasitism exists in the donkey population of Ethiopia. Particularly, the finding of cestodes and trematodes with high infection prevalences showed that they are not accidental or unusual finding in donkeys, as has been previously suggested. Considering the absence of care and management of donkeys in Ethiopia, the poor body condition of the animals, the high strongyle faecal worm egg counts (data not shown), and the high prevalence of parascarid infection, it is very unlikely that they have received any recent anthelmintic treatment. Moreover, donkeys have never been treated against cestodes and trematodes in Ethiopia. It seems therefore, that the prevalences of all species of helminth parasites and faecal egg counts represent the natural levels in the absence of helminth control.

CHAPTER FOUR

THE PREVALENCE AND ABUNDANCE OF GASTROINTESTINAL PARASITES OF WORKING DONKEYS IN ETHIOPIA: A RETROSPECTIVE STUDY OF CESTODES, TREMATODES, ASCARIDS AND ARTHROPODS.

4.1 Introduction

Equids are hosts to more than 75 species of helminths belonging to 28 genera of nematodes, 8 species in 4 genera of trematodes, 4 species in 3 genera of cestodes, and 10 species of arthropods belonging to 2 genera (Lichtenfels, 1975; Soulsby, 1982; Krecek, et al, 1987, 1994). Studies of helminth parasites in donkeys in Africa (Graber, 1970; Vercruysse et al., 1986; Pandey and Eysker, 1990; Khallaayoune, 1991; Mattioli, et al., 1994; Pandey et. al., 1994; Karanja et al., 1994; Lewa et al., 1997; Getachew, 1999; Matthee et al., 2000, 2002) and elsewhere (Tolliver, et al., 1985; Burgu et al., 1995; Gonenc, 1999) have uncovered a diversity of helminth species. These studies have shown that helminths in working donkeys are not only highly prevalent but infection intensities are also very high. Heavy internal parasite burden can adversely affect the health of equids, particularly when an animal is called upon to work and, as is often the case, is undernourished and stressed (Svendsen, 1994, 1997b; Krecek and Guthrie, 1999). Most of these studies were, however, focused on strongyles and few attempts have been made to study the non-strongyle helminths of donkeys. No studies have been conducted on internal parasites of working donkeys of Ethiopia based on necropsy findings except to identify the different species of strongyles and their relative abundance (Getachew, 1999). The few case reports available have been based on a small number of donkeys and most of the information was from coprological studies (Feseha et al., 1991; Yilma, et al., 1991; Feseha, 1998; Getachew, 1999). These studies were semi-quantitative. Quantitative studies on the gastrointestinal parasites of donkeys, particularly the non-strongyle helminths, cestodes, trematodes and ascarids is lacking.

Therefore, information regarding species composition, abundance, prevalence, and site distribution of helminth parasites is limited in Ethiopian donkeys. The lack of information on necropsy findings is related to the fact that donkey meat is not consumed or processed to animal feed due to religious beliefs in Ethiopia. For this reason there is no donkey slaughtering in abattoirs thus obtaining data is not simple.

The current study was based on an accumulation of data from necropsied donkeys at the Donkey Health and Welfare Project (DHWP) Hospital, Faculty of Veterinary Medicine, Addis Ababa University (AAU). This was part of the on-going study in parasite control which includes, documenting helminth biodiversity, their prevalence, abundance, pathogenic effect, economic significance and also developing strategic treatment and control programmes in working donkeys of Ethiopia. The present chapter presents part of this long-term study with particular reference to the relative abundance and prevalence of cestodes, trematodes, ascarids and bot larvae recovered from post-mortem examinations of donkeys. Some gross pathological lesions believed to be caused by these parasites are also presented. Data on strongyles and other non-strongyle helminths is reported elsewhere (Getachew, 1999).

4.2. Materials and methods

4.2.1. Animals

A total of 122 donkeys were examined for several species of internal parasites at necropsy at the Faculty of Veterinary Medicine, AAU, Ethiopia. However, only 112 donkeys fully examined for cestodes, trematodes, ascarids and bot larvae were considered in the present study. The rest 10 animals had been either treated with ivermectin while they were hospitalised at the clinic, or had not been examined for the above-mentioned parasites and hence were excluded from the study.

The 112 donkeys represented 61 males and 51 females ranging in age from 1 to 20 years (9 ± 4 yr) (mean \pm sd). They had originated from different sources within the vicinity of the Faculty of Veterinary Medicine, Debre Zeit. There were also some donkeys abandoned by their owners with serious injuries due to car accidents and predator bites, and transported to the hospital from the nearby cities/towns or villages within 70 km radius from the hospital. Information on clinical history and cause of death had shown that the donkeys either died of various clinical ailments or were euthanased because of poor prognosis with no chance of recovery. Specific history on the use of parasiticides in these animals showed no prior use of anthelmintic except the abandoned donkeys for which information was not available. However, considering the usual practice of care and management of donkeys in Ethiopia, the poor body condition of the animals and their parasitic fauna at necropsy, it is very unlikely that they had received any drug treatment. However, caution is needed in interpreting the result, as these animals were not sampled from a population of known frame and there could be a bias.

Data were collected from 1995 to 2004 at different times of the year. Breakdown by year of the number of donkeys examined is: 1995 (8), 1996 (11), 1997 (11), 1998 (7), 1999 (5), 2000 (16), 2001 (11), 2002 (9), 2003 (17), and 2004 (17). Each donkey was subjected to routine pathological necropsy procedures (Klei and Torbert, 1980; Malan et al, 1981a) and the gastrointestinal tract was opened in its entirety to identify lesions and collect parasites. A complete examination of the contents and mucosal lining of each portion of the gastrointestinal tract was conducted to recover any mature adult worms. Stomach, small intestine, caecum, ventral and dorsal colon, and small colon/rectum were portions of gastrointestinal tract examined. Organs of the abdominal and thoracic cavities, particularly, liver and lungs were examined for liver flukes and lungworms, respectively. Details on worm recovery procedures and identification are outlined in Chapter Two and Appendix VI.

4.2.2. Statistical analysis

Because of the skewed nature of the worm counts generalized linear model fitted with negative binomial distribution and linked with identity function was employed. Multiple binary logistic regressions were conducted for the risk of having the different types of parasite infections. All statistical analysis was performed in R Version 2.0.1 (R Development Core Team, 2004), whereas descriptive statistics and graphs were produced in Microsoft Excel data analysis tool. The significant levels for all statistical tests were set to $P < 0.05$.

4.3 Results

A total of 43 species of parasites, consisting of 34 species of nematodes, 3 species of trematodes, 3 species of cestodes and 3 species of arthropods were recovered from the autopsied donkeys. These included 24 strongylids (17 species of small and 7 species of large strongyles), two anoplocephalids, 1 anoplocephaloid (paranoplocephalid), two fasciolids, 1 paramphistomatid, 1 ascarid, 3 spirurids (habronematids), 1 oxyurid, and 2 gasterophilids. In addition, 1 attractid (cosmocercid), 1 onchocercid, 1 strongyloidid, 1 dictyocaulid, 1 trichstrongylid and 1 oestrid species were recovered. Only data on cestodes, trematodes, ascarids and bot larvae are reported here and information on the rest of the parasites has been reported elsewhere.

The prevalences, mean intensities and ranges of the different species of cestodes, trematodes, ascarid and bot larvae are shown in Table 4.1.

Table 4.1 Prevalence, mean intensity and range of the different species of cestodes, trematodes, ascarid and bot larvae recovered from donkeys at necropsy, Ethiopia (n=112).

Helminths	N ^o of donkeys Infected %		Mean N ^o of parasites per infected host	Range of N ^o of parasites per infected host
Anoplocephalidae				
<i>A. perfoliata</i>	19	17.0	36	4-203
<i>A. magna</i>	1	0.9	8	8
<i>A. mamillana</i>	2	1.8	5	4-5
Fasciolidae				
<i>Fasciola</i> spp*	47	42.0	30	3-79
Paramphistomatidae				
<i>G. aegyptiacus</i>	34	30.4	502	3-2523
Ascarididae				
<i>P. equorum</i>	61	54.5	14	3-77
Gasterophilidae				
<i>G. intestinalis</i>	108	96.4	103	2-816
<i>G. nasalis</i>	110	98.2	123	14-570

* *F. hepatica* and *F. gigantica*.

4.3.1. Anoplocephalids.

A. perfoliata was found in 17% of the donkeys autopsied while *A. magna* and *A. mamillana* only in 1 and 2 donkeys, respectively. All age groups of donkeys examined were infected. The donkeys with *A. perfoliata* infection were on average 11 years old (range 2-20) while donkeys without *A. perfoliata* 9 years old (range 1-20). Infection rate of *A. perfoliata* by sex were 8.0% for males and 8.9% for females. Statistical analysis showed no significant differences, both in infection prevalence and intensity of infection,

between the different age groups as well as between sexes ($P>0.05$). The number of *A. perfoliata* specimens per infected animals varied from 4 to 203. Eight (42.1%) had less than or equal to 10 worms, 5 (26.3%) had between 10 and 50 worms, 3 (15.8%) had between 50 and 100 and 3 (15.8%) had more than 100 worms. The number of tapeworms found varies and only a few donkeys had a high worm burden and many donkeys had few worms, and it seem that *A. perfoliata* infection follows aggregate distribution in the donkey population (Fig. 4.1).

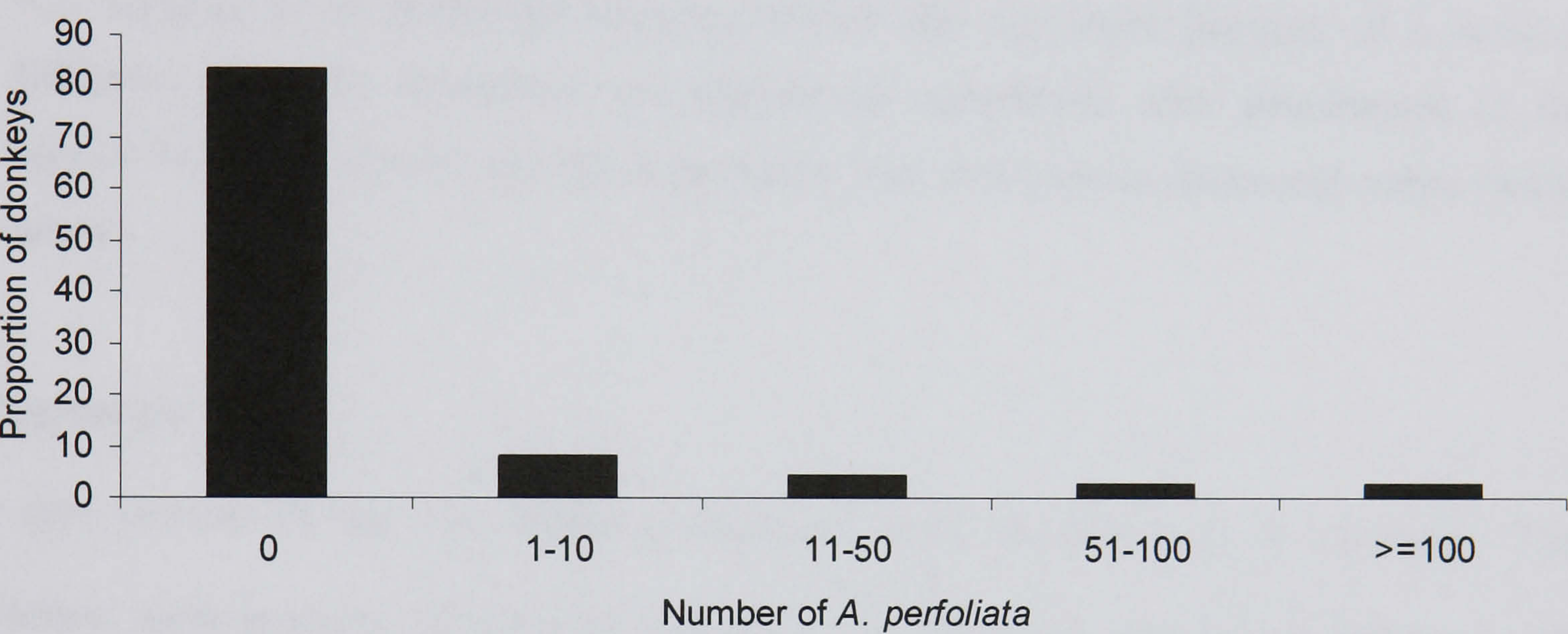


Fig. 4.1 Frequency distribution of the number of *A. perfoliata* in 112 donkeys autopsied, Ethiopia.

Most of the donkeys infected with few *A. perfoliata* had no lesions or only slight lesions. Donkeys with large numbers of tapeworms had mucosal lesions of varying degrees of severity. In most cases mucosal lesions at the ileocaecal junction are more severe than the caecum. The lesions comprised mucosal thickening, oedema, hyperaemia, ulcerations and diphtheritic membranes (Fig 4.2.)

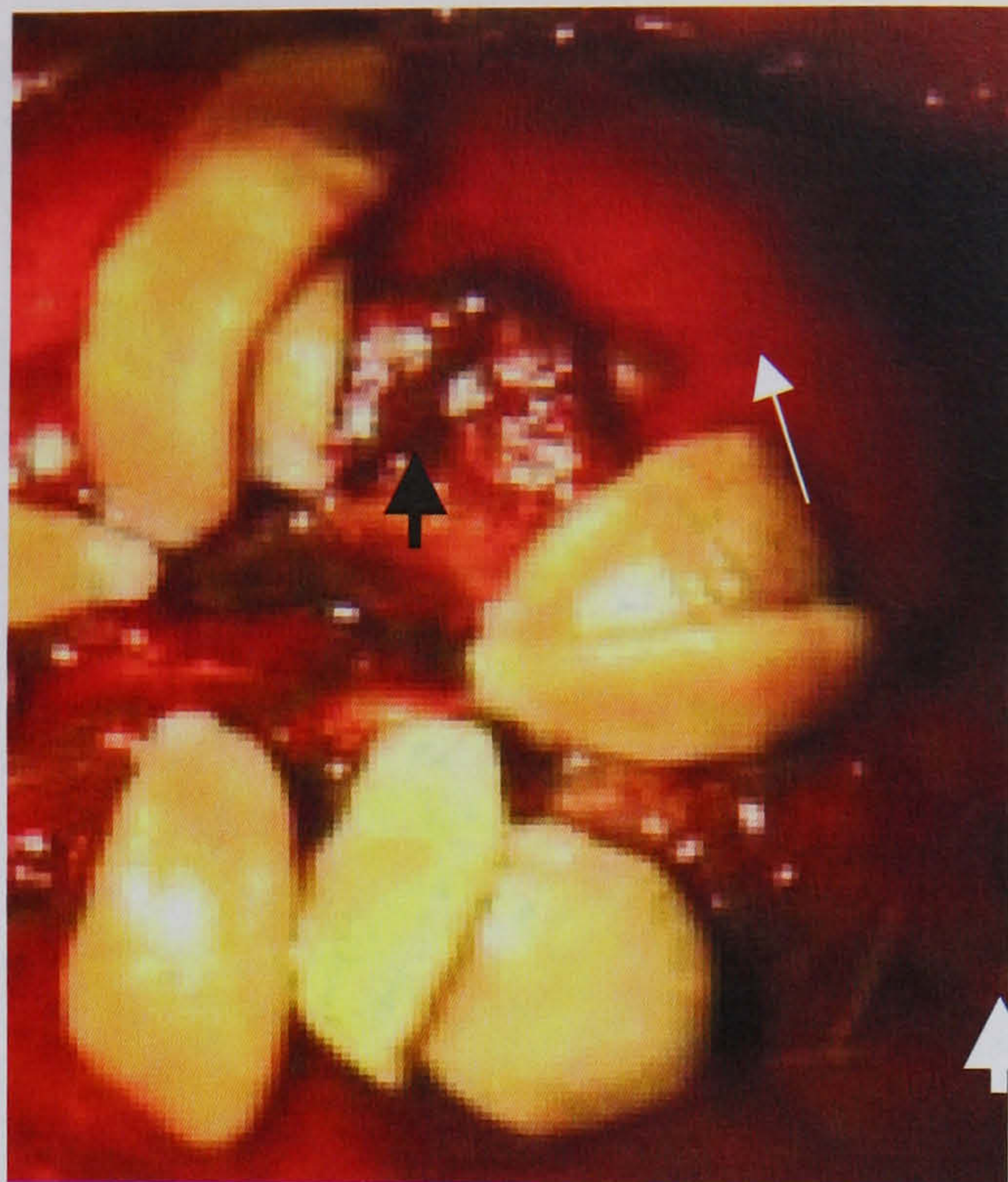


Fig. 4.2. Clumps of *A. perfoliata* attached around the ileocaecal junction of a donkey, Ethiopia. Note the ulceration and diphtheritic membrane after detachment of the tapeworm (black arrow) and the hyperaemic and oedematous ileocaecal valve (white arrow)

4.3.2 Ascarids

Fifty five percent of the 112 donkeys examined were infected with *P. equorum*. The prevalence, mean intensity of infection and ranges of infections are shown in Table 4.1. All age groups were infected. Eighty seven percent (13/15) of the donkeys less than or equal to 4.5 years old, and 50% (48/97) of the donkeys greater than 4.5 years old were infected (Table 4.2). There were no statistically significant differences in infection prevalences between the different age groups ($P>0.05$). Although the mean intensity of infection seems to decrease in older donkeys, no significant differences were observed between the different age groups ($P>0.05$). The highest intensity of infection was 77 worms in a four-year-old donkey (Fig. 4.3). The overall mean intensity of infection was 14 worms. The proportion of infected donkeys with less than 10 worms was 51% and more than 10 was 49%.

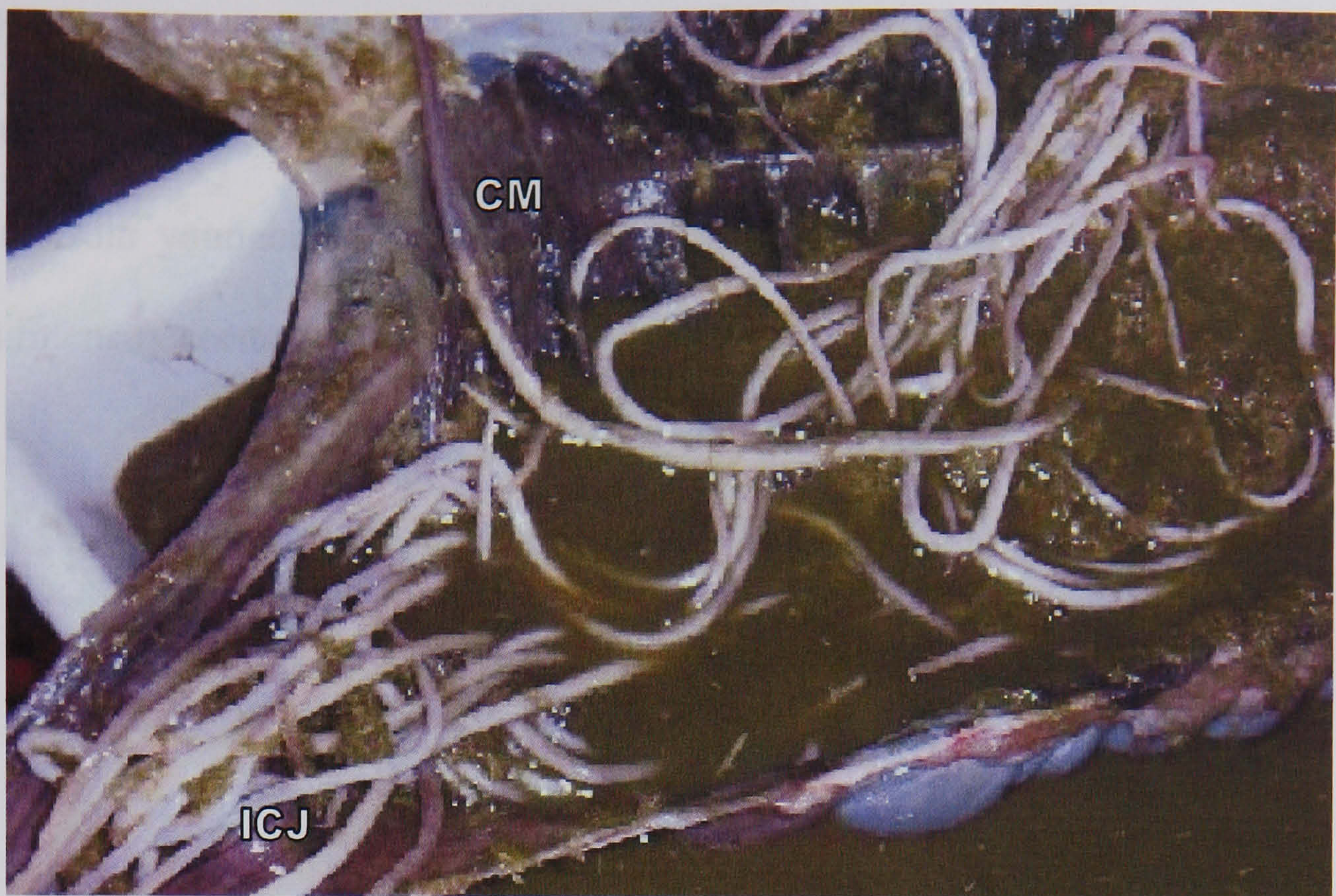


Fig.4.3. Mature *P. equorum* in a four-year-old donkey, which died of colic after anthelmintic treatment. Most of the worms were found blocking the ileocaecal junction (ICJ), in the caecum (CM) and few in the small intestine.

Table 4.2. Number and proportion of infected donkeys with *P. equorum* in different age groups.

Age groups (years)	I/N*	%	Mean intensity	Intensity Range
<= 2.5	6/6	100	42	13-77
2.5-4.5	7/9	78	14	9-32
4.5-8	10/24	42	9	3-32
>=8	38/73	52	11	3-37

*I/N -Number infected /number examined, i.e., age specific prevalence.

4.3.3 Fasciolids

Of the 112 donkeys autopsied 42% were infected with *Fasciola*. The mean infection intensity for the infected donkeys was 30 flukes ranging from 3 to 79. Eighteen percent and 24% of the donkeys had worm counts between 1-20 and 21-100, respectively (Table 4.3).

Although no significant differences ($P>0.05$) were observed between age groups up to 8 years, the mean infection intensity in 8 years old and above donkeys was statistically significant from younger donkeys of age less than or equal to 2.5 years ($P<0.0001$). The infection intensity seems to increase with age of the donkeys (Fig 4.4).

Table 4.3 The infection levels of flukes in 112 autopsied donkeys, Ethiopia.

Groups of donkeys with	Number	% of total examined	% of infected
0	65	58.0	-
1-10	10	8.9	21.3
11-20	10	8.9	21.3
21-50	17	15.2	36.2
51-100	10	8.9	21.3

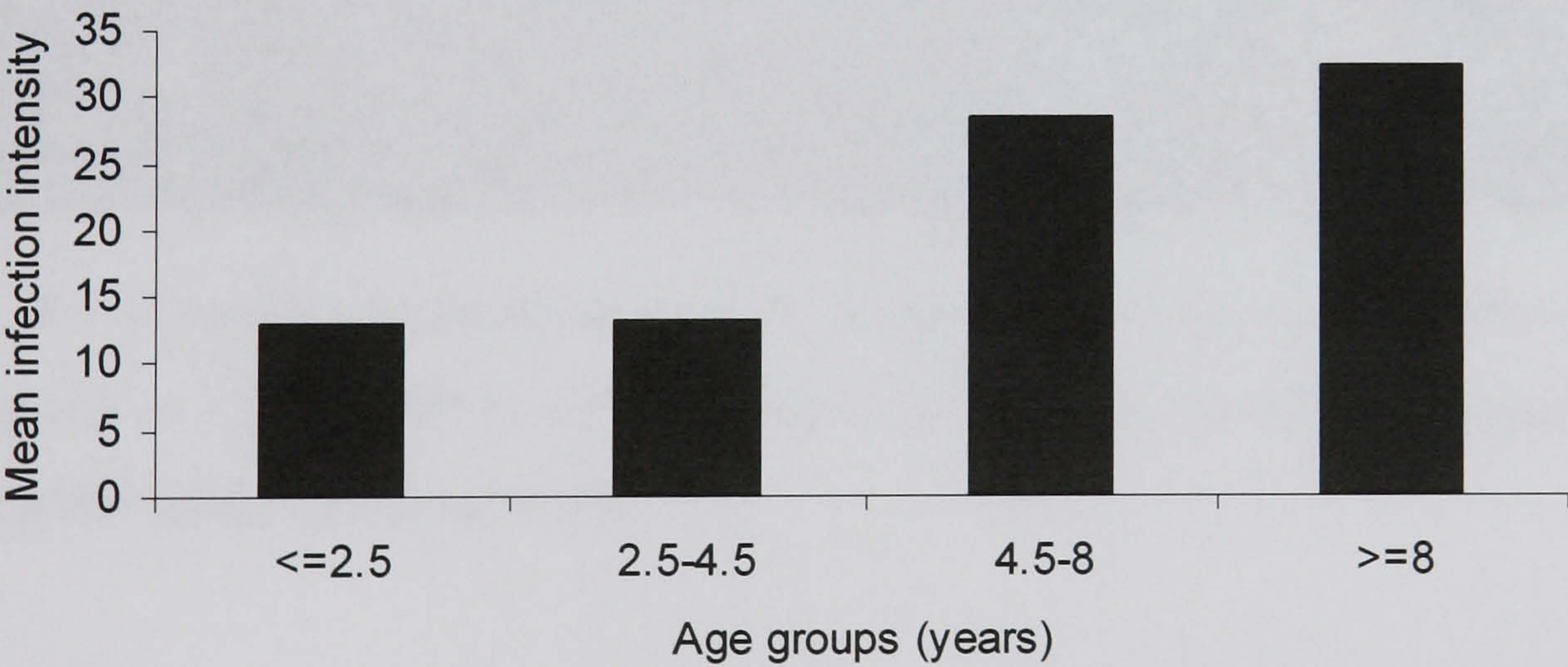


Fig. 4.4. The mean infection intensity of flukes in different age categories of the 112 autopsied donkeys, Ethiopia.

An attempt was made to identify the species of *Fasciola* recovered from donkeys using morphologic and morphoanatomic study. Considering the general shape and size, some

worms resembled *F. hepatica*, others *F. gigantica* and intermediate forms. But morphoanatomic study has revealed both *F. hepatica* and *F. gigantica* (see Appendix VI for details).

Gross pathological lesions of hyperplasia/thickening of the bile ducts and brownish grey nodules or patches 1-3 mm in diameter were observed in some of the donkeys examined infected with large number of flukes (Figs 4.5 and 4.6). In no cases were calcified bile ducts observed, nor the cirrhosis commonly encountered in cattle.

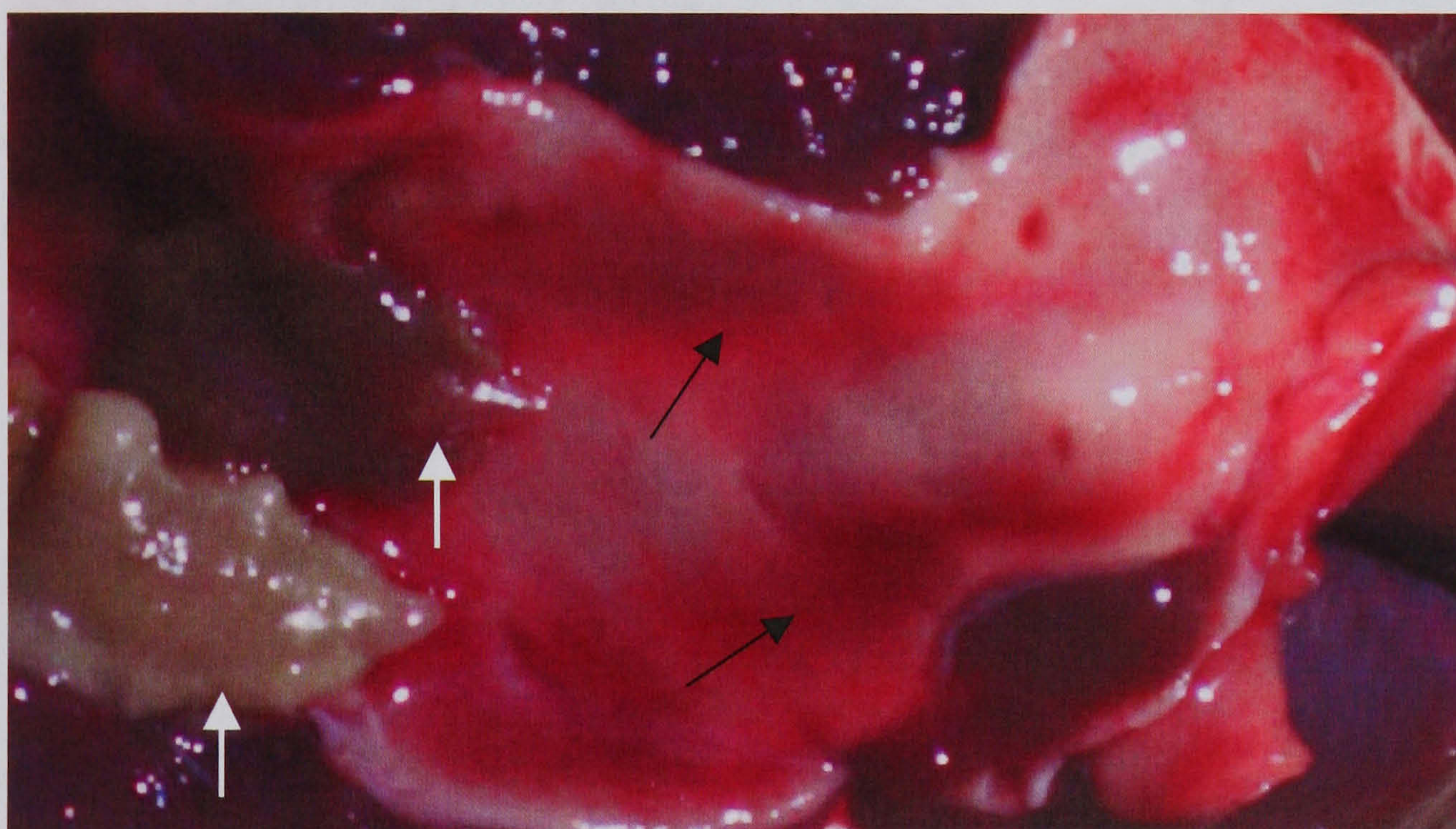


Fig. 4.5. Hyperplasia (thickening) of the main bile duct due to fluke infection. Note the two flukes (white arrows) lodged in the bile duct and the haemorrhagic/hyperaemic epithelial lining (black arrows).



Fig 4.6. Brownish grey nodular or patches obstructing branches of the main bile duct (arrows).

4.3.4. Paramphistomatids

The paramphistomatid trematode, *G. aegyptiacus*, was present in 30.4% of the 112 donkeys examined. Fifty three percent of the infected donkeys harboured more than 200 worms while 18% had more than 1000 (Table 4.4). The mean infection intensity was 503 with a maximum of 2523 flukes from a 14 year-old male donkey. The parasite was present in all age groups. It occurred in 20% of the donkeys under 4.5 years old and 32% of older donkeys. The infection prevalence was not significantly different between the different age groups but the mean intensity of infection was significantly higher ($P < 0.0001$) in donkeys 8 years and above compared to those less than 8 years old. The infection prevalence was significantly higher in females ($P = 0.02$). On the other hand, males had significantly higher infection intensity compared to females ($P < 0.0001$).

The only prominent pathologic lesions observed were severe haemorrhagic and oedematous colitis in the 14 year-old male donkey infected with more than 2523 flukes.

Table 4.4 The infection levels of *G. aegyptiacus* in 112 autopsied donkeys, Ethiopia.

Groups of donkeys with	Number	% of total examined	% of infected
0	78	69.6	-
1-10	5	4.5	14.7
11-50	5	4.5	14.7
51-200	6	5.4	17.7
201-500	7	6.3	20.6
501-800	3	2.7	8.8
801-1000	2	1.8	5.9
>1000	6	5.4	17.7

4.3.5. Gasterophilids (bot larvae)

Examination of the stomach and the first part of the duodenum of 112 donkeys revealed two species of bot larvae: *G. intestinalis* and *G. nasalis*. *G. intestinalis* larvae were found clustered in groups mainly in the saccus caecus of the oesophageal region and margo plicatus of the stomach. A few larvae attached to the glandular region of the stomach were also observed in some animals. *G. nasalis* were mainly attached to the pylorus and dorsal region of the first part of duodenal ampulla (Fig 4.7). Substantial numbers of *G. nasalis* were recovered from the caudal part of rectal mucosa, ampulla recti, of some donkeys. A few loose larvae were also recovered from the stomach and posterior parts of the small and large intestines. A total of 24,564 larvae were collected and examined in the present study, of which 11,054 (45%) were identified as *G. intestinalis* and 13,510 (55%) as *G. nasalis*. Both

second and third stage larvae of each species were recovered from their respective predilection sites. No attempts were made to recover the first or second stages from the buccal cavity.



Fig. 4.7. Large masses of *G. nasalis* attached to the pylorus (thick black arrow) and *G. intestinalis* along the margo plicatus (thick white arrow). Note the pyloric opening (thin white arrow) completely surrounded by the larvae.

G. intestinalis larvae were recovered in 96.4% while *G. nasalis* were present in 98.2% of the donkeys examined. The average number of larvae per infected donkey was 103 for *G. intestinalis* (range 2-816), 123 (range 14-570) for *G. nasalis*, and the combined total mean infection intensity was 222 (range 16-951). No evidence of any association was observed in the infection prevalence and infection intensity between age and sex of the donkeys ($P>0.05$). The majority of donkeys, 93.5% and 86.4%, harboured less than or equal to 200 larvae of *G. intestinalis* and *G. nasalis*, respectively. When the two species were combined, over 78% of donkeys had between 100 and 500 larvae and 3.6% over 500 larvae. This shows an aggregated distribution of the larvae in donkeys in which many donkeys harbour few larvae and few donkeys harbour many larvae (Table 4.5).

Table 4.5. Frequency distribution of the number of bot larvae in infected donkeys

Infection size	Number and (%) of donkeys infected with		
	<i>G. intestinalis</i>	<i>G. nasalis</i>	Both species
1-100	63 (58.3)	49 (44.6)	20 (18.0)
101-200	38 (35.2)	46 (41.8)	37 (33.3)
201-300	4 (3.7)	9 (8.2)	34 (30.6)
301-400	1 (0.9)	5 (4.6)	12 (10.8)
401-500	1 (0.9)	0	4 (3.6)
501-600	0	1 (0.9)	1 (0.9)
601-700	0	0	1 (0.9)
>700	1 (0.93)	0	2 (1.8)

Attempts were made to identify the type of lesions the larvae caused. Macroscopic lesions such as multiple erosive crater-like lesions and well-circumscribed ulcerated mounds caused by *G. intestinalis* were common findings. In 9.8% of the donkeys quite extensive ulcers in the oesophageal region were observed. Small prominent circular and funnel-like or punctiform lesions associated with an inflamed duodenum and pylorus characterised lesions created by *G. nasalis*. At higher levels of infection multiple lesions of coalescent and crater-like lesions, which were smaller in size, were evident at the duodenal ampulla and pylorus. In most cases the number of mucosal lesions was higher than the number of larvae detected at the sites. No evidence of larval perforation of the stomach or duodenum was found. The other important effect of *Gasterophilus* observed was that they were the main cause of rectal prolapses in donkeys (Fig 4.8). Larvae recovered from the rectal prolapses were mainly *G. nasalis*.



Fig 4.8 Prolapsed rectal mucosa due to irritation and tenesmus from the gasterophilus larvae in donkeys: **a)** swollen and oedematous mucosa. **b)** extensively oedematous and ruptured rectal mucosa with the larvae attached to it.

4.4 Discussion

The current study has shown high prevalences of the non-strongyle intestinal helminths of cestodes, trematodes, ascarids and bot larvae in the donkey population of Ethiopia. To the best of our knowledge this is the first study made in Ethiopia to determine the extent of infection by these helminths in donkeys. No work on these parasites has been reported from

equids in Ethiopia except some case reports by Yilma et al (1991), Feseha (1997) and Getachew (1999).

4.4.1 Anoplocephalids

The relatively high prevalence of *A. perfoliata* as compared to other cestode species in the present study is consistent with the findings reported from South Africa (Matthee et al., 2000, 2002), Turkey (Burgu et al., 1995) and Kenya (Lewa et al. 1998). This species of equine tapeworm is also highly prevalent in horses and ponies in different geographical regions (English, 1979c; Owen, et al., 1986; French and Chapman, 1992; Pearson, et al., 1993; Yoshihara et al., 1993; Fogarty et al., 1994; Nilsson et al., 1995; Williamson et al., 1997; Lyons et al., 2000). Although many studies on the prevalence and intensity of infection of *A. perfoliata* based on post-mortem investigation have been performed in horses in many countries, similar studies made in donkeys are very limited. Moreover, the few studies available have not reported the intensity of infection to compare with the present findings. The finding of all three species in the preset study was consistent with that of Khallaayoune (1991). On the other hand Graber (1970) from Burkina Faso and Vercruysse et al. (1986) from Chad reported only *A. magna* with prevalences of 27% (n=30) and 6% (n=183), respectively. The high prevalence of *A. magna* as compared to the present findings and absence of other cestode species in these studies may indicate variation in the physical environment conditions or geographical distribution.

Although there was uneven age distribution among the autopsied donkeys, it seems that all age groups are potentially susceptible to *A. perfoliata*. However, the high worm burden seen in older donkeys may indicate the absence of acquired immunity and/or absence of treatment leading to the accumulation of worms through time. No study has been made in donkeys to compare such association. However, studies made in horses and ponies have shown that

horses of all age groups appear to be affected and there seems to be no apparent acquired immunity or age resistance to *A. perfoliata* (Bain and Kelly, 1977; Lyons et al., 1984; Tolliver et al., 1987; Owen et al., 1988; Benton and Lyons, 1994; Fogarty et al., 1994; Ihler et al., 1995; Nilsson et al., 1995).

The present study revealed that the majority of donkeys had low levels of infection, however there were individuals with high numbers of worms, which predisposed them to varying degrees of mucosal lesions. The relationship between the number of tapeworms and the severity of associated lesions was demonstrated in the present study, particularly at the ileocaecal junctions where severe necrotic ulcerations and diphtheritic membranes were seen. This association between extensive pathological changes and a high burden of *A. perfoliata* is well documented in horses and ponies (Pearson et al., 1993; Fogarty et al., 1994; Williamson et al., 1997).

4.4.2. Ascarids

The present study shows that 55% of donkeys were infected with *P. equorum*. Of these 43% were above 4.5 years old. Similar findings have been reported from studies made in other Africa countries (Graber, 1970, Vercruysse et al., 1986; Khallaayoune et al., 1991; Matthee, et al., 2000, 2002; Pandey and Eysker, 1990) and elsewhere (Burgu et al., 1995). These studies however, lack information on the intensity of infection and age related prevalence or worm burden to compare with the present study.

Even though the mean intensity of infection seems to be higher in young donkeys less than 2.5 years old, there was no statistically significant difference either in infection prevalence or mean infection intensity between the different age groups. Tolliver et al (1985) in their study recorded *P. equorum* only from one out of eight donkeys necropsied. They speculated that

this could have been because the donkeys were too old to have harboured this parasite. On the other hand, Vercruysse et al., (1986) reported an infection prevalence of 43% (n=30) from adult donkeys, similar to our finding. The coprological study (Chapter 3) also supports this finding, as most adult donkeys were shedding ova at more than 200 epg. Both from coprological and necropsy findings it would therefore appear that, unlike horses, it is not uncommon to find *P. equorum* in adult donkeys. This is in disagreement with the general opinion that *P. equorum* is a parasite of young horses. It has been shown that *P. equorum* is a highly antigenic and immunogenic parasite of young horses and ponies, and even in the absence of previous exposure significant age-dependent immunity develops by six months of age (Clayton and Duncan, 1979b; Bello, 1985). Most horses stop passing eggs by one year of age (Bello, 1985; Austin et al., 1990). The high infection prevalence and intensity of infection in some adult and older donkeys may suggest that unlike horses, donkeys may not develop acquired immunity or else may show immuno-suppression or be immuno-compromised due to the high level of stress the adult donkeys are experiencing because of mismanagement, and other concurrent diseases and malnourishment (Svendsen, 1994; Krecek and Guthrie, 1999).

All examined donkeys were 1 year old and above and this did not enable us to determine the level of ascarid infection in foals or suckling animals where the problem of small intestinal impaction with ascarid worms is quite well recognised in the horse (Austin et al, 1990; Clayton, 1978, 1986; Southwood et al., 1998). In a retrospective study (Southwood, et al., 1998), the median age of foals with ascarid impaction was 5 months (4-24 months). In the present study the parasite burden in most of the cases was low and unlikely to cause impaction. However, the frequently-seen cough and mucoid to purulent nasal discharge, which clear after treatment with ivermectin; and the observation of widely scattered white foci on the liver of most of the donkeys examined at present led us speculate that adult

donkeys may also suffer from the migrating effect of the larvae through the lungs and liver. However, it is difficult to rule out lungworms and other parasites. The epidemiological role the adult animals play in contaminating the environment is important, considering the high fecundity of female *parascaris* coupled with the ability of the eggs to remain infective for a long time in the environment (Clayton, 1986; Austin et al., 1990; Southwood, et al., 1998). Even if we believe that adults do not suffer from clinical disease, they could represent the main risk factor for the infection of young animals.

4.4.3 Fasciolids

Although fasciolosis is one of the major parasitic problems in domestic ruminants in Ethiopia (Gemechu and Mamo, 1979; Mulugeta et al., 1989; Ngategize et al., 1993; Yilma and Malone, 1998), no study has determined the extent of fluke infection in equids. Apart from some case reports (Feseha et al., 1991, Getachew, 1999) this is the first study to determine the prevalence and abundance of fluke infection based on post-mortem examination of donkeys. Even worldwide, few studies have determined the prevalence of equine fasciolosis, particularly in donkeys. Alcaino et al. (1983) from Santiago, Pandey (1983) from Morocco, Fahmy and El-Attar (1990) from Egypt, Burgu (1995) from Turkey, Trawford and Tremlett (1996) from England had reported prevalences of 28.3% (n=219), 5.9% (n=422), 17% (n=60), 8% (n=14) and 20% (n=10), respectively from donkeys. The 42% infection prevalence obtained in the present study was consistent with the coprological result of 45% (Chapter 3) but quite high compared to the results of the previous studies. None of the above mentioned studies determined the infection intensity. Even though no quantitative figures were given, Green et al. (1968) and Kearney (1974) have reported heavy fluke infections in donkeys on post mortem examination. A mean infection intensity of 27 with a maximum of 87 worms can be calculated from the study made by Pandey (1983),

which is similar to our findings. Alcaïno et al. (1983) reported that 65% of the 100 infected horses had less than 20 *F. hepatica* with a mean of 21 and a range of 1-146 worms.

All the autopsied donkeys came from low-to-mid altitude areas (1500-2300 masl). Most of these areas are not suitable for the establishment of snails and the transmission of the free-living and infective stages of *Fasciola* species. The most likely sources of infection are grasses on the banks of irrigation canals, permanent water bodies and ponds found in these areas and some areas of low-lying lands subjected to flooding during the main rainy season (June-September). The other possible explanation for such a high infection prevalence is the trade of donkeys by which most of the lowlanders and midlanders buy their donkeys from the highland *Fasciola*-endemic areas.

The distribution of the number of flukes in donkeys of different ages was interesting. Only 1 donkey less than 2.5 years old was infected. There was no difference in the grazing management practice between the young and old donkeys to account for the difference observed. The differences in duration of exposure to the infection could be one reason. There appeared to be a progressive accumulation of infection throughout the life of the donkeys. The other possible explanation could be that most of the young animals were born in lowland or midland areas and might have had a lower level of infection than their parents which probably came from the endemic highland area. There is no data available to compare to our results concerning age-related infection prevalence or intensity. However, contrary to our findings, Fischer (1982) and Alcaïno et al. (1983) found higher prevalences of fluke infection in horses younger than 5 years of age. According to Alcaïno et al. (1983), the low prevalence in horses often recorded might be due to the fact that the majority of horses killed at abattoirs are apparently older than 5 years and they usually show lower infection rates than younger animals. However, in our study the majority of donkeys were above 5 years (more

than 65% are 8 years and above) and it is within these age groups that the prevalence was high. It is possible that factors such as poor nutrition, which decrease the immune status of donkeys, may be involved in the differences observed. Grelck et al. (1977) for example, suggested that horses with heavy nematode infection and poor body condition are more easily infected with *F. hepatica* than animals in good condition. The low number of young donkeys examined in our study may not be sufficient to draw a definitive conclusion at present, and further studies are needed.

As far as pathogenecity of *Fasciola* in equids is concerned, there are contradictory opinions. Some authors found no clinical signs or pathologic effect and suggested that equids are resistant to fluke infection (Boray, 1969, Fischer, 1982; Alves et al. 1988). Others reported various clinical signs and pathological changes in horses (Moisant et al., 1972; Owen, 1977; Krawiecki, 1986; Perler et al., 1997). These workers found, among others, signs of lowered performance, poor condition, diarrhoea, a pronounced loss of weight and anaemia. Similar clinical signs have also been reported in donkeys (Collins, 1961; Pankhurst, 1963; Green et al., 1968). We also observed similar clinical signs in most of the donkeys coming to our clinic, but because of the problems of polyparasitism and absence of treatment it was difficult to attribute the observed clinical signs only to fluke infection. However, gross pathological changes such as hyperplasia (thickening) of bile ducts, brownish grey nodules and patches of 1-3 mm in diameter were observed in some of the donkeys examined. Similar findings have been reported by Fahmy and El-attar (1990). Along with hyperplasia of the epithelial lining, Fahmy and El-attar (1990) also observed an increased amount of fibrous connective tissue in the portal area of livers of donkeys that they examined. Collins (1961) reported complete gross and histopathological findings of livers of donkeys, which died of *F. hepatica* infection. His post-mortem findings demonstrated greyish white thread like scars over the entire surface of the liver extending into the hepatic parenchyma with thickening of

the hepatic ducts, while his histopathologic findings were of a great deal of connective tissue. The connective tissue was collagen in nature, with denser and more matured bundles in areas of the larger interlobular bile ducts and the liver parenchyma. He also described more diffused inflammatory cells, primarily lymphocytes, plasma cells and polymorphonuclear leukocytes.

Garlanda (1958), on the other hand, reported an uncommonly severe hepatitis and cholangitis in horses heavily infected with *F. hepatica*. Similarly, Dorchies et al. (1990) in their controlled experimental work, of 11 ponies inoculated with 800 *F. hepatica* metacercariae at 7-9 months of age reported that two died after 6-8 weeks of infection. Celano et al. (1991) reported gross abnormal dilation of the bile ducts. The pathological findings of calcification of the bile ducts or cirrhosis commonly seen in cattle were not observed in the present study and a similar finding was reported by Pandey (1983). The absence of such pathological conditions may support Boray's (1969) classification of equids in his delayed resistance group, in which the disease is self-limiting or in the category of hosts in which, according to Ross (1967), the life span of *Fasciola* is very long because of the absence of calcification of the bile duct. However, Boray (1969) considered that the life span of *Fasciola* in equids is short. In the absence of experimental infections these two contradictory views cannot be reconciled.

Although equids are generally considered more resistant to fasciolosis than domestic ruminants (Boray, 1969; Nansent et al., 1975; Alves et al., 1988) it is difficult to define the resistant status of donkeys. Alkahane et al (1970, 1974) made comparative morphological studies of *Fasciola* species from cattle, horses, goats and pigs and concluded that equids are as suitable as cattle for liver fluke. Boray (1969) classified the hosts of *Fasciola* into three groups: (i) early resistance groups (such as the pig), in which the infection is self-limiting without harming the host, (ii) delayed resistance group (cattle, horse donkey, man), in which

the disease is self limiting but may cause severe pathogenic lesions; (iii) low resistance group (sheep, goat etc..) in which the disease is highly pathogenic.

The observed high infection prevalences and infection intensity, coupled with some of the evidence of gross pathological lesions observed in the present study, together with previous reports that donkeys (Collins, 1961; Pankhurst, 1963; Green et al., 1968) and horses (Owen, 1977; Moisant et al., 1972; Montes et al., 1984; Krawiecki, 1986; Dorchies et al., 1991; Celano et al., 1991; Toll Vera et al., 1995; Perler et al., 1997) can be clinically affected indicate that equids can be susceptible to fasciolosis. Sub-clinical infections may occur in which the parasites can still have a significant effect on production and performance. This could particularly be the case as donkeys are undernourished and highly stressed animals due to mismanagement. They could also play a significant epidemiological role in contributing to the continuous contamination of pasture and the residual infection derived from this contamination may be sufficient to produce infection in domestic ruminants and humans, particularly in endemic areas.

4.4.4 Paramphistomatids

Gastrodiscus aegyptiacus occurred at various levels of infection intensity among the different age groups of donkeys. This is the first study made in Ethiopia to determine the extent of infection in equids apart from the case report in a single horse by Bracegirdle (1973). The prevalence of 30.4% from autopsied donkeys and the coprological findings of 17.4% to 58.2% (Chapter 3) from different regions indicate the wide distribution of this parasite in the donkey population. There are varying reports concerning the occurrence and prevalence of this paramphistomatid in equids. Prevalences from 10% (n=14) in Zimbabwe (Pandey and Eysker, 1990) to 62.5% in South Africa (Matthee et al., 2000, 2002) have been recorded. Similar findings were reported by Graber (1970), Vercruysse et. al. (1986) and

Hasslinger and El-Seify (1995) with prevalences of 34% (n=183), 57% (n=30) and 21.8% (n=156), respectively from donkeys. This parasite has also been reported from horses (Graber, 1970; Azzie, 1975; Islam, 1986; Applewhaite and Ruiz, 1983).

Although the infection prevalence was not significantly different among the different age groups, the parasite burden was significantly higher in older donkeys and it seems that infection intensity increases with age of the donkeys probably due to lack of immunity leading to accumulation of the parasites. In the present study 18% of the infected donkeys were harbouring more than 1000 worm with a maximum of 2523 *Gastrodiscus* in a 14-year male donkey. Worm burdens as high as 2,552, 5598 and 7430 were reported by Matthee et al (2000, 2002) from adult donkeys. Wells et al (1998) also suggested that older donkeys harbour increasing numbers of *Gastrodiscus*. Our results also showed that female donkeys have significantly higher infection prevalences but significantly lower infection intensities. But the reason for this is not readily apparent. A similar finding was reported by Wells et al. (1998) in which Jennies had significantly higher egg counts than jacks and geldings.

Because of the fragmentary knowledge of this paramphistomatid of equids, some aspects of its occurrence, pathology and clinical symptoms are not well known. However, contrary to common belief, *Gastrodiscus* can be pathogenic to equids. Although it is very difficult to rule out the concomitant effects of other parasites, the observed severe haemorrhagic and oedematous colitis associated with large numbers of *Gastrodiscus aegyptiacus* in one of the donkeys autopsied indicate the potential pathogenicity of this parasite in donkeys. In this particular case the donkey was highly emaciated and anaemic, and there were no other parasites where the flukes were found attached. Similar disease was reported by Applewhaite and Ruiz (1983) in horses. Per-acute colitis associated with thousands of immature and mature *Gastrodiscus aegyptiacus* have also been reported in adult horses (Azzie, 1975).

According to Azzie (1975), the infected mare had not been exposed to pasture for 3 years, which shows that it harboured the parasites for at least this length of time and hence indicates the longevity of the parasites. Similarly, Bracegirdle (1973) reported a case of severe parasitism by *G. aegyptiacus* associated with incoordination and collapse of a horse in Ethiopia. He reported a 16-year-old gelding found with thousands of *Gastrodiscus aegyptiacus* concentrated within a small area of the wall of the colon. He observed no other pathology or other parasites in significantly large numbers and he suggested that the horse was clinically affected by a massive infestation of *Gastrodiscus*. According to Soulsby (1982), the immature stages of paramphistomes are responsible for severe pathological changes in domestic ruminants. These are embedded in the mucosa and are plug feeders, drawing pieces of mucosa into suckers, which they then pinch off causing necrosis and haemorrhage. Associated with these lesions are anaemia, hypoproteinaemia, oedema and emaciation. According to Horak (1971), acute diseases by paramphistomes are usually seen only in young animals; previous infection and age of the host afford some protection against reinfection in ruminants. Whether this also holds true in equids is not known.

4.4.5. Gasterophilids

Although there are 8 species known to infect equids (Zumpt, 1965), the present study revealed only two species of *Gasterophilus*: *G. intestinalis* and *G. nasalis*. These are the two major bot larvae commonly reported worldwide (Pandey et al., 1994). Other species are rarely reported from donkeys. *G. ternicinatus* and *G. inermis* have been reported only from Burkina Faso (Kaboret et al., 1986) and Tunisia (Kilani et al., 1986), respectively. Khallaayoune (1991) and Kaboret et al. (1986) reported *G. haemorrhoidalis* while *G. pecorum* was reported by Kilani et al. (1986), Kaboret et al. (1986), Graber and Gruvel (1964) and Matthee et al. (2000) from donkeys.

To the best of our knowledge no work on gasterophilus larvae have been reported from equids in Ethiopia except for case reports by Feseha (1997) and Getachew (1999). The present study showed high prevalence of *Gasterophilus* in donkeys. Similar infection prevalences of both species from Morocco (Pandey et al., 1992b) and *G. intestinalis* from Burkina Faso and Egypt (Kaboret et al., 1986; Hilali et al, 1987) have been found in donkeys. On the other hand, lower infection prevalences of *G. intestinalis* (Mukbel et al., 2001) and *G. nasalis* (Kaboret et al., 1986; Kilani et al., 1986; Hilali et al, 1987; Mukbel et al., 2001) have also been reported. Unlike the present finding, Matthee et al. (2000, 2002) reported only *G. intestinalis* from South Africa, while Karanja et al (1994) from Kenya reported only *G. nasalis*.

Pandey et al. (1980) reported that 51% (n=94) of horses were infected with up to 300 larvae of *G. intestinalis* and *G. nasalis* and four horses had a combined burden of >1000 larvae. In our case 82% of donkeys were infected with up to 300 larvae of both species and the maximum number of larvae recovered was 951. Compared to the present finding, Mukbel et al. (2001) reported very low infection intensities with an overall mean number of 26 *G. intestinalis*, and 6 *G. nasalis* in 105 examined donkeys. Although there was no difference in infection prevalence between the two species in the present study, the mean infection intensity of *G. nasalis* was higher per infected donkey. Fifty six percent of donkeys were infected with over 100 *G. nasalis* larvae as compared to 40.8% of donkeys with *G. intestinalis*. Moreover, 13.7% and only 6.5% of donkeys harboured over 300 *G. nasalis* and *G. intestinalis*, respectively. Studies made in Egypt (Hilali, et al, 1987) and Jordan (Mukbel et al, 2001) on the other hand, showed that *G. intestinalis* was the dominant species with higher prevalences and intensities of infection.

These differences in infection prevalence and/or infection intensity, and species of bot larvae in different localities could be attributed to differences in climatic conditions, geographical locations, animal husbandry or management aspects and probably the strain of the parasites themselves. Warmer climate, which is favourable for the development and survival of the free-living stages could be the reason for the higher infection prevalences and intensity of infection in most tropical countries. According to Pandey et al. (1980), survival of free-living stages extends over a much longer period in warmer climatic conditions, and second and third stage larvae can be found simultaneously in the stomach during nearly every month of the year. The high prevalence in the present study may therefore indicate the absence of treatment in donkeys and the favourable climatic condition for the development and survival of the free-living stages.

Even though there were no data on donkeys to compare our results with, the absence of age or sex related differences in infection prevalence and intensity of infection in the present study is consistent with the findings of Edwards (1982) and Pfister and Brodcard (1996) in horses. Pandey et al. (1980), however, reported that younger horses had higher infection levels than older animals, which he thought might be due to the development of immunity. Unlike our result, Bernard et al. (1994) and Agneessens et al. (1998) found significantly higher infection prevalences in mares. It is not likely that such a difference is genetically determined, but it may be a reflection of different management conditions for both sexes. Age or sex related infection intensities need further investigation.

The observed substantial aggregation of bot larvae, which seems to follow a negative binomial distribution, in which many larvae were found in few animals and many animals were infected with few larvae, was also interesting. Acquired protective immunity in some of the hosts is not an entirely satisfactory explanation because of the absence of age-related

changes in larval burden. Individuals, however, may develop some protective immunity and subsequently develop tolerance under conditions of continued exposure. Non-uniform innate resistance in host species and behavioural differences that increase an individual's rate of exposure can cause an aggregated distribution of parasites (Maizels et al. 1993). The other possible explanation in this particular instance is the behaviour of the adult female flies and the grooming behaviour of the donkeys. According to Cogley and Cogley (2000), in their observational study female flies resolutely stayed with horses once they had located them and hence lay as many eggs as possible on that host.

Although these parasites are considered to be well tolerated by their hosts, they have been incriminated in inducing gastric erosion, ulcers, abscesses, ruptures and peritonitis (Rooney, 1964; Waddell, 1972; Pandey et al., 1980; Dart et al., 1987). In the present study the raised gastric tissues into well-circumscribed ulcerated mounds surrounded by distinct rims and erosions were common macroscopic lesions seen in donkeys autopsied which were infected with *G. intestinalis*, whereas duodenal lesions associated with *G. nasalis* were flatter and much less circumscribed and lacked distinctive localized proliferation of tissue in their immediate surroundings. Similar lesions were reported by Cogley and Cogley (1999) and Sequeira et al. (2001) in horses.

Histopathological findings by Cogley and Cogley (1999) and Sequeira et al (2001) revealed that stomach tissue affected by *G. intestinalis* exhibited chronic ulcerative gastritis of the non-glandular portion of the stomach advancing into the underlying mucosae. These studies also revealed that the areas of duodenum affected by *G. nasalis* exhibited a multifocal chronic ulcerative duodenitis with predominantly intensive fibrosis of tunica submucosa that extended from beneath the ulcer towards the tunica muscularis and laterally beyond the borders of the ulcers. According to Cogley and Cogley (1999), the gastric lesions produced

by *G. intestinalis* were more destructive than the duodenal lesions produced by *G. nasalis*. More importantly, they found that proliferation of tissue below the lesions associated with the larvae prevents larval penetration into the stomach and duodenal walls. Whether such tissue proliferation takes place or follows a different pathologic pattern in donkeys is unknown. Moreover, such protective mechanisms may not hold true in immuno-compromised or immuno-deficient equids, particularly donkeys, which are highly stressed and undernourished, and may lead to the breakdown of the host's inflammatory response and inadequate or absence of tissue proliferation.

The other interesting observation was that bot larvae were found to be an important cause of rectal prolapse in working donkeys. They re-attach temporarily to the rectal mucosa after passing through the digestive tract causing irritation and inflammation of the rectum. This creates an intense tenesmus leading to prolapse. According to Soulsby (1982), this re-attachment at the rectal mucosa is the main behaviour of *G. haemorrhoidalis*. However, our observations showed that all rectal prolapses due to bot larvae were caused by *G. nasalis* with a few *G. intestinalis* sometimes found together. Similar lesions to those found in the pyloric and duodenal ampullae were also observed on the rectal mucosa, and raised lesions where larvae were attached can easily be felt in live animals.

4.4.6. Overview.

Generally the present retrospective study has revealed that donkeys are hosts for many parasites confirming the case for polyparasitism. The high infection prevalence of the non-strongyle intestinal helminths of donkeys obtained in the present study particularly shows that these parasites are not accidental findings as it has been suggested in previous studies. Moreover, the observed pathological lesions, particularly due to *Fasciola*, *A. perfoliata*, *G. aegyptiacus* and *Gasterophilus* larvae showed their potential pathogenicity. The study has

also revealed that parasites have an overdispersed distribution, in which many donkeys harbour few parasites and few donkeys are infected with many parasites. The finding of high infection prevalence and infection intensity irrespective of the age of the donkeys was also interesting as this shows that there seems to be no apparent acquired immunity or age-dependent resistance to these parasites. However, experimental studies based on mono-specific infection of the donkeys would need to be conducted to determine the extent of pathological conditions, and the immune status of donkeys towards the infection of these parasites. The interaction between parasites would be another interesting area to be explored.

CHAPTER FIVE

A SERO-EPIDEMIOLOGICAL STUDY OF EQUINE CESTODOSIS

5.1 Introduction

Anoplocephala magna, *A. perfoliata* and *Anoplocephaloides mamillana* (formerly, *Paranoplocephala mamillana*) are the three common cestode species found as adults in equidae (Soulsby, 1982). In the early 1900s infections by *A. magna* and *A. mamillana* were the most common in horses and ponies, and *A. perfoliata* was a rare finding (Hall and Koskins, 1918; Olsen, 1938; French and Chapman, 1992). However, recent studies have shown that *A. perfoliata* is the dominant equine tapeworm with prevalences ranging from 18-87% in various geographical regions of the world (Owen et al., 1988; Pearson, 1993; Yoshinara et al., 1993; Fogarty et al., 1994; Bucknell et al., 1995; Nilsson et al., 1995; Ihler et al., 1995; Williamson et al., 1997; Lyon et al., 2000; Chapman et al., 2002).

Equine cestodes were considered for many years to be accidental findings and were regarded as harmless. During the past two decades, however, they have been the subject of several epidemiological and pathological studies, following reports of clinical diseases associated with *A. perfoliata* infection (Owen et al., 1989; Proudman and Edwards, 1993; Pearson et al., 1993; Fogarty et al., 1994; Williamson, 1997; Proudman et al., 1998; Proudman and Trees, 1999; Rodriguez-Bertos et al., 1999).

Diagnosis of cestodosis in live animals depends upon the identification of cestode eggs in faeces. This is, however, both time-consuming and of poor sensitivity (Proudman and Edwards, 1992; Williamson et al., 1998; Meana et al., 1998). Serological approaches to ante-mortem diagnosis have provided a tool for epidemiological studies. ELISA-based methods employing soluble antigen from a number of different sources have been reported. Somatic

antigen derived from mechanically disrupted protoscolex or from whole worm extract, and excretory/secretory (E/S) antigen derived from *in vitro* culture of cestodes have been found diagnostically useful (Hoglund et al, 1995; Hoglund et al, 1998; Proudman and Tree, 1996a, 1996b). Studies by Proudman and Trees (1996a, 1996b) in comparing E/S and somatic antigens demonstrated the superior sensitivity of E/S antigen as a capture layer to detect antibodies to *A. perfoliata* in horses. Western blot analysis of the antibody response to E/S antigens revealed a strongly immunogenic doublet of proteins at 12 and 13 kDa. Furthermore, they were able to demonstrate that the antibody response to this antigen doublet was isotype restricted to IgG (T). The affinity purified anti-12/13 kDa IgG (T) ELISA was then developed for the sero-diagnosis of equine *A. perfoliata* (Proudman and Trees, 1996b).

Previous studies of *A. perfoliata* infection have been restricted to horses. The objective of the present work was therefore to conduct a sero-epidemiological study on naturally acquired *A. perfoliata* infection in the donkey population kept under an extensive traditional management system in Ethiopia. The present chapter presents the results of this study.

5.2. Materials and Methods

5.2.1 Animals

A total of 797 donkeys from four geographical regions were included in the study. The donkeys were naturally infected and had never been treated against cestode infection. Animals represented both sexes and ranged in age from 6 months to 20 years and above. The life expectancy of donkeys in Ethiopia is estimated to be around 9 to 13 years as compared to 37 years in the UK (Svendsen, 1994).

5.2.2 Blood sample collection and preparation of sera

The study was conducted between June 2003 and May 2004 in Ethiopia. Blood samples were collected directly from jugular veins and transported in an ice pack to the Donkey Health and Welfare Project Laboratory, Addis Ababa University, Faculty of Veterinary Medicine, Ethiopia. They were allowed to clot overnight at room temperature. Sera were then prepared after centrifugation at 800g for 5 minutes and stored at -20°C (Proudman and Trees, 1996a; Hoglund et al., 1998) until used for immunodiagnosis.

5.2.3 Enzyme-linked immunosorbent assay (ELISA)

To ensure compatibility between assays, antigen preparations were tested for purity by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) and detection of the anti-12/13 kDa IgG(T) was optimised by chequerboard titration at the University of Liverpool, Faculty of Veterinary Medicine, equine division diagnostic laboratory, Diagnosteq. It was this affinity-purified 12/13-kDa antigen that was employed as a capture layer to detect, IgG(T), antibody of *A. perfoliata*. This ELISA was then used for serological assay in our study. Prior to using this ELISA kit developed for the detection of *A. perfoliata* antibody in horses' sera, a test was made and proven to detect *A. perfoliata* antibody in donkey sera (see Appendix V for detail). The same batches of antigen and control sera were used throughout the study. Running positive and negative controls at fixed positions on each ELISA plate ensured interbatch comparability. Protocol of assay to detect anti-12/13 kDa IgG(T) is outlined in Chapter Two. Briefly, the assay employed a coating step of affinity purified 12/13 kDa antigen at a concentration of 5 $\mu\text{g/ml}$ in a carbonate coating buffer (0.1M, pH 9.6); 100 μl per well was incubated on Immulon 4 plates (Dynatech laboratories, Billingham, West Sussex, USA). Non-specific binding sites were then blocked by incubating 100 μl of 2% skimmed milk powder/phosphate buffered saline (SMP/PBS) per well followed by incubation of 100 μl of test serum, diluted 1/100 in 2% SMP/PBS. The first

detector antibody, goat anti-equine IgG(T) hyperimmune serum (ICN, Biochemicals, Ohio, USA), diluted 1/400 in 2% SMP/PBS was incubated, 100 µl per well. 100 µl of peroxidase conjugated anti-goat monoclonal antibody (Sigma Chemical Co., Missouri, USA), diluted 1/1000 in 2% SMP/PBS, was then incubated. All the above incubations were at room temperature for one hour, and each incubation step was followed by three washes using 2% SMP/PBS containing 0.01% Tween-20. Three final washes were followed by incubation of 100 µl of ABTS (azino-*bis* 3-ethylbenzthiazoline-6-sulphuric acid) (Sigma Chemical Co) at a concentration of 0.4 mg/ml in citrate buffer (pH 4.2) in each well for 15 minutes in a dark place. The plates were automatically read when the positive controls reached reference optical density (OD) of 1.796 (mean value from 11578 samples developed at Diagnosteq, Liverpool University) at 405 nm in a Titertech Multiscan Plus MK II spectrophotometre (Flow Laboratories, Germany).

5.2.4 Interpretation of the results

The negative cut-off OD value of 0.20 was calculated for the affinity purified 12/13 kDa antigen ELISA from helminth naïve ponies (Proudman and Trees, 1996b). However, they observed false negatives in some animals with a very low level of infection intensity. It was therefore difficult to differentiate between low infection intensity and the truly negative animals. Although this was the case, the assay was found to consistently recognise those with moderate and high infection intensities. Using this method they were able to classify results into low/negative ($OD \leq 0.2$), moderate ($0.2 < OD < 0.6$) and high ($OD > 0.6$) infection intensity. Even though infection intensity in terms of absolute numbers has not been validated, according to Proudman (personal communication) OD values of 0.2-0.6 represent burdens of approximately 1-200 worms while OD values > 0.6 might have a burden up to 2000 worms. Our results were therefore, interpreted accordingly. However, caution is needed in interpretation as there is no infection intensity work done in donkeys.

5.2.5 Statistical analysis

All anti-12/13kDa IgG(T) ELISA results were quoted as the mean optical density (OD) minus the mean background OD. Background ODs were measured using blank wells incubated with diluents without serum. Anti-12/13kDa IgG(T) in equine serum has been demonstrated to be well correlated (correlation coefficient, $r_s = 0.63$) with infection intensity (Proudman and Trees, 1996b). The analysis presented is based upon the immunological response as measured by ELISA. This in turn represents infection intensity.

Because of the skewed nature of the data, the presence of negative values and non-integer numbers, fitting the data into different parametric tests such as Poisson, Beta or negative binomial generalised linear models were not appropriate. Therefore, non-parametric tests were applied as necessary. Kruskal-Wallis and Wilcoxon pair wise rank sum tests were used to test the hypothesis of no difference in the ELISA OD values between age groups, sex, region, body condition and seasons. Spearman's rank correlation and Kappa measure of agreement (Petrie and Watson, 1999) were used to test if there was any association or agreement, respectively, between serological and coprological assays in detecting cestode infection. Tests of independence in the frequencies of intensities of infections as determined by ELISA OD between different age groups, sex, body condition score, regions and seasons were assessed using a Chi-square test.

Multiple binary logistic regression was conducted for the risk of having *A. perfoliata* infection, which was defined as an ELISA OD > 0.2. Descriptive statistics and graphs were performed using data analysis tools in Microsoft Excel and Minitab statistical software, whereas statistical analyses in R (R Foundation for Statistical Computing, Version 2.0.1) and Minitab for windows, release 12.21. The significance level for all statistical tests was set to $P < 0.05$.

5.3. Results

5.3.1. Serological responses

A range of ELISA OD values were obtained from serological assays of donkey sera. The wide range of OD values may indicate an overdispersion of *A. perfoliata* in the donkey population, in which most animals harbour few parasites and a few donkeys are infected with large number of parasites (Fig 5.1). Our assay result indicated that 26% and 8% of the donkeys were moderately and highly infected, respectively. The rest, 66% had either low infection intensity or were negative for *A. perfoliata* infection. Considering OD values, which indicate medium and high infection intensity as sero-positive, the assay showed at least 34% sero-prevalence of *A. perfoliata* infection in donkeys.

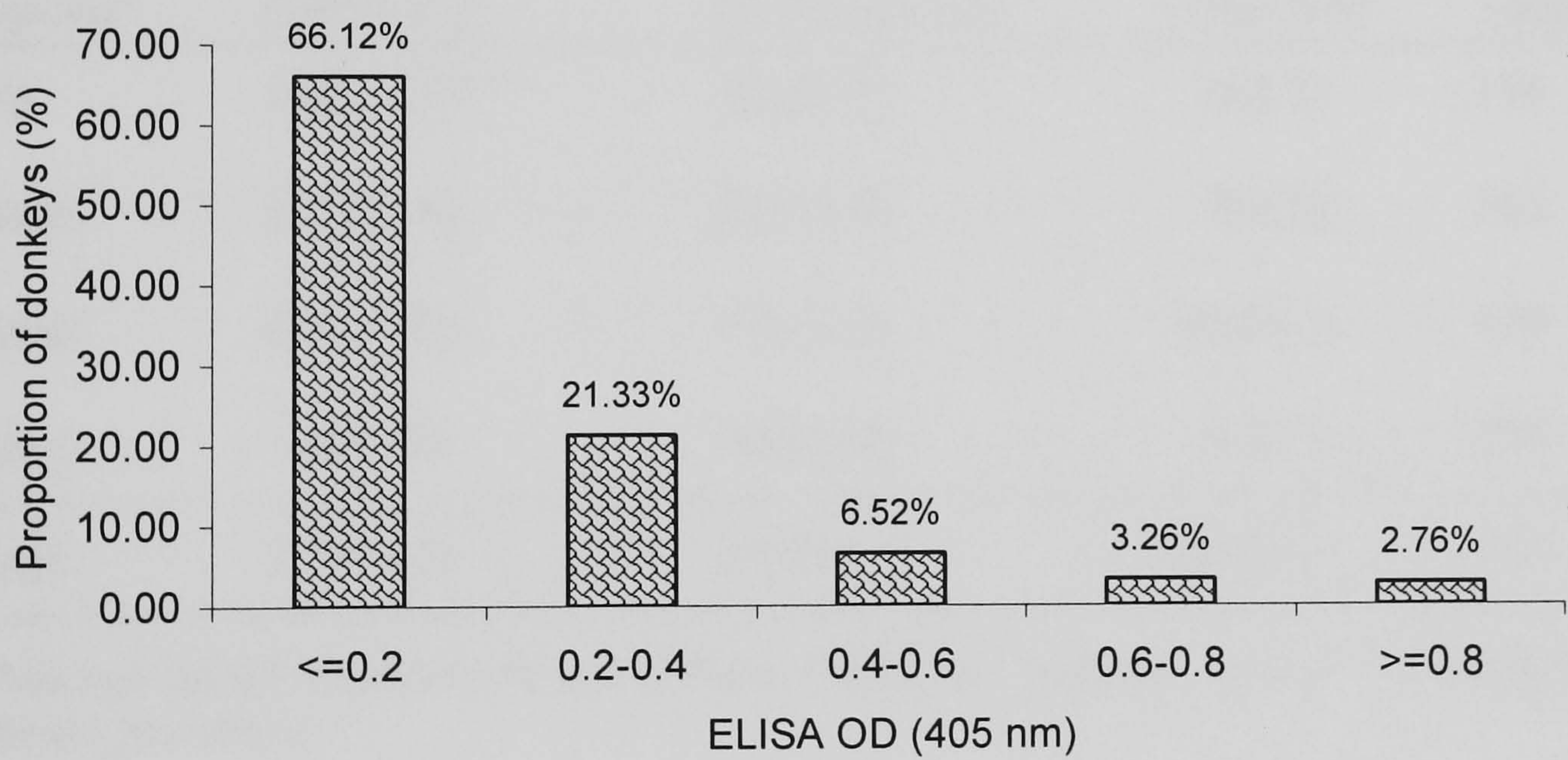


Fig 5.1 The distribution of ELISA OD to anti-12/13 kDa IgG(T) of *A. perfoliata* in donkeys (n=797).

Step wise multiple binary logistic regression analysis of region, age, sex and body condition as factors for the presence of *A. perfoliata* infection defined as ELISA OD>0.2 revealed region as the main risk factor for high sero-prevalence ($P<0.0001$). Pair wise contrast between the different regions showed that Bereh was significantly different ($P<0.0001$) from other regions with odds ratio (OR) of 8.98 ($P<0.0001$, 95% CI= 5.52-14.55). Forty one

percent of moderately infected and 67% of highly infected donkeys were found in Bereh (Table 5.1). Moreover, Kruskal-Wallis rank sum test between ELISA OD and region, age, sex and body condition revealed region as a significant factor (Chi-Sq= 168.302, P<0.0001) and the rest had no significant effect on the antibody level. Results of pair wise comparison using Wilcoxon rank sum test between the different regions and the ELISA OD showed statistically significant differences in the antibody responses between each region (P<0.0001) except between Akaki and Boset (P>0.05).

Table 5.1 Number of donkeys examined and the proportion with different levels of infection intensities of *A. perfoliata* in different regions, Ethiopia.

	Low/zero	Medium	High	
Regions*	(OD<=0.2)	(0.2<OD<0.6)	(OD>0.6)	Total
Ada	146(75.3)**	42(21.7)	6(3.1)	194
Akaki	133(81.6)	22(13.5)	8(4.9)	163
Bereh	43(25.32)	87(51.2)	40(23.5)	170
Boset	205(76)	59(21.9)	6(2.2)	270
Total	527(66.1)	210(26.4)	60 (7.5)	797

* Ada and Akaki -midlands, Boset- lowland and Bereh - highland region. ** Numbers in parentheses indicate prevalence.

The Chi-square test on the frequency of infection intensity (as determined by ELISA OD) revealed statistically significant differences between regions (Chi-Sq = 183.93, P<0.0001). From the Chi-square table (Table 5.2), there were more donkeys with both medium and high infection intensities but less with low/zero than expected in Bereh as compared to other regions. This region contributed over 75% of the total Chi-square value calculated for the whole table. Therefore, this demonstrates that Bereh was the region where higher proportions of donkeys were either moderately or highly infected with *A. perfoliata* compared to all other regions.

Table 5.2 Chi-square test in the analysis of the frequencies of infection intensities of *A. perfoliata* in different regions, Ethiopia.

	Ada	Akaki	Bereh	Boset	Total
Low/zero	146 128.28*	133 107.78	43 112.41	205 178.53	527
Medium	42 51.12	22 42.95	87 44.79	59 71.14	210
High	6 14.60	8 12.27	40 12.80	6 20.33	60
Total	194	163	170	270	797

Chi-Sq = 2.448 + 5.901 + **42.858**** + 3.924 +
1.626 + 10.218 + **39.770**** + 2.072 +
5.070 + 1.487 + **57.818**** + 10.097 = 183.289
df = 6, P-Value = 0.000

*Expected counts are printed below observed counts. **The largest contribution of Chi-square came from Bereh.

5.3.2. Relationship between serological and coprological assays

Attempts were made to see if there was any relationship between serological and coprological assays in detecting cestode infection. Spearman’s rank correlation coefficient between ELISA OD values and the McMaster cestode faecal egg counts revealed a statistically significant correlation coefficient ($r_s = 0.0916$, $t= 2.594$, $P=0.01$, 95% CI= 0.023, 0.160) indicating that the two techniques are weakly associated. However, the scatter diagram does not show any clear-cut relationship (Fig 5.2).

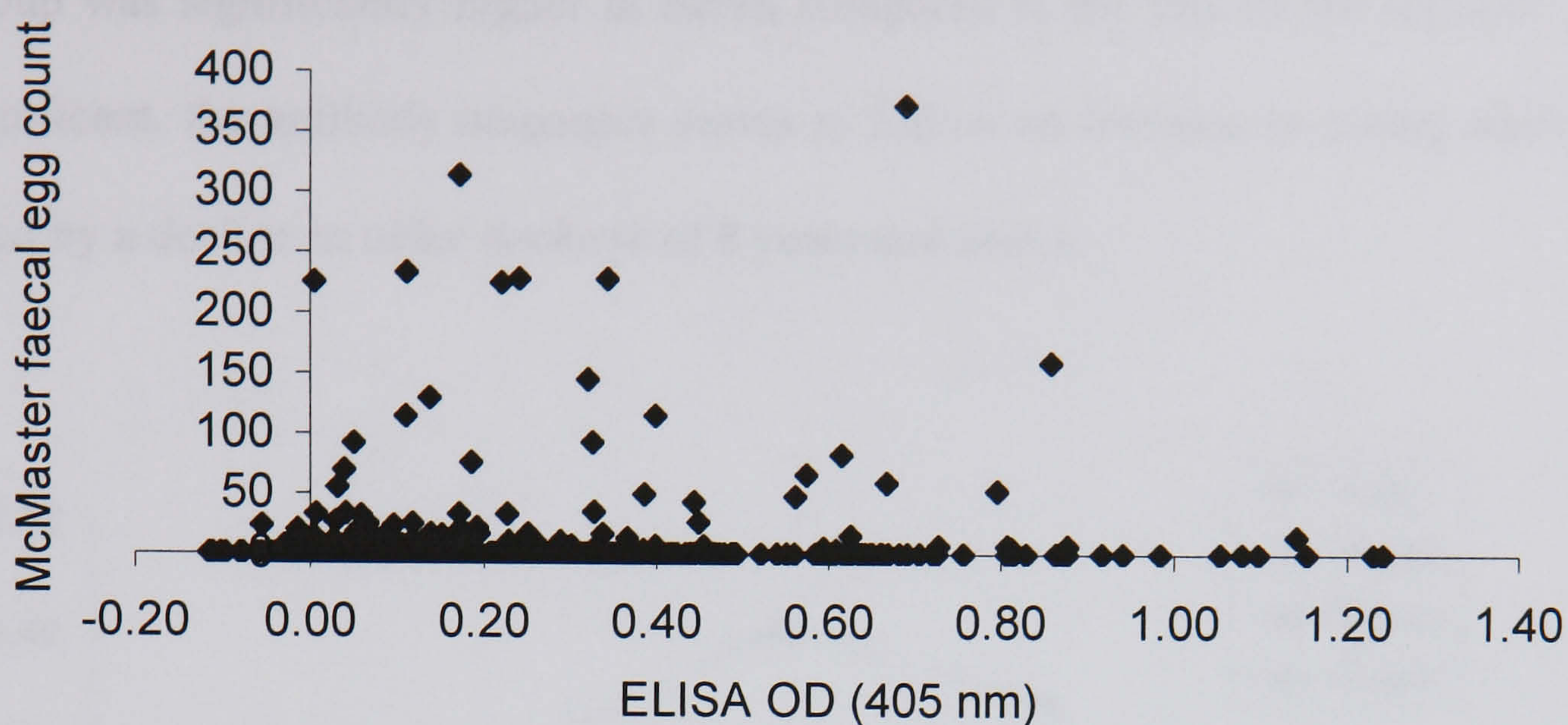


Fig. 5.2. Scatter diagram of the relationship between ELISA OD and McMaster cestode faecal egg count.

Kappa measure of agreement between serological assay and the centrifugation/flotation technique in detecting cestode infection revealed Cohen's kappa coefficient, $\kappa = 0.161$. This measurement was also calculated for each region to see if there were any differences, but similar results with the overall data were obtained. Kappa coefficient of only 0.161 indicates a poor agreement between the two diagnostic methods. McNemar's test on the equality of the proportion of positive samples by the two diagnostic tests showed that ELISA detected a significantly greater proportion of animals (Chi-Sq = 23.1, $P < 0.0001$, 95% CI= 0.061, 0.143).

5.3.3 Age dependency of serologically-determined infection intensity

It was found that age had no significant effect on the frequency of intensity of infection (Chi-Sq= 4.708, $P = 0.582$). The IgG(T) response to 12/13kDa antigen as a measure of median infection intensity in the different age groups is shown in Fig. 5.3. Kruskal-Wallis one-way analysis of variance showed no significant differences (Chi-Sq=1.89, $P=0.596$) between the overall median OD and the different age groups. A similar result was obtained for each

region. However, as can be seen from Fig 5.3, the magnitude of the median OD value in each age group was significantly higher in Bereh compared to the rest of the regions. Although not significant, the antibody responses seems to follow an increase in young adult donkeys followed by a decline in older donkeys of 8 years and above.

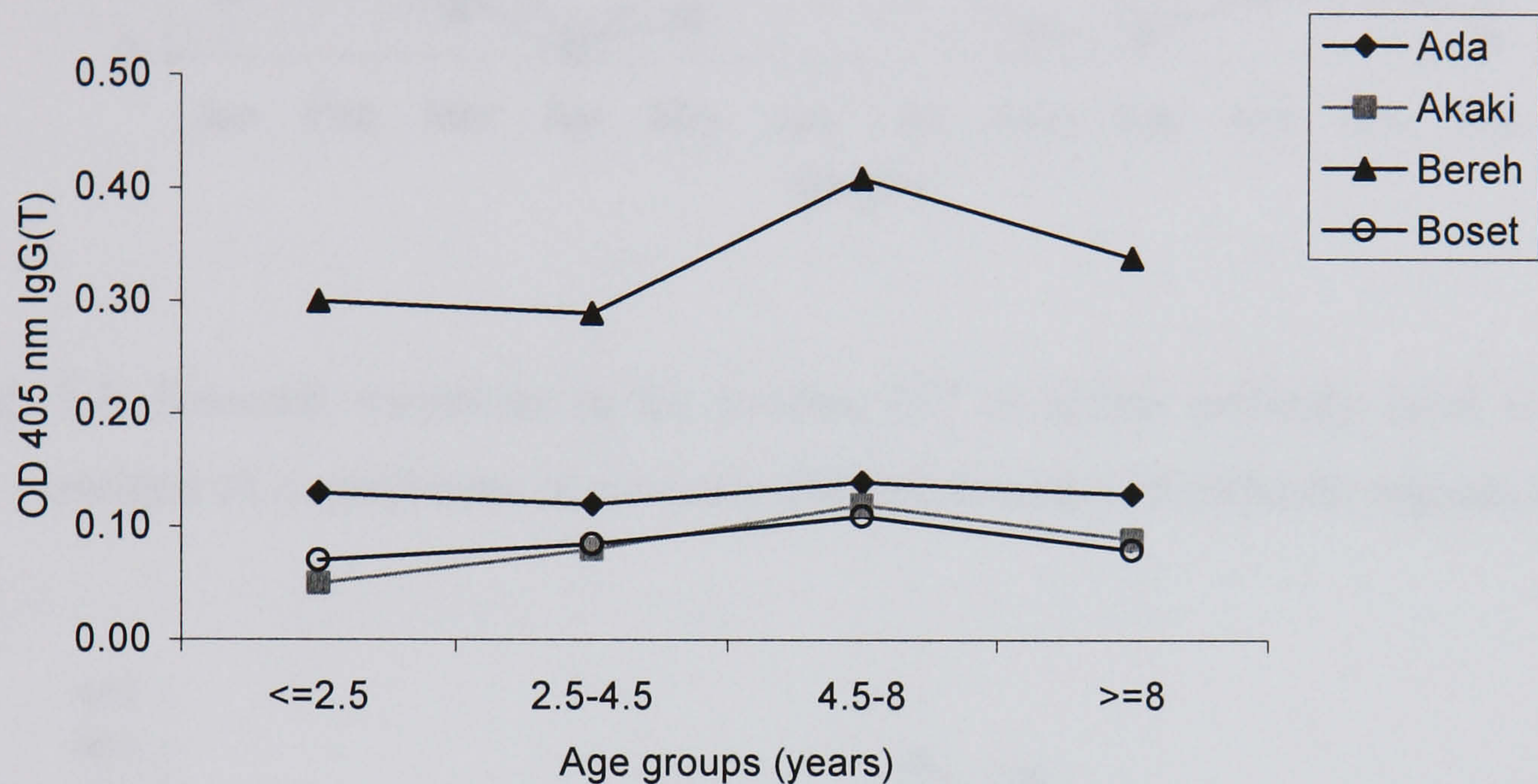


Fig. 5.3 IgG(T) response to 12/13 kDa antigen as a measure of infection intensity in different age groups. ELISA results are expressed as median optical density (405nm) for each age group in each region.

5.3.4 Temporal variation in serological responses

A statistically significant monthly variation in the overall serum antibody levels was observed (Chi-Sq= 23.85, P=0.013). Similar results were obtained for each region. The rise in antibody response seems to follow rainy seasons although there were variations between the regions (Fig 5.4). The rainy seasons are March-April, and June-September (Fig 5.5). The bimodal pattern of antibody response was particularly obvious in Bereh.

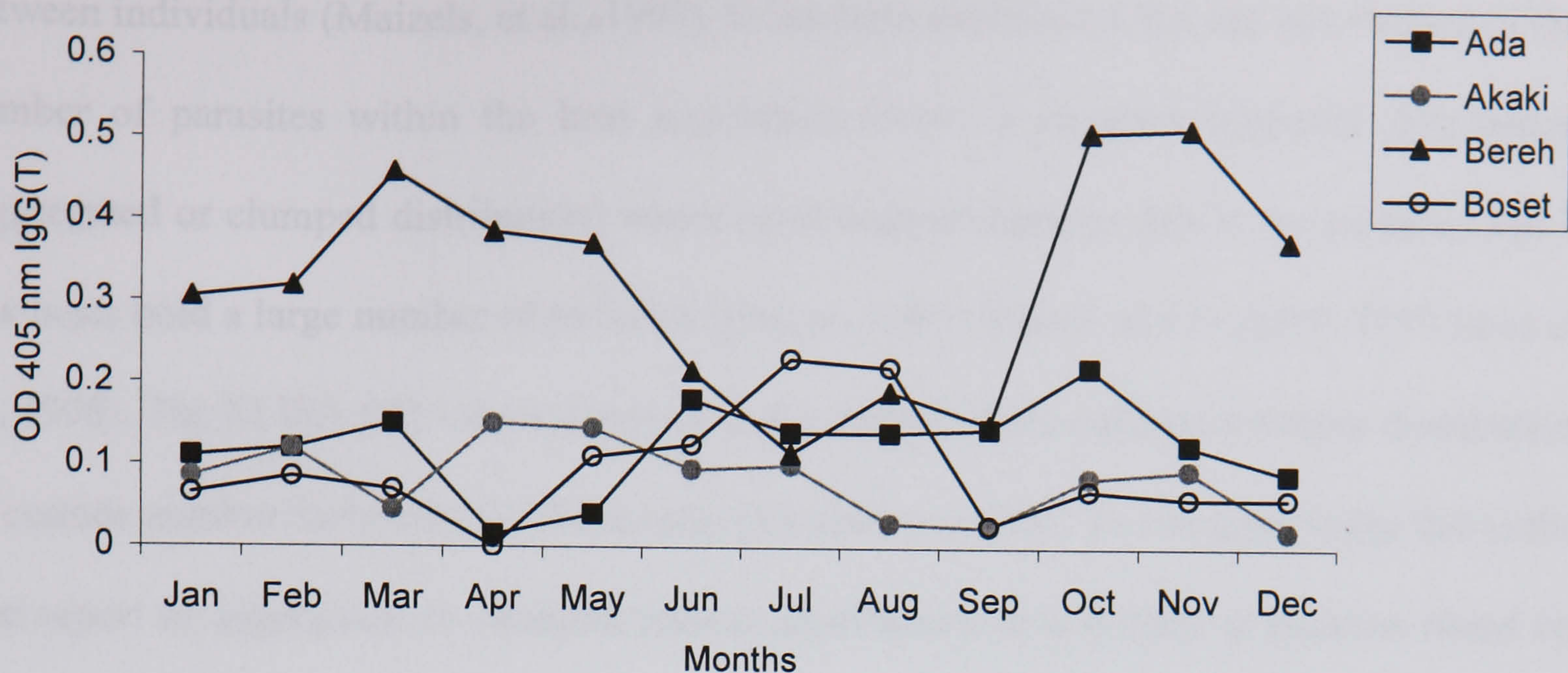


Fig. 5.4. Seasonal variations in the median OD of serum antibody level to a 12/13 kDa antigen of *A. perfoliata* in naturally infected donkeys of different regions, Ethiopia.

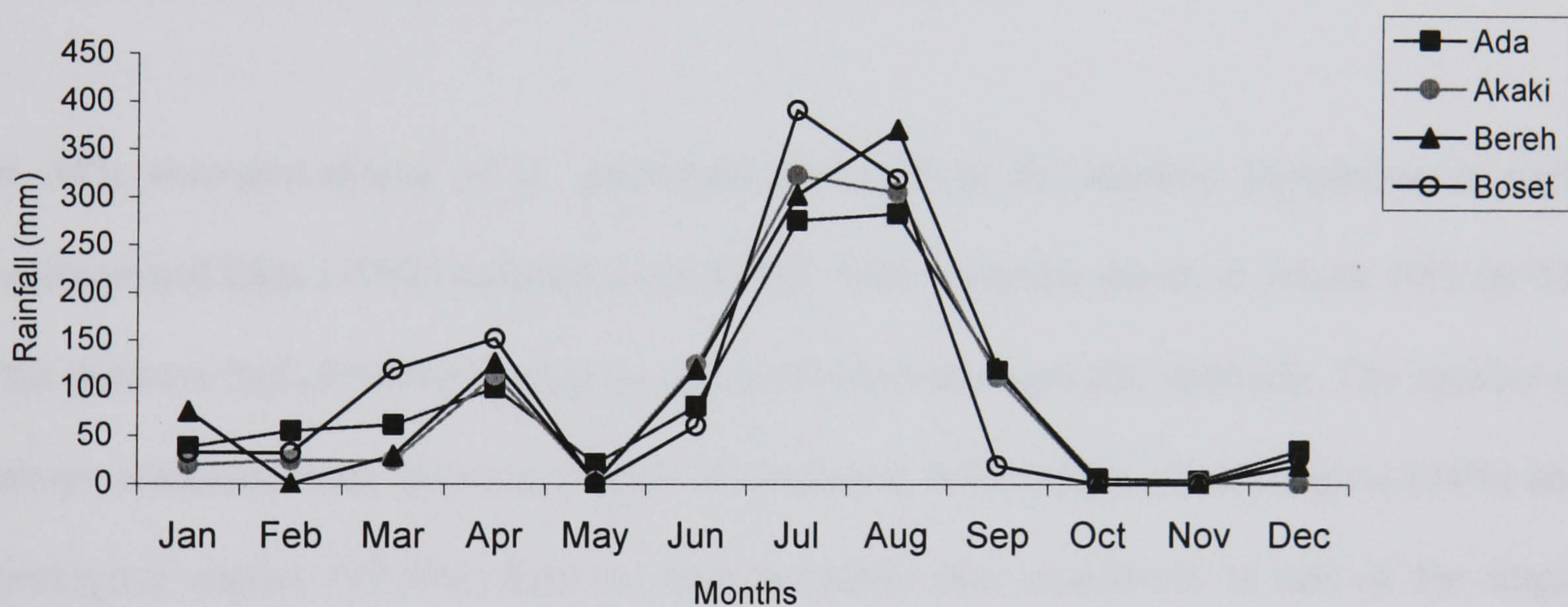


Fig 5.5 Monthly rainfall distribution of each region, Ethiopia.

5.4 Discussion

5.4.1 Serological responses

The present study has revealed a range of OD values indicating different levels of infection intensities in individual animals. The epidemiology of helminth infection is characterised by a long parasite lifespan and repeated reinfection throughout the host's own life, which varies

between individuals (Maizels, et al., 1993). It has been established that the distribution of the number of parasites within the host population follow a negative binomial distribution (aggregated or clumped distribution) where most animals harbour few or no parasites and a few hosts hold a large number of parasites (Barger, 1985; Wilson and Grenfell, 1997 Stear et al., 1998). The ELISA OD values obtained in the present study indicate a similar distribution of cestode number harboured by the donkey population studied. To our knowledge this is the first report of aggregated or clumped cestode distribution in a donkey population based on serological findings. The aggregated distribution of *A. perfoliata* obtained by direct measurement of parasite burdens on post mortem examination of donkeys supports this finding (Chapter 4). However, the relationship between serum antibody level and infection intensity of *A. perfoliata* in donkeys needs further investigation.

The 34% sero-prevalence of *A. perfoliata* obtained in the donkey population is high. Proudman and Ellis (1992) reported a pilot study with a similar result, in which 40% (n=15) of the donkeys had clear serological evidence of tapeworm-specific antibody. The number of donkeys examined was, however, small. Considering both results of serological (34%) and coprological assays (27.9%) there is clear evidence that cestodosis is one of the major problems in the donkey population of Ethiopia.

The high correlation coefficient between anti-12/13 kDa IgG(T) and infection intensity in horses makes the ELISA a good predictor of worm burden in this species and it may be used as a measure of infection intensity (Proudman and Trees, 1996b). In common with most intestinal helminth infections, morbidity and mortality to *A. perfoliata* is related to the intensity of infection (Pearson, et al., 1993; Fogarty, et al., 1994, Williamson, et al., 1997). A study by Proudman et. al. (1998) revealed that 22% of spasmodic colic cases (n=103) and 81% of ileal impaction colics (n=20) in horses were associated with *A. perfoliata* infections.

Moreover, serological diagnosis was also associated with an increased risk of spasmodic colic. If we can extrapolate from these findings to the donkey population, donkeys are at high risk of intestinal disorders or diseases associated with *A. perfoliata* infection.

There were variations in antibody response and infection intensity as determined by ELISA OD between the regions. With OR of 8.96 (95% CI of 5.52-14.55) Bereh, the highland region, is a high-risk area for anoplocephalosis. Similarly, a Chi-square test on the frequency of infection intensity revealed Bereh as a region where the majority of donkeys were either moderately or highly infected. The main factors for such difference in sero-prevalence or infection intensity between regions is likely to be the favourable environmental conditions for the survival and development of oribatid mites, as well as for cestode eggs. Unlike Bereh the mid-lowland regions do not have a defined permanent pastures, which have been used for many years, and hence play crucial role in supporting many mites. This might lead to a lower force of infection, and hence to low worm burden and low antibody response (see Chapter 3 for detail).

In endemic areas many animals will be exposed to tapeworm infection during their lifetime without necessarily being infected at the time of sampling. This is particularly true where cestocidal medications are administered. Treatment will reduce the specificity of the test due to persistent antibodies after the death of parasites (Proudman and Trees, 1996b; Hoglund et al., 1998). In the present study this might not be a problem because donkeys are unlikely to have been treated against cestode infection.

5.4.2. Relationship between serological and coprological assay

Even though the Spearman's rank correlation revealed the presence of a weak association between the McMaster faecal egg count and ELISA OD, the association was small and no

clear-cut pattern of relationship was apparent on visualising the data. Moreover, faecal egg recovery is of known low sensitivity and there is only a low correlation between faecal worm egg count and worm burden (Proudman and Edwards, 1992; Nilsson et al., 1995; Williamson et al., 1998). However, the many animals with zero faecal egg counts and low OD values could be the reason for the significant but weak relationship observed. There were also several animals with positive faecal egg count, which had moderate or high ELISA OD.

The statistically significant difference obtained between serological (OD>0.2) and coprological assays in detecting the presence of cestode infection indicates that the serological assay could be a better diagnostic tool than the McMaster or centrifugation/flotation technique despite its inability to recognise low infection intensities. However, caution is needed in interpretation as there might be serologically false positives, particularly in previously treated animals. The serological assay detected 34% infection prevalence as compared to 24% by centrifugation/flotation technique, a difference of 10% (95% CI = 6% to 14%). Since it is difficult in practice to differentiate eggs of *A. perfoliata* from *A. magna*, it was difficult to tell whether these two assays were looking for the same thing. Cohen's kappa coefficient (κ), for measure of agreement between the two techniques in detecting cestode infection showed a poor agreement ($\kappa=0.161$). The serological assay, however, detects antibody specific to *A. perfoliata* and no cross-reactions were found with other cestode species or other helminths (Proudman and Trees, 1996b; Hoglund et al., 1995). Therefore, the superiority of ELISA in detecting cestode infection might indicate that *A. perfoliata* was the dominant cestode species in the examined donkey population. The dominance of *A. perfoliata* obtained from donkeys on post-mortem examination (Chapter 4) coupled with egg measurements in which the majority were within the range of that of *A. perfoliata* (Appendix IV) supports this suggestion.

5.4.3 Age dependency of serologically-determined infection intensity

The magnitude of the median OD values in each age group was higher in the highland region, Bereh, as compared to the other regions. The antibody response, however, seems to follow a similar pattern in all regions although it is more pronounced in Bereh. Lack of difference in antibody response between the different age groups of donkeys was inconsistent with the result of the study made by Proudman et al (1997). According to Proudman et al. (1997), there was a clear indication of age dependency of IgG(T) response to 12/13kDa antigen. In their study the maximum antibody response was observed in horses aged six months to two years, falling to a lower plateau level until 15 years, then rising again in older animals. The first peak of antibody response was attributed to the high level of exposure of young horses to infection, because they believed that this age group is the one usually kept on pasture all year round. Horses of 3-15 years are most likely to be stabled for recreational and competitive uses and therefore encounter less chance to become infected. The subsequent increase in antibody response in older horses was attributed to their exposure to infection, because they believed that most older horses are those retired from work and would be expected to spend much of their time grazing rather than being stabled. A further factor that they thought influenced the mean worm burden and hence antibody response was the use of anti-cestodal therapy, which they thought might be more regularly used in horses aged 3-15 years leading to a decreased worm burden (Proudman et al., 1997).

Although the age-dependent pattern of exposure to *A. perfoliata* was not studied, it would be reasonable to suggest that exposure to infection would be high in animals that spend most of their time on pasture. Hoglund, et al. (1995) reported high worm burden in horses slaughtered at the end of summer grazing period irrespective of their age supporting our suggestion. In Ethiopia donkeys of all age groups spend their time on open grazing/pastureland all year round (except during the night time, when they are kept in

kraals). They are exposed to a similar force of natural infection throughout their lifetime, although this varies between regions. Moreover, neither pasture management nor parasite control strategies are implemented that might decrease worm burden. In endemic areas where donkeys spend their entire life on pasture with no stabling or treatment, the rate of replacement of worms due to reinfection could be high. This may be the case particularly in the highland region where the transmission potential is high compared to other regions.

The different age-specific pattern of antibody response observed in donkeys compared to that of horses (Proudman et al., 1997) may represent the different management systems adopted, which in turn, influences exposure of the animals to different forces of infection. Although not statistically significant, there was a steady rise in antibody response starting from 4.5 years until 8 years followed by a decline in older donkeys of 8 years and above. The change in mean worm burden for many helminths follows a convex curve, in which the age of peak intensity depends in part on the life expectancy of the parasites (Maizels et al., 1993). The different pattern of antibody response in our case may indicate the absence of acquired immunity, the build up of worm load in adult donkeys and the parasites' long life expectancy. Post-mortem studies by Lyons, et al. (1984), Owen, et al. (1988), Fogarty, et al. (1994) and Ihler et al. (1995) revealed a similar number of *A. perfoliata* present irrespective of age, indicating that neither acquired protective immunity nor age resistance to *A. perfoliata* seems to be of any importance. Moreover, the demonstration of a positive correlation between specific antibody, IgG(T), response to the 12/13kDa antigen and infection intensity (Proudman and Trees, 1996b) suggests a non-effector role in parasite-specific acquired immunity.

Different responses to an individual antigen among animals or humans with similar exposure and force of infection are likely to be caused by genetic, stress-related, nutritional and

behavioural components (Maizels, et al., 1993). According to Quinnell, et al (1995), high antibody levels may be protective in older hosts and reduce worm burden; alternatively, high worm burdens may be immunosuppressive and reduce antibody levels. In endemic areas donkeys are expected to harbour worms for most of their lifetime owing to repeated exposure to reinfection and absence of treatment. Donkeys are likely to be neglected, highly stressed from mis-management, different disease ailments, and malnourishment; in particular older donkeys are likely to suffer most (Starkey, 1994a,b; Svendsen, 1994, 1997b). Therefore, immuno-suppression or immuno-incompetence, which affects the animals' ability to respond to parasite antigen and hence produce antibody, might explain the decline in antibody level in older donkeys rather than parasite-stimulated immunity, which reduces parasite burden. The immuno-epidemiological study of Proudman et al. (1997) was based on a population of well-managed domestic horses and it seems that age-related exposure due to different management practices influenced antibody level rather than age *per se*. Therefore, exposure to different levels of force of infection and immunological status of the animal seems to govern the antibody level against *A. perfoliata* infection in the equine population. The fact that all regions show similar differences although each region is very different is strong evidence for this.

5.4.4 Temporal variation in antibody response.

The rise in antibody response following rainy seasons was observed in most regions although there were variations both in magnitude and pattern. The bimodal pattern of antibody response in Bereh was interesting. This is the highland endemic region where the sero-prevalence and infection intensity were highest and where conditions are best for the survival and development of the oribatid mites and cestode eggs providing high transmission potential. In other regions the absence of permanent pasture, important in maintaining the

oribatid mites, create gaps in the infection cycle and may have given rise to the irregular pattern of seasonal antibody response.

Although it is not possible to determine the exact time between primary infection and sero-conversion, the rise in antibody levels following rainy seasons may indicate the animals' greater exposure to infection during these times of the year. It has been suggested that the development of a proportion of worms may be inhibited over the winter period (Sanda and Tsukada, 1985; Hoglund, 1995) and it has generally been accepted that the prepatent period is longer than 6-10 weeks (French and Chapman, 1992). Consequently, it is possible that cysticercoids acquired at the end of a long rainy season might have been inhibited during the long dry season (October to February) and contributed to the observed rise in antibody level during or following the short rainy season. This might have been important in Ada, Akaki and Boset where there is a long dry season, which is unfavourable for the survival and development of the parasites. The low antibody levels observed in April in Ada and Boset, and July in Bereh followed by an increase may indicate the turnover of worms and the dependency of antibody level on the newly acquired infection. A similar bimodal seasonal pattern in antibody response to the scolex E/S antigen and in the number of detectable intestinal tapeworms in horses in endemic areas was observed by Hoglund et al (1995) and Hoglund et al. (1998). They suggested that the antibody response was related more to the establishment of newly acquired infections than to the presence of a small or moderate number of old worms in the intestine. There is no information on the turnover of worms, and the half-life of equine IgG(T) in serum is not known. This makes it difficult to interpret the seasonal variation of serum antibody level of equine IgG(T). More detailed sero-epidemiological studies would be needed to fill such information gaps and fully understand seasonal antibody responses.

5.4.5. General conclusion.

The present sero-epidemiological study has shown a substantial serological evidence that donkeys are potentially infected with *A. perfoliata*. The risk of infections, both in sero-prevalence and intensity, as determined by ELISA OD, were high in the highland area where pastures are low-lying and wet, and a permanent pasture management is regularly practiced. Studies conducted on horses and ponies have shown that intensity of infection and severities of the intestinal lesions are closely associated (Williamson, et al., 1997; Proudman and Trees, 1999). Post-mortem examination of donkeys infected with *A. perfoliata* showed a similar result (Chapter Four). The high positive correlation of serum antibody level with intensity of *A. perfoliata* infection allows its use as a measure of infection intensity and hence severity of intestinal lesions (Proudman and Trees, 1996). Therefore, the present findings of 8% of the total and 24% of the highland examined donkeys, respectively, with high ELISA ODs, clearly indicate that donkeys are at high risk of cestodosis, warranting the consideration of cestodes in the anthelmintic treatment programme.

Even though it is difficult to determine seasonal variation in serum antibody level from the present study, the findings of serological and coprological assays indicate the best time for treating donkeys appears to be at the beginning of the rainy season to reduce pasture contamination during the subsequent rainy season in mid-lowland regions of Ada, Akaki and Boset. The chance of getting reinfected is low during the long dry season in these regions. On the other hand, in the high-land region, Bereh, where favourable environmental conditions for the survival and development of oribatid mites and cestode eggs prevails almost throughout the year and permanent pasture management is regularly practiced, two cestode treatment doses, one at the beginning and another at the end of rainy season are recommended depending on owners ability to afford cost of the drug.

CHAPTER 6

FIELD EFFICACY OF PRAZIQUANTEL ORAL PASTE AGAINST NATURALLY ACQUIRED CESTODES OF DONKEYS IN ETHIOPIA.

6.1 Introduction

Parasitic infections cause significant losses to the equine population, either directly as a result of colic or indirectly by reducing condition and performance (Barrett et. al., 2004). Parasites are the main cause of early demise in working equids, in general, and donkeys, in particular, in the developing countries (Svendsen, 1994a, 1997). Although they are still major problems of equids in the developing world, modern anthelmintic use has decreased the prevalence of large strongyles in most of the developed world, which has, in turn, shifted the focus of parasitologists to the pathogenic importance of the small strongyles, tapeworms, and other parasites. The equine tapeworms, particularly *A. perfoliata*, have become very common, having a worldwide distribution and causing significant gastrointestinal disorders and diseases (French and Chapman, 1992; Proudman and Trees, 1996b; Proudman et al., 1998; Lyons et al., 2000). According to a case-control study (Proudman et. al., 1998), tapeworm infection is a significant risk factor for both spasmodic colic and ileal impaction and virtually all ileocaecal intussusceptions. It has now become common practice to treat tapeworm infection in horses and ponies in the developed world.

The present coprological, serological and post-mortem investigations have revealed high prevalence of tapeworm infection in donkeys in Ethiopia (Chapters 3, 4 and 5). The high infection prevalence and intensity of infection observed in the highland region in particular indicates that a cestode treatment programme could result in improved welfare of donkey.

In previous years, treatment of tapeworm infections has not been investigated extensively and no commercial paraciticides were licensed for the treatment of tapeworms in equids in general, and donkeys in particular. Consequently, control programmes for these parasites were often speculative. Pyrantel pamoate at 13.2 mg/kg and 19.8 mg/kg (2 and 3 times the recommended dose level) have been reported to be effective against tapeworm infections in horses (Slocombe, 1979; Lyons et al., 1989). Because of the increased reports of clinical disease associated with cestode infections, drugs such as Praziquantel (Equitape Horse Paste, Fort Dodge) or a combination of praziquantel and ivermectin (Equimax, Verbac Animal Health) or praziquantel and moxidectin (Combo Care Gel, Farnam) have been developed for the treatment and control of single and mixed parasitic infection in horses. These have been found effective (Grubbs et al., 2003; Rehbein et al., 2003; Marley et al., 2004; Barrett, et al., 2004). Even though some reports indicated that pyrantel embonate (Strongid P, Pfizer) was effective under field conditions in reducing cestode faecal egg counts when twice the recommended dose for strongyles was given to the donkeys (Trawford, 1997), no drug efficacy studies have been conducted in donkeys.

The aim of the present study was, therefore, to evaluate the efficacy of Praziquantel (Equitape, Fort Dodge) against naturally acquired cestodes of donkeys. Its efficacy was measured in terms of its ability to reduce faecal egg counts. Change in the estimated risk of disease after treatment was assessed by serological diagnosis using ELISA.

6.2. Materials and methods

6.2.1 Animals

Forty-four donkeys, aged 2 to 20 years old and above, of both sexes with evidence of tapeworm infection were randomly selected from the cestode positive donkeys. Positivity for tapeworm infection was confirmed both by faecal egg recovery and ELISA OD>0.2. The

selected donkeys were then randomly allocated into treatment (n= 22) and control (n=22) groups. None of the donkeys had ever received cestode treatment before. Body weight of each animal was estimated from heart girth and height measurements and using the heart girth nomogram (Appendix I). The treatment group was treated with both praziquantel (Equitape Horse Paste, Fort Dodge) and ivermectin per os (Ivomec injectable, Merial) at the recommended dose rate for horses of 1 mg/kg and 200 µg/kg, respectively, while the control group was treated only with ivermectin. Both groups continued to receive ivermectin at 8 weekly intervals to eliminate the majority of intestinal parasites other than tapeworm, a practice implemented to facilitate isolation and identification of tapeworm eggs. Great efforts were made to assure that each animal received the appropriate dosage of each treatment. All donkeys were maintained on similar pasture throughout the study period. During the treatment trial period three donkeys were sold, one was stolen and two were eaten by predators, all from the control group. Complete data were therefore obtained and presented from only 38 donkeys.

6.2.2 Faecal and blood sample collection and analysis

The study was conducted from February to July 2004. Faecal samples were collected by manual removal from the rectum of donkeys before and 2, 4, 8, 12, and 16 weeks after initial treatment. The centrifugation/flotation technique (Proudman and Edwards, 1992) was used for detection of tapeworm eggs. Two coverslips were examined per sample prepared from 30 gram of faeces. Although the technique of centrifugation/flotation is essentially qualitative, a semi-quantitative estimate can be made by counting the number of cestode eggs observed under the two coverslips. For the convenience of calculation and analysis total egg count was expressed as total number of eggs per 30 gram of faeces. This enabled any partial reduction in the number of eggs to be assessed after treatment. The results were expressed as a reduction in the arithmetic mean egg count for the treatment group at each sampling period

compared to the control group at the same sampling period. Blood samples were collected before and 8 and 16 weeks after initial treatment from all donkeys. Sera were analysed using ELISA according to the method of Proudman and Trees (1996b). Details of the procedures and protocols for the analysis of both faecal and sera samples are detailed in Chapter Two.

6.2.3 Statistical analysis

Data for pre-treatment and post-treatment faecal egg counts and the tapeworm antibody ELISA ODs were compared graphically and the differences between the samples were evaluated with two-sample Wilcoxon test (Mann-Whitney) for non-parametric data. The percentage efficacy was calculated from the faecal egg count reduction, using the arithmetic mean egg count with the following formula: % Efficacy = (mean egg count control - mean egg count treated)/mean egg count control*100 (Coles, et al., 1992). The relationship between the risk of colic and the intensity of tapeworm infection as determined by the anti-12/13 kDa IgG(T) ELISA is described by the equation: odds ratio (OR) = $e^{2.576*ELISA\ OD}$. This was determined for the horse (Proudman and Trees, 1998). Assuming it also holds true in donkeys, this equation was used to calculate a change in the predicted risk of colic before and after treatment. All statistical computations were performed using R, version 2.0.1 and graphics and tables in Microsoft Excel 2000 and Minitab Statistical Software, version 12.21. Significance levels were set at $P < 0.05$.

6.3. Results

The mean and median egg counts and percentage efficacy of praziquantel at each sampling period are shown in Table 6.1. Prior to initiation of the treatment, there was no statistically significant difference between the median egg counts of the treatment and control groups ($p=0.096$). Compared to the control group significantly higher egg reduction was however observed in treated donkeys ($P < 0.0001$) throughout the study period for 16 weeks. The

average faecal egg count was reduced from 22.7 (± 42.4) to 0 in 100% of the donkeys 4 weeks post-treatment. Donkeys in the control group continued shedding eggs although some of them were negative at some sampling dates and positive at others. Apart from one donkey, which was positive for cestode eggs two weeks after treatment and became negative in all subsequent samplings, all treated donkeys remained negative throughout the trial period (Fig. 6.1).

Table 6.1 The mean and median faecal cestode egg count and percentage efficacy of praziquantel administered to donkeys infected with cestodes.

Weeks in trial	Treatment group (n=22)		Control group (n=16)		% efficacy
	Mean (sd ^a)	Median	Mean (sd ^a)	Median	
Week-0	22.7 (± 42.2)	6	9.0(± 16)	4.5	-
Week-2	0.1(± 0.3)	0*	2.7(± 2.8)	2.0*	97
Week-4	0(-)	0*	6.0(± 8.5)	2.5*	100
Week-8	0(-)	0*	4.0(± 6.6)	2.5*	100
Week-12	0(-)	0*	7.6(± 13.2)	3.5*	100
Week-16	0(-)	0*	15.6(± 34.5)	4.5*	100

*Data within the same row are significantly different ($P < 0.0001$); Wilcoxon test for non-parametric data. ^asd: standard deviation.

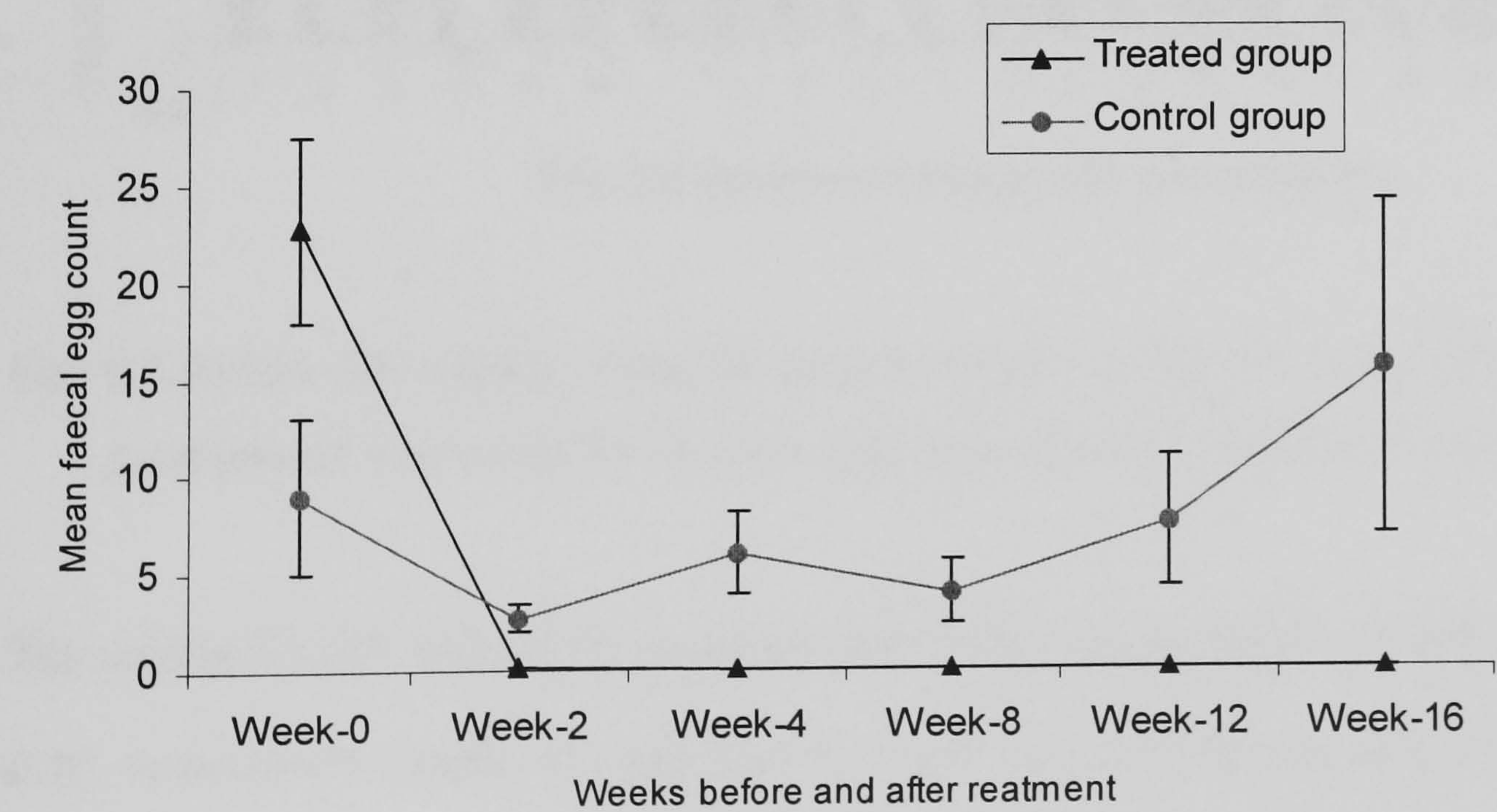


Fig 6.1. The mean faecal egg counts in the treated and control groups at each sampling period. Week-0 is before treatment and Week-2 to weeks-16 after treatment. Error bars indicate mean \pm se (standard error).

Eight weeks following praziquantel therapy a marked decrease in ELISA ODs was observed in most of the donkeys treated with praziquantel. The level of decline in serum antibody however varies between individual donkeys (Fig 6.2). Eight weeks after dosing with praziquantel 55% of 22 donkeys with OD values between 0.2 to 0.6 before treatment had OD values less than 0.2 (the negative cut-off point), while 41% of donkeys with OD values between 0.6-1.0 and one donkey with an OD value of 1.43 before treatment had OD values between 0.2-0.6 and 0.74. These values continued to decrease with time and 16 weeks after treatment only two donkeys with original OD values of 1.43 and 0.98 pre-treatment still had OD values of 0.40 and 0.25, respectively.

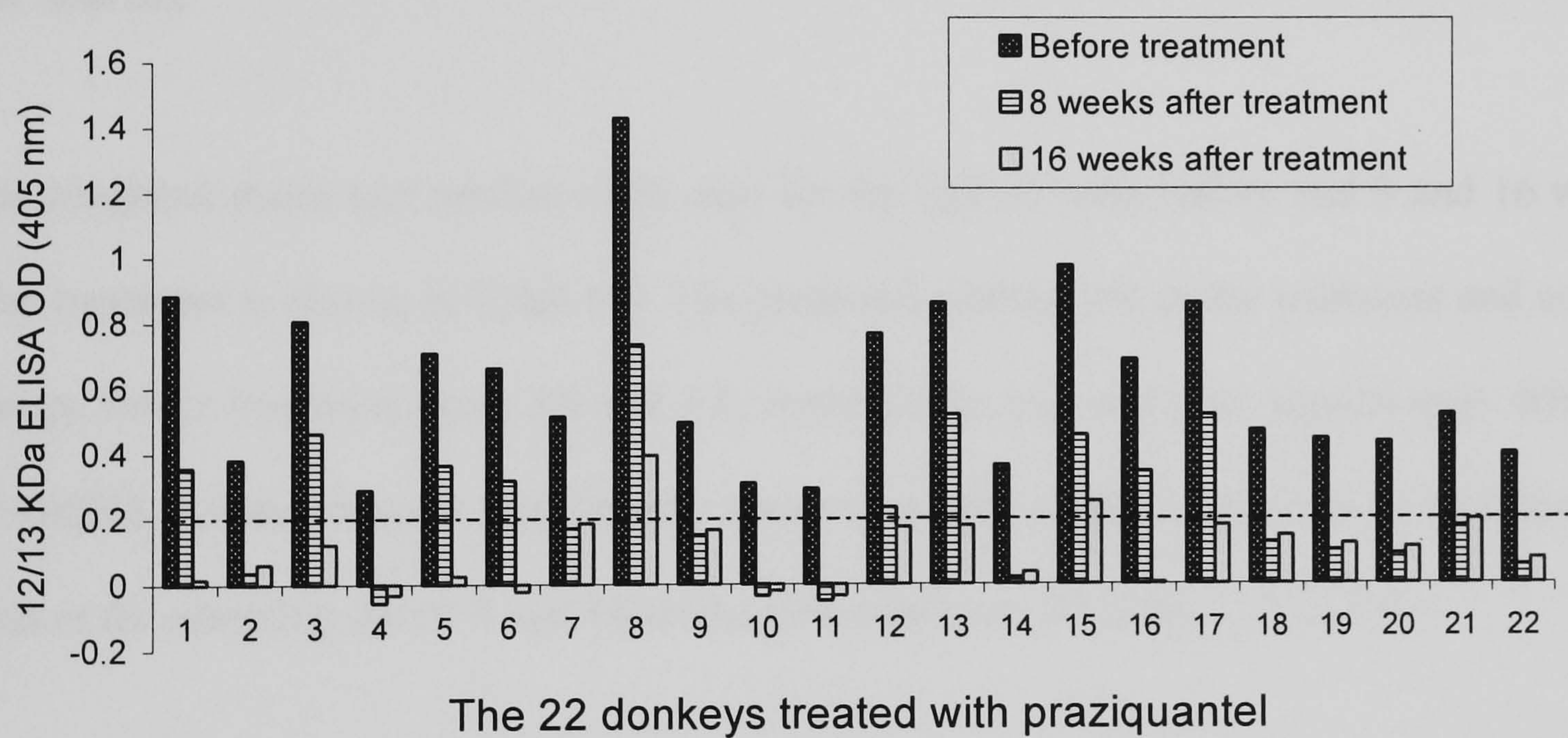


Fig. 6.2 ELISA OD values of the 22 donkeys before, 8 and 16 weeks after treatment with praziquantel. Each number on the x-axis represents an individual donkey.

The median ELISA ODs of the treatment and control groups before treatment were 0.52 and 0.50, respectively (Table 6.2 and Fig. 6.3) and were not significantly different ($P=0.63$). However, the median OD values of the control group were consistently significantly higher than the treatment group ($P<0.0001$) at both sample times: 8 and 16 weeks after treatment (Table 6.2 and Fig 6.3).

Table 6.2 Summary statistics for ELISA OD values and predicted odds ratio for the risk of having colic in 22 praziquantel treated and 16 control donkeys infected with tapeworms.

	BT*	Weeks after treatment		Control		
		8	16	BT*	8	16
Minimum OD	0.29	-0.06	-0.04	0.24	0.49	0.49
Maximum OD	1.43	0.74	0.40	1.32	1.23	1.25
Mean OD	0.62	0.23	0.11	0.59	0.81	0.74
Median OD	0.52	0.17	0.12	0.50	0.74	0.65
Mean OR ^a	6.80	2.10	1.40	6.30	9.80	8.60
Median OR	3.80	1.60	1.40	3.60	6.80	5.40

*BT: Before treatment commenced

^aOR: odds ratio

The predicted mean and median odds ratio for the risk of colic before and 8 and 16 weeks after treatment is shown in Table 6.2. The predicted median OR in the treatment and control groups before treatment were 3.8 and 3.6, respectively, and were not significantly different (P=0.626). It was however significantly lower in treated donkeys than the control group at each of the sampling dates: 8 and 16 weeks post-treatment (P<0.0001) (Fig 6.4).

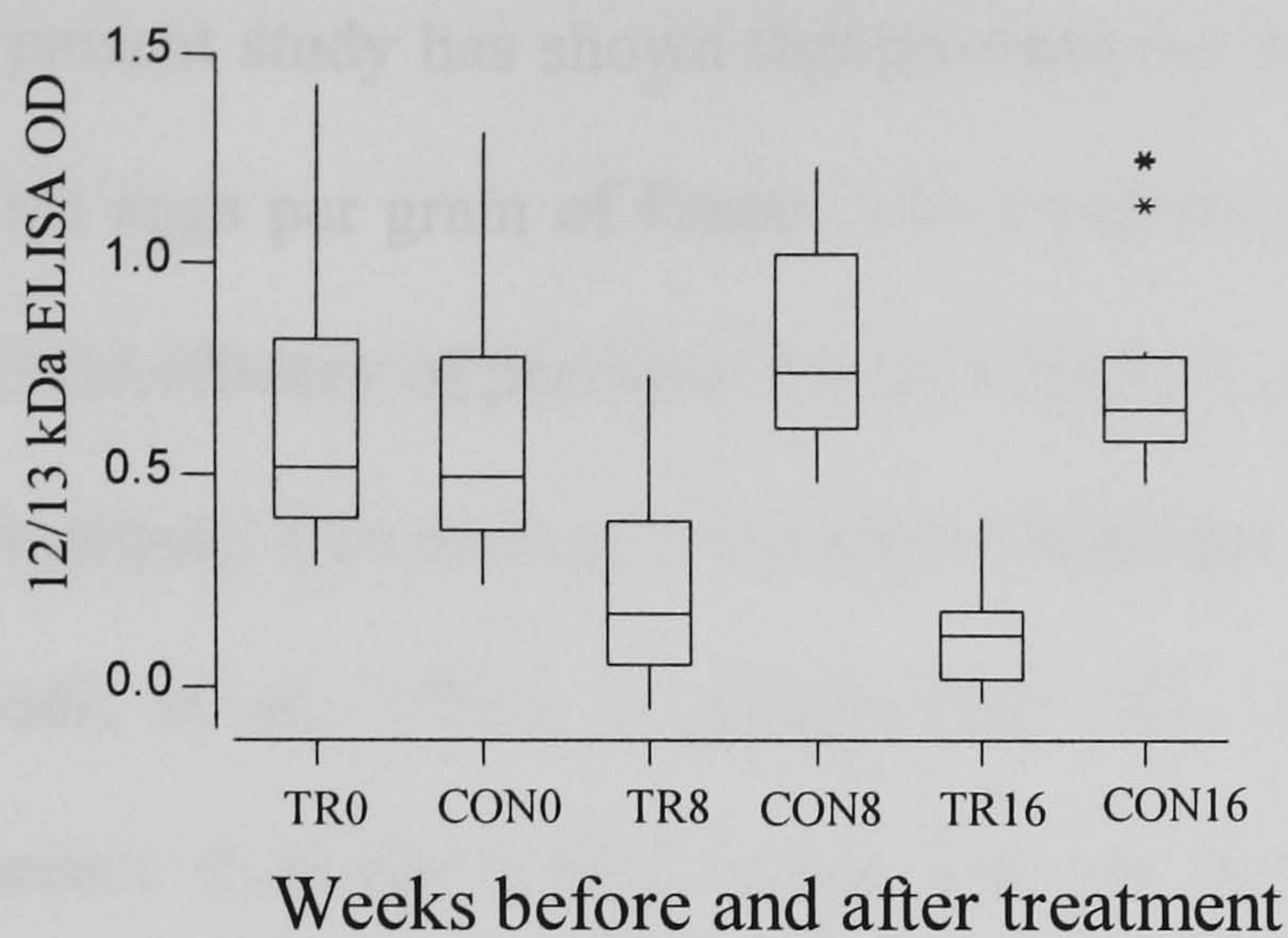


Fig. 6.3. Box and whiskers plot of the median serum antibody levels of *A. perfoliata* infected donkeys before and 8 and 16 weeks after treatment with praziquantel. TR0 and CON0 treatment and control groups before treatment; TR8 and CON8 treatment and control groups after 8 weeks treatment; TR16 and CON16 treatment and control groups after 16 weeks of treatment, respectively.

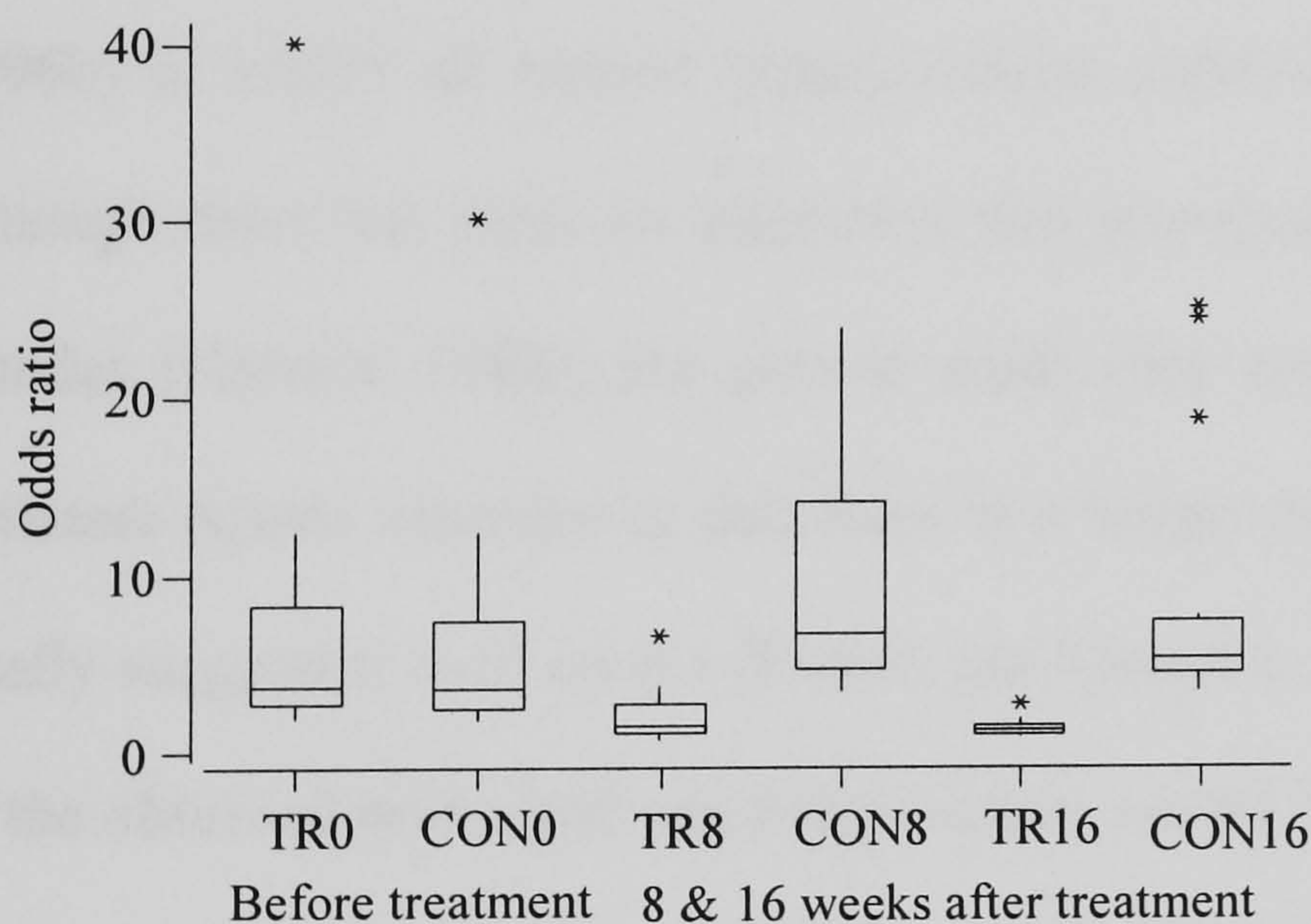


Fig. 6.4. The predicted OR for the risk of spasmodic colic before, and 8 and 16 weeks post-treatment in treated and control groups. TR0 and CON0 treatment and control groups before treatment; TR8 and CON8 treatment and control groups after 8 weeks treatment; TR16 and CON16 treatment and control groups after 16 weeks of treatment, respectively (Odds ratio = $e^{2.756 \cdot \text{ELISA OD}}$).

6.4 Discussion

The present study has shown that praziquantel had high efficacy in reducing the number of cestode eggs per gram of faeces. The treatment suppressed faecal egg counts for 16 weeks. The field efficacy of praziquantel, as outlined in the World Association for the Advancement of Veterinary Parasitology (WAAVP) guidelines for evaluating the efficacy of anthelmintics (Woods, et al., 1995), is greater than 90% until day 112 with statistically significant difference from the control group. The one donkey positive after two weeks of treatment may be attributed to poor drug administration (spitting of the drug) or cross contamination while processing the faecal samples. The other possible explanation is that the killing of tapeworm by praziquantel might have been prolonged followed by delayed release of eggs from cestode segments. There was no egg reappearance after 16 weeks of treatment in all donkeys treated with praziquantel. A similar result was reported by Proudman and Trees (1996b) in which all treated horses remain coprologically negative for up to 136 days. Although there has been an indication that praziquantel is not effective against immature cestodes (Harnett, 1988), the present study may indicate the activity of the drug against immature equine cestodes or that there is a longer prepatent period of the worms than that usually suggested: 6-10 weeks (French and Chapman, 1992). The other possible explanation for the observed prolonged egg reappearance period is that the treatment trial was conducted during the dry season, during which time the environmental conditions are not favourable for the survival and development of cestode eggs and the oribatid mites, and hence there was a low probability of reinfection.

In the control group there were individuals, which were positive at one sampling date and negative at others. These results were not unexpected due to the low sensitivity of the centrifugation/flotation techniques (Proudman and Edwards, 1992) and its low repeatability. At each of the sampling date from week 2 through to week 16 nine to thirteen (56-81%)

donkeys in the control group had cestode egg counts, substantiating continuous tapeworm infection in this group. This can be compared to the treatment group where no cestode eggs were recovered within this treatment period. To overcome the low sensitivity of the flotation technique in recovering cestode eggs, a critical test, which depends on the recovery of worms after treatment at necropsy, is recommended (Duncan, et. al., 2002).

The immunological assay showed a marked decrease in the serum antibody levels in the treated group. The observed variation in the decline of antibody levels among individual donkeys may be attributed to their natural antibody level and infection intensity before treatment. Donkeys with high ODs take longer time for their OD to come down below the negative cut-off point (0.2). The continuous decline in antibody level may indicate that either praziquantel is effective against the immature forms of equine cestodes or that the immature forms are not affected by praziquantel, as suggested by Harnett (1988) but do not trigger antibody production. This supports a hypothesis that cestodes exhibit a long prepatent period for the immature form to develop to adults before stimulating antibody production. However, whether only adults are responsible for the antibody production is not yet known.

The persistently high ELISA ODs obtained in two of the donkeys 16 weeks after treatment is consistent with the results of Barret et al. (2004) in which some horses with high OD values before treatment still had values above 0.6 by 12 weeks post treatment, but continued to decrease thereafter. Barrett, et al (2004) attributed this high antibody response to the age of horses in which peak worm burden occurs in young and old animals (Proudman et al., 1997). This may not hold true in donkeys as the present study showed no such association (Chapter 5) although the two donkeys with high ODs were aged (14 and 20 years). One possible explanation is that they might have had naturally high antibody levels. Another possibility is that they may have been reinfected, triggering a subsequent immune response.

In the present study the decline in antibody level seems relatively rapid in that 91% of the donkeys had ELISA OD less than 0.2 by 16 weeks post treatment, as compared to the study made by Barrett et al. (2004) in horses. This may indicate inherent differences between donkeys and horses or could be attributed to the differences in the level of antibody production against the antigen of *A. perfoliata*. Alternatively there may be strain differences in *A. perfoliata* infecting donkeys and horses, or possibly differences in sensitivity of the ELISA to horse and donkey IgG(T).

Antibody level is highly correlated with *A. perfoliata* infection intensity (Proudman and Trees, 1996b). In addition, as ELISA OD increases there is an increased risk for horses to develop colic (Proudman and Edwards, 1993; Proudman, et al., 1998 ; Proudman and Trees, 1999). The significantly reduced antibody level in the current study after praziquantel therapy clearly indicated a reduced risk of tapeworm-associated colic or any other tapeworm-associated gastrointestinal disorders or diseases in donkeys. The predicted mean odds ratio of 6.8 pre-treatment in the treatment group and 6.3 to 9.8 in the control group implies donkeys were approximately 6 to 10 times more likely to suffer from spasmodic colic than uninfected donkeys. After 16 weeks post-treatment the predicted mean odds ratio of the treatment group had decreased to 1.4 indicating that they were approximately 5 to 7 times less likely to suffer from colic than they had been before treatment or than the control groups, respectively.

Praziquantel (Droncit, Injectable, Miles Inc.) at the dose rates of 1, 1.5, and 2 mg/kg (Lyons, 1992; Slocombe, 1997) was 98-100% effective in removing *A. perfoliata* in horses. Proudman et al (1995) indicated the activity of praziquantel (Droncit, Injectable) at a dose rate of 1 mg/kg in horses against *Anoplocephaloides mamillana*. Even though the present study has shown high efficacy of praziquantel as a single product against cestode infection in donkeys, specific combinations of ivermectin and praziquantel (Coles et al., 2003; Rehbein

et al., 2003; Barrett et al., 2004; Marley et al., 2004) and praziquantel and moxidectin (Grubbs et al., 2003) were found to be highly effective against cestodes, reducing infection by more than 99% and provided a significant reduction in nematode infections of treated horses. It is possible that the use of ivermectin or moxidectin alone may allow tapeworms to flourish by removing nematodes, and the use of these combined products is useful for the simultaneous removal of a range of helminth parasites instead of using them separately.

In Ethiopia polyparasitism is a common problem (Feseha, 1997; Getachew, 1999). The treatment of equids with a combination of ivermectin and praziquantel is a simple and effective way to control both nematode and cestode infections and is highly recommended. This is a rational option when diagnostic tests have shown that both types of parasites are present. This is particularly the case in the highlands of Ethiopia where nematodes and cestodes are both endemic and cause major problems.

CHAPTER SEVEN

MATHEMATICAL APPROACH IN MODELLING SEASONAL VARIATION OF STRONGYLE FAECAL EGG COUNTS UNDER TROPICAL WEATHER CONDITION IN DONKEYS, ETHIOPIA.

7.1. Introduction

Gastrointestinal parasites, particularly strongyles, are one of the major health problems of equids worldwide, causing intestinal disease. It has been increasingly recognized that small strongyles, cyathostomins, have become the principal parasitic pathogens of grazing equids causing cyathostomiasis and colic (Herd, 1990a; Paul, 1998, Love, 1995; Love et al., 1999). Infections with these nematodes typically involve very large populations and numerous species. More than 52 different species have been identified in equids (Lichtenfels et al., 1998). Similar species of cyathostomins to those found in horses have been described in donkeys by many workers (Matthee et al., 2000; Matthee et al., 2002; Burgu et al., 1995; Tolliver et al. 1985; Getachew 1999; Eysker and Pandey, 1989). However, at least 10 species have been reported only from donkeys and zebras (Lichtenfels et al., 1998).

Cyathostomin eggs are by far the most plentiful nematode eggs found in the faeces of horses, often comprising over 95% of the strongyle ova in horse faecal samples (Reinemeyer, 1986; Uhlinger, 1991). Mfitilodze and Hutchison (1988) have also shown that more than 75% of the recovered larvae from the deposited faecal pellet on pasture were cyathostomins under tropical environmental conditions. Similar studies made in donkeys by Wells et al. (1998) and Getachew (1999) have revealed that cyathostomins make up over 92% of the strongyle ova in donkey faecal samples. It is therefore reasonable to assume that a high proportion of the equine faecal egg count and larvae on pasture are cyathostomins.

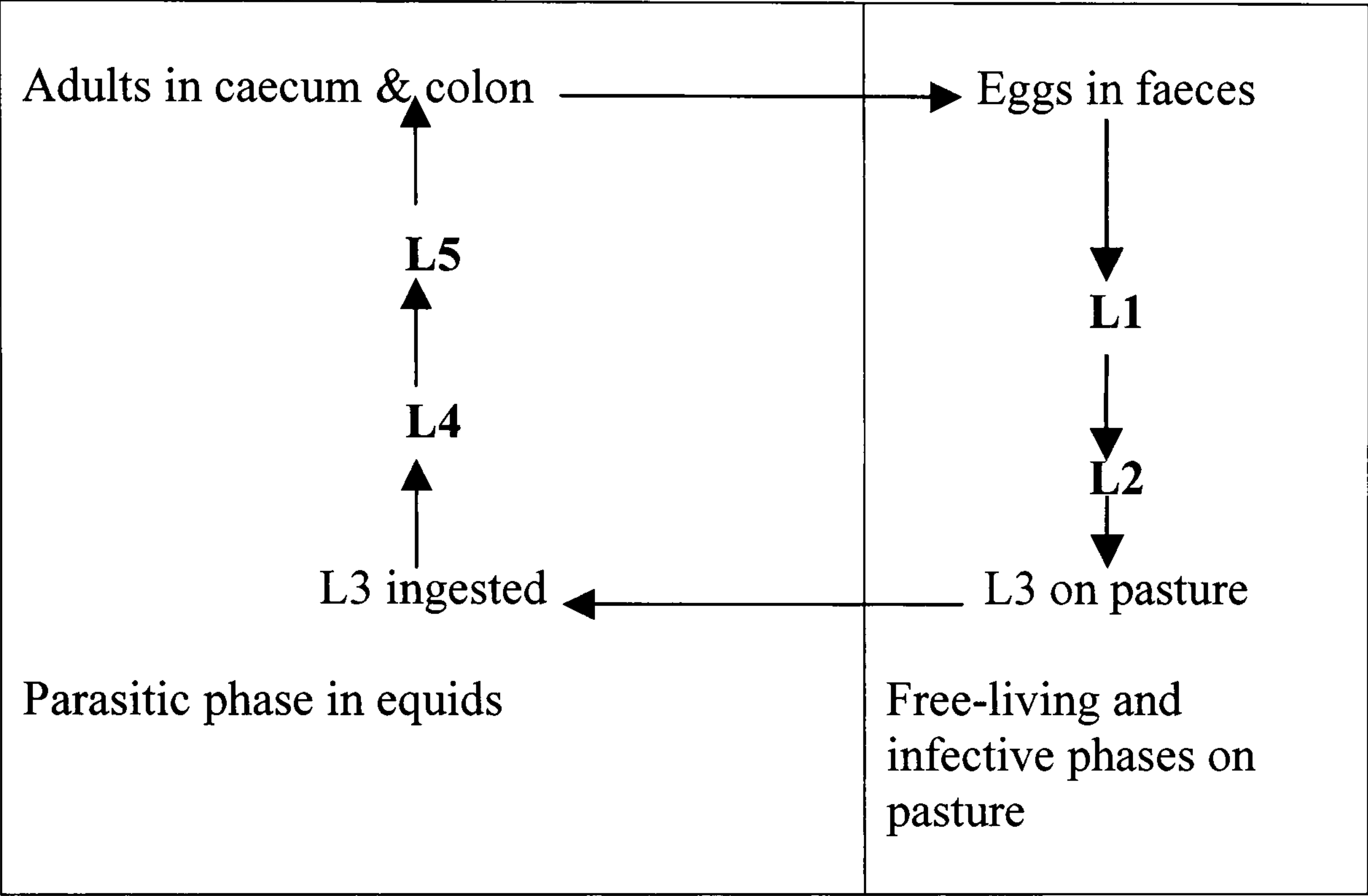
Despite widespread use of epidemiological or mathematical modelling to study the different aspects of human and domestic ruminant diseases, their application in the study of equine health problems in general and parasites, in particular, is not well developed. Mathematical modelling of the population biology of *Ostertagia ostertagi* is one of the well-studied epidemiological models of veterinary parasitology (Gettinby et al., 1979; Grenfell, et. al., 1987; Paton, et al., 1984; Smith and Guerrero, 1993). Prediction of the prevalences of various parasitic diseases of ruminants using biological dynamics and indices of climate has been successfully demonstrated by many workers (Ollerenshaw and Rowlands, 1959; Gettinby et al., 1974; Thomas, 1978; Paton, et al, 1984; Paton, 1987). These models enabled to plan different control strategies and ways to prevent anthelmintic resistance.

The population biology and dynamics of cyathostomins is not fully investigated and no attempts have yet been made to model any aspects of these equine parasites. Multiple logistic regression modelling to assess the risk factors associated with the diagnosis of cyathostomiasis in horses, comprises the only quantitative epidemiological work, on cyathostomins infection (Reid et al., 1995). The current work was therefore undertaken to predict peak seasonal fluctuation of strongyle faecal worm egg counts and infective larval availability on pasture under the mid-lowland Ethiopian tropical weather condition using a mathematical modelling approach. In addition the effect of timing and frequency of anthelmintic control strategies on the parasitic worm burden was simulated. The model was based on a mathematical representation of the dynamics of the various developmental stages of the parasite life cycle. Particular attention was given to the influence of rainfall on larval development, dispersion to pasture and larval survival.

7.1.1. Basic life cycle of cyathostomin

Adult worms live in the colon and caecum of equids and have a non-migratory life cycle. The adult females produce eggs, which are transmitted via the faeces onto pasture (Fig. 7.1).

The parasites hatch from eggs and pass through first (L1) and second (L2) non-infective larval stages to reach the infective third (L3) stage (Soulsby, 1982; Paul, 1998). Equids are infected with infective L3 while grazing. After ingestion, the infective larvae undergo exsheathment in the small intestine and migrate to the caecum and colon for the rest of their development.



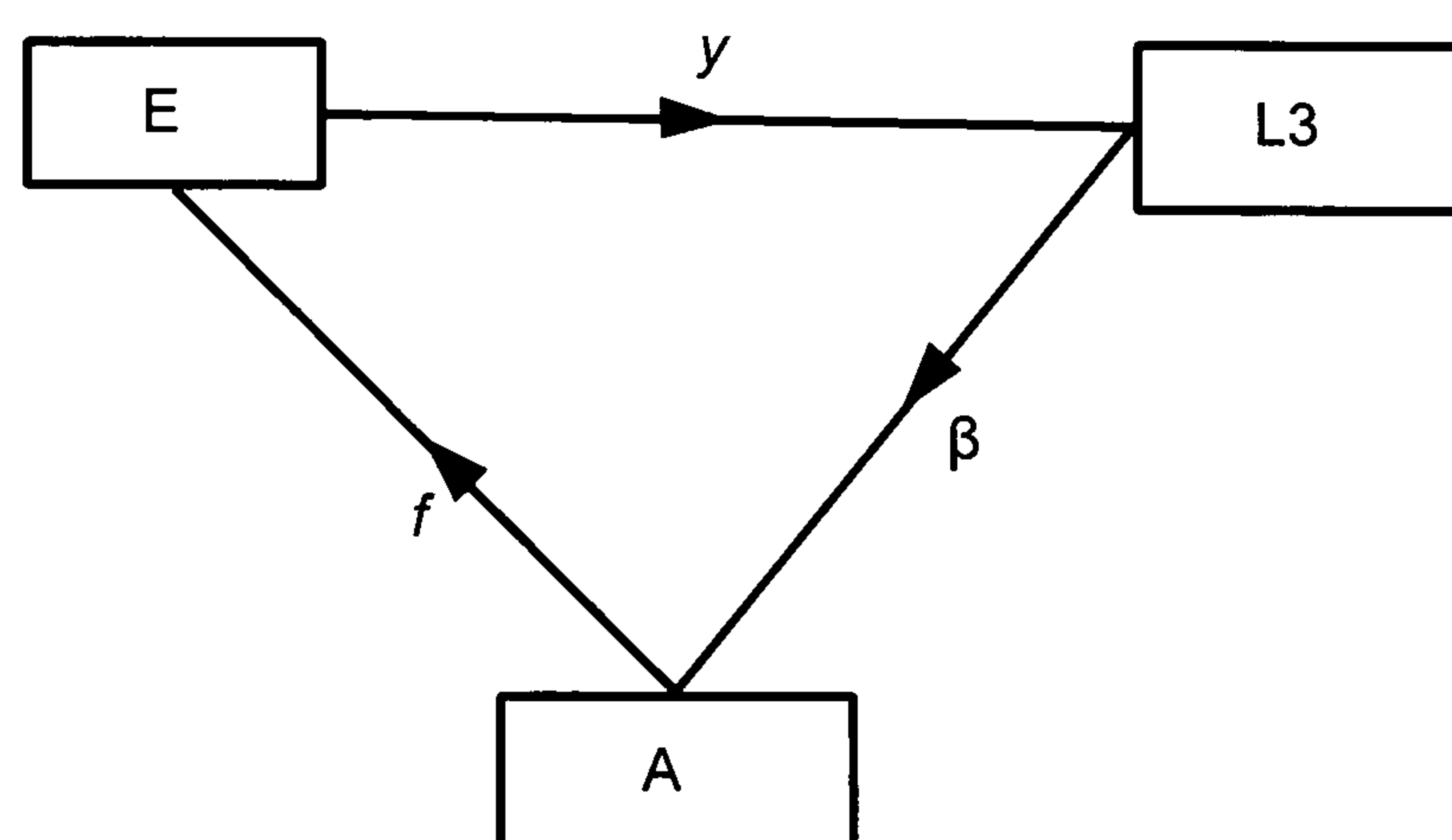
F.g.7.1. The life cycle of cyathostomins in equids. Arrested larval development is not considered here.

Source: Adapted from Paul (1998).

Parasitic third stage larvae penetrate the mucosa and, in some species, the submucosa. Here, larvae become encysted by host fibroblasts and moult to fourth stage larvae (L4). L4 emerge from the tissue cysts and resume development to the fifth stage (L5) in the lumen of the large intestine and then into egg-laying adults completing the cycle (Soulsby, 1982; Paul, 1998). Some larvae undergo arrested development (hypobiosis) at the early third stage (EL3) but this was not considered in our model construction, because it is not thought to occur in donkeys (Eysker, 1987).

7.1.2. Simple description of the model.

A first approach to modelling is to produce a visual representation of the system. One useful method of visualising a modelling problem is to produce a network diagram. A network diagram consists of blocks connected by lines (Fig.7.2). The lines represent the flow of individuals between the blocks. The blocks represent the states in which an individual can exist. Such diagrams are useful, not only because they represent a concise method of defining a model of our understanding of the system under investigation, but also because they lend themselves to a number of analytical techniques (Innocent, 1998).



F.g.7.2. A network representation of a simplified lifecycle of cyathostomins. E, number of eggs, L3, number of infective larvae, and A is number of adult parasites. y , β and f are larval yield, ingestion rate and rate of egg production, respectively.

If a typical lifecycle of the cyathostomins is considered it can be interpreted as a simplified network as in Fig. 7.2. For simplicity we choose three developmental stages of the cyathostomin lifecycle (E, L3, and A) for model construction. These are state variables of the model, because they are the variables, whose values change through the model run and describe the state of the model at any point in time. y , β and f are parameters of the model because they represent constant values.

We can follow the flow through the diagram, starting from E, eggs on pasture. The eggs are produced by adult female parasites at a rate of f eggs per female per day. Therefore, the total number of eggs produced per day will be fA . These will then develop to infective larvae (L3) at the rate of y , making the total number of L3 produced per day yfA . Assuming no mortality of L3 on pasture and they are ingested with an ingestion rate of β , and all the ingested L3 develop to adult, the total number of adult produced per day becomes $\beta L3$. These flows of one developmental stage to the other will continue and is cyclical. But this is not always the case and there are factors, which determine the rate of development, survival time and abundance of each developmental stage. These factors are considered in the detailed model description in section 7.5.

7.2 Parameters, their definition and functional forms used in the model

A number of the aspects of the population dynamics of equine cyathostomins are not known, particularly in donkeys, but need to be accounted for by the model. The data used in parameter estimation were from field studies in tropical weather conditions in horses. The functions and complete sets of parameters of the model, which define the development and mortality rate of the free-living and parasitic stages of cyathostomins, are described in Table 7.1. Most of the model parameters are dependent upon the biology of the parasites and the environmental conditions. These are briefly explained in the subsequent subsections, and detailed explanations are found in the literature review section of Chapter One.

Table 7.1 Model parameters and functional forms (all rates per capita instantaneous daily rates).

Parameters	Estimated values	function	Definition*
y	0.24	constant	Probability that an egg develops to infective larvae (% larval yield).
τ_1	7	constant	Time (days)for maturation of eggs to infective larvae (L3).
μ_1	-	$R/S_w+1-R/(S_d)$	Mortality rate of infective larvae on pasture (see subsection 7.2.3).
β	0.002	constant	Rate at which infective larvae are ingested by a single host (see subsection 7.2.4).
s	-	$\begin{cases} 1 & A < p \\ p/(A+1) & A \geq p \end{cases}$	Rate that an ingested infective larvae develop into adult (see subsection 7.2.5).
p	5000	constant	estimated threshold worm burden up to which all infective larvae able to develop into adults (see subsection 7.2.5).
τ_2	60	constant	Time (days) for maturation of ingested larvae (L3) to Adult
τ_3	180	constant	Life span of adult cyathostomins in the gut (days).
f	12	constant	Initial rate at which adult female cyathostomin produce eggs per day.
S_w	84	constant	Maximum survival days of infective larvae on pasture during wet season.
S_d	5	constant	Maximum survival days of infective larvae during dry season.
R	0	dry season	a function used to model mortality rate of L3 (see subsection 7.2.2).
	1	wet season	

*The value and functional forms of each parameter and their justifications are described in subsections 7.2.1 to 7.2.8

7.2.1 Development of eggs to infective larvae (L3)

There is always sufficient thermal energy from the available temperature throughout the year and temperature is never below the minimum requirement, 4°C (Ogbourne, 1972) for eggs to develop to infective larvae in tropical weather condition (Hutchinson, et al., 1989). Because of this, larval development was assumed to depend on moisture/rainfall unlike in temperate regions where temperature is the main factor (Ogbourne, 1972).

Studies by Mfitilodze and Hutchinson (1988), Onyali et al. (1990) and Waruiru et al (1998) have shown most strongyle eggs completed their development to L3 seven days following the deposition of dung on pasture during the wet warm season of the tropics, whereas high temperature and low humidity during the dry hot season bring about rapid faecal desiccation resulting in high mortality of pre-infective larvae. Therefore, one week was taken as an estimate to model the maturation time of eggs to L3 (τ_1)

Data on absolute measurement of survival rate of eggs and the free-living stages were not available. However, studies have shown that on average 24% of deposited eggs developed to the infective stage during favourable wet, warm tropical weather conditions (Mfitilodze and Hutchinson, 1988; Hutchinson, et al., 1989). This was taken as an initial estimate of larval yield (y) or the probability that an egg develops to L3 in the model.

7.2.2 Rainfall as a determinant factor for larval translation to pasture

Development of L3 is only partially indicative of infective potential. There is always development of eggs to L3, although at different rates during different times of the year. It is the number of infective larvae that are able to translate to the herbage, and the length of time they remain viable on pasture that is of greater epidemiological importance. These were considered to be the important considerations for the model development.

Rainfall is assumed to be necessary before newly-formed infective larvae are available for consumption. This condition is justified on two accounts: firstly, rainfall is frequently followed closely by the appearance of a large number of infective larvae on pasture. Secondly, migration from faecal masses into a consumable position on the sward is necessary, and this will require the presence of a film of moisture (English, 1979b). Many studies have shown that larval translation to pasture and pasture larval recovery vary greatly and is generally limited to the wet season, and little or no larval translation onto pasture were observed during most of the dry hot seasons of the tropics (English, 1979b; Hutchinson, et al., 1989; Mfitilodze and Hutchinson, 1988; Onyali, et al., 1990; Waruiru, et al., 1998, 2002). Absence of rainfall coupled with scarcity of herbage during the dry season makes even the few larvae that survive unavailable for the animals to ingest. Similar weather conditions to those studied prevail in mid to lowland regions of Ethiopia. Generally, animals have a high probability of acquiring infection from pasture during, and immediately following wet seasons during which high faecal egg counts were consistently observed in donkeys (Feseha et al., 1991; Feseha, 1998; Getachew, 1999).

Rainfall alone is not sufficient to fully describe the ability of the parasite to contaminate the environment. It is also necessary to consider the amount of herbage cover. The greater herbage density the greater the capacity of the surface layer of the pasture to retain moisture over a long period of time, enhancing the survival of larvae and their translation to pasture. According to this herbage rainfall relationship, and based on observations made and data on rainfall and herbage coverage from the MOA (2004) in the study regions, the following criteria were developed to model the translation and availability of infective larvae on pasture:

Monthly rainfall of 70-76 mm for 5 to 15 days per month with minimum herbage coverage, and 110-325.4 mm for 15-27 days per month with sufficient available herbage coverage onto

which larvae can translate, characterise the small (March and April) and main rainy seasons (June to September), respectively, of the mid-lowland regions of Ethiopia. These are suitable seasons for the development and survival of the free-living and infective stages of the parasites and for larval translation to pasture. Wet seasons (March and April, and June to September) were assigned a value of 1 for rainfall (R) in the function used to model the availability and mortality rate of L3 on pasture as defined and described in Table 7.2 and subsection 7.2.3.

On the other hand, the dry season (October to February and the month of May) of the mid-lowland regions is characterised by dry hot weather with usually 0 mm monthly rainfall. Occasionally there may be an unexpected rainfall but this is usually only for short periods of time, rarely more than a day or two. Although there might be a rapid larval development during this time of the year, larval desiccation precedes development in most cases due to the high temperature and low humidity. Even the developed infective larvae may die, either because there is no rainfall, thus larvae cannot translate to the meagerly available herbage, e.g. at the end of rainy season, or herbage coverage is so scarce and their mortality rate is quite high even when rainfall occurs. Therefore, the dry season was assigned a 0 value for the rainfall (R) in the function used to model larval availability and their mortality rate as defined and described in Table 7.2 and subsection 7.2.3. The values of R were set using a lookup table of the ModelMaker controlled by the variable 'Months' as shown in Table 7.2 and Fig.7.3.

Larval migration from faecal masses to herbage is likely to occur in waves coincident with falls of rain (English, 1979b; Hutchinson, et al., 1989) and is difficult to measure. Studies have shown that as many as 79% of eggs deposited can reach the infective stage (Hutchinson, et al., 1989) but all may not disperse successfully on to available pasture. In the

present model we assume that 75% of the developed L3 are able to disperse to herbage during the wet season and none during the dry season.

Table 7.2. Rainfall data and herbage coverage for the study year (1996) and its interpretation in the model.

Months	Number of rainy days	Monthly rainfall (mm)	Herbage coverage	<i>R</i>
January	2	16.85	none*	0
February	1	7.52	none*	0
March	5	76.38	low**	1
April	13	77.62	low*	1
May	3	50.95	none*	0
June	18	149.92	low**	1
July	26	249.35	high	1
August	27	258.08	high	1
September	21	116.95	high	1
October	2	47.80	low**	0
November	1	8.57	none*	0
December	1	2.78	none*	0

Source: Adapted from MOA (2004). * Animals depend on crop residues and straws. ** Short rainy seasons and beginning and end of long rainy season during which herbage coverage is very low.

7.2.3 Survival and mortality rate of L3 on pasture

Climate strongly influences the free-living and parasitic stages. Their development and survival is affected by both temperature and humidity. Under wet and warm tropical conditions large numbers of infective larvae (L3) survive for up to 12 weeks (84 days) but do not survive under hot and dry conditions (Hutchinson et al., 1989; Onyali, et al., 1990; Waruiru et al., 1998). We arbitrarily use a survival period of 5 days in our model during the dry season to account for possible rainfall that may allow survival and 84 days for the wet season.

The mortality rate of L3 (μ_1) was modelled as a function of rainfall and their survival on pasture during different time of the year as follows:

$$\mu_1 = R/S_w + (1-R)/S_d. \quad 7.1$$

where μ_1 is mortality rate, R represents rainfall and takes a value of 0 during the dry and 1 during wet season as described in subsection 7.2.2 and Table 7.2. S_w and S_d are the survival times of L3 on pasture during the wet and the dry season, respectively (Table 7.1). Substituting the value of R depending on the season, $\mu_1 = 1/S_w = 0.012$ during the wet season and $\mu_1 = 1/S_d = 0.2$ during the dry season. The model assumes that all the translated larvae have an equal chance of survival.

7.2.4 Larval ingestion rate (β)

Larval distribution on pasture is not random, but follows the distribution of faecal masses (Crofton, 1954). The concentration of the infective larvae close to the faecal masses is high and there appears to be little migration beyond 30 cm (English, 1979b; Herd and Willardson, 1985; Hutchinson et al., 1989). Although horses (Taylor, 1954) and donkeys (author's observation) acquire an abhorrence of faeces, it is only transient. This is particularly true in Ethiopia where there is a scarcity of grass, and as the grazing season progresses animals are forced to graze the roughs, the portion on which there is a high concentration of infective larvae. Moreover, donkeys, with other animals, spent their entire daytime on small areas of pasture during the wet season, which increases both the density of infective larvae and the chance of exposure to many larvae.

Data on the rate of larval intake are very difficult to obtain. The ingestion rate is usually estimated based on daily grazing rate by an animal and the available herbage density (Kao, et al. (2000). It was estimated that an adult donkey on average consumes 2% of its body weight per day in dry matter (DM) (Pearson, 2005). An adult Ethiopian donkey weighs, on average

approximately 120 kg (Pearson, 2001). Therefore on average a donkey consumes 2.4 kg DM/day. Since there is no pasture management and animals graze following the immediate growth of the grass, it is very difficult to estimate the carrying capacity of the land and to estimate the available herbage per unit area. Herbage density in the mid-lowland regions is relatively poor but covers most of the grazing land during the mid wet grazing summer season and its availability can roughly be estimated at 1000 kg/km². Assuming a random distribution of larvae on pasture the rate of ingestion of infective larvae (β) by a donkey was estimated as daily grazing rate/available herbage, which is approximately equal to 0.002 (see Chapter One section 1.6.4 for detail). During the dry season the ingestion rate was assumed to be zero as survival of infective larvae is negligible and animals mostly depend on crop residues and straws, and availability of grass is very scarce posing little or no danger of acquiring infection.

7.2.5 Establishment rate of the ingested infective larvae (s)

It is not known how the immune response of donkeys is affected during parasitic infection. The high worm burden and high levels of faecal egg counts frequently reported (Eysker and Pandey, 1989; Getachew, 1999, Wells et al., 1998; Mathee, et al., 2000) might be associated with their level of stress due to overworking, other diseases and malnourishment, and may suggest that they might be immuno-compromised or may have a high threshold capacity to withstand such high worm burden compared to horses. Studies by Hong, et al. (1987) and Seaton et al. (1989) suggested that incoming infective larvae of *T. circumcincta* develop and establish to replace the existing adult population. Gopal, et al (1999) and Adams (1982) have shown the establishment of 23% and 21% of infective larvae of *T. circumcincta* and *H. contortus*, respectively, in previously exposed lambs. In an experiment to determine the larval uptake of *T. colubriformis* in sheep, Dobson, et al. (1990b) found that highest establishment of infective larvae occurred in sheep with worm burdens less than 400,

remained constant in sheep with worm burdens 400-3500, and the lowest establishment occurred in animals with over 3500 worms after 42 days of larval intake.

Such data are not available in equids; however, there are indications that adult horses might develop immunity against cyathostomins (Klei and Chapman, 1999). A Study by Love and Duncan (1992) in ponies showed that previous exposure to cyathostomins resulted in a reduction in the size of the acquired worm burden. Assuming that a similar establishment mechanism might work in donkeys with cyathostomins, a function that determines/regulates the number of ingested larvae, which develop into adults was fitted to the model. Considering the large number of adult cyathostomins usually present in donkeys (Eysker and Pandey 1989; Getachew, 1999; Mathee, et al., 2000), an estimated threshold value of 5000 worms was used up to which value ingested infective larvae could establish. Thereafter there was a decline in the proportion able to develop into adults. The establishment rate was modelled as follows:

$$s = \begin{cases} 1 & A < p \\ p/(A+1) & A \geq p \end{cases} \quad 7.2$$

Where s is establishment rate, A is total number of worm burden at any time interval, p is the threshold worm burden up to which all larvae ingested are able to develop into adults.

7.2.6 Development time of L3 to egg laying adults (τ_2)

The development of parasites from the infective larval stage to egg laying adults within the host animal was assumed to proceed uninterrupted. Cyathostomins are known to undergo inhibited development in horses (Eysker et al., 1990; Love, 1992; Paul, 1998) but this was not taken into account during model construction since there is no evidence that this occurs in donkeys (Eysker, 1987). No work has been undertaken to determine the prepatent period

in donkeys. Assuming that a development rate similar to that in horses and ponies may prevail in donkeys, an average of nine weeks (Love and Duncan, 1992) was used as an initial estimate for the development period to patency.

7.2.7 Survival time of adult cyathostomins (τ_3)

Death of adult parasites in the alimentary canal of animals is due either to senescence or to some protective activity on the part of the host (Poynter, 1954). It is difficult to separate the acquired immune-mediated death rate from the death rate in the absence of immunity. There is no evidence for how long adult cyathostomins can live in equids. According to a study by Reinemeyer (1986), gravid females were most common from late spring to late summer and he suggested that spent females died and were replaced by the succeeding generation of immature worms in the following spring. Thus indicating that the longevity of adult cyathostomins may be several months. Similar studies by Eysker and Pandey (1989) and Eysker (1987) in Zimbabwe have shown that cyathostomins in donkeys survive the long unfavourable dry winter condition mainly as adults and not as inhibited larvae. This also suggests that the survival time of adult cyathostomins will be at least several months. An initial estimate of 6 months was used in the model to model the life span of adult cyathostomins (τ_3) in donkeys.

7.2.8 Fecundity (f)

Paton et. al. (1984) found that lambs, which experienced early larval challenge from contaminated pasture, had a heightened immune response and consequently were infected with worms of lower fecundity. In a study of natural infection in which *T. circumcincta* predominated, egg counts were found to increase linearly with worm burden early in the year, but were reduced under higher worm burden later in the year (Boag and Thomas, 1977). Density-dependent fecundity and fecundity associated with worm length were also

reported by Stear, et al. (1995), Stear and Bishop (1999) and Bishop and Stear (2000) in sheep infected with *T. circumcincta*.

In contrast, Coyne, et al. (1991) has shown no evidence for density-dependent regulation of parasite fecundity in *H. contortus*, *Nematodirus* species and *Oesophagostomum venulosum*. Although the effects of immune response on some parasites of domestic ruminants are well established, it seems that there is a variation among different species of parasites. The absence of strong density-dependent constraints of fecundity in some parasites may indicate that the natural regulation of these parasites is through density-dependent constraint on parasite survival, which could be directed at the newly ingested larvae, or adult parasites already in the gastrointestinal tract or both (Smith, 1988).

No study has been reported on the fecundity of equine cyathostomins and whether it is density dependent or not is yet to be determined. Miller (1953) indicated that the lower rate of egg production is characteristic of cyathostomins and suggested that female cyathostomin can lay eggs at rates varying from 10 to 12 eggs per female per day.

We assumed density independence in modelling fecundity of cyathostomins in donkeys and estimated from the available data as follows: The daily faecal out put of an adult donkey is approximately 3 kgs. Studies by Feseha (1998) and Getachew (1999) have shown that the mean faecal egg count of a donkey in Ethiopia is 1200 epg. Therefore, a donkey with a daily faecal output of 3000 g and a mean epg value of 1200 will seed the pasture with approximately 3,600,000 eggs per day. Studies have shown that cyathostomin ova in donkeys comprise approximately 92% of the whole strongyle ova (Wells, et al., 1998, Getachew, 1999). Therefore 92% of the produced eggs, 3,312,000, were assumed to be those of cyathostomins. From the studies by Eysker (1987), Eysker and Pandey (1989), Getachew

(1999) and Matthee, et al. (2000) an average worm burden of 48,211 per donkey was taken as an estimate. Assuming a 1:1 sex ratio, the fecundity (f) becomes 137 eggs/female per day. This value and the above estimate by Miller (1953) were used in modelling the fecundity of cyathostomins in the present model. In the final model development, however, f was assigned a value of 12 estimated by Miller (1953) (Table 7.1).

7.3. Materials and methods

7.3.1 Meteorological data

Meteorological data for the study period were obtained from the regional Ministry of Agriculture (MOA. 2004). Weather patterns markedly alter pasture larval contamination and it has been shown that there is a high correlation between climate and the risk of parasitic diseases (Ollerenshaw, et al., 1978). The current model involved climate data, particularly rainfall, to predict seasonal variation in contamination of pasture by infective larvae, which develops from the eggs contained within faeces.

7.3.2 Faecal worm egg count

Monthly faecal worm egg count data for the year 1996, corresponding to the meteorological data, was obtained from the study made by Getachew (1999) at the Donkey Health and Welfare Project, Addis Ababa University, Faculty of Veterinary Medicine, Ethiopia. Faecal samples collected and analysed for egg recovery from 1498 donkeys of mid-lowland regions, Ada and Akaki, were used in either: validating the model or: to tune parameter values so that the output of the model followed observed results in the absence of control.

7.4. Detailed model description

The simple network diagram produced in Fig. 7.2 represents the flow of the numbers of each developmental stage between the state variables of the model. But the model does not take in

to account the different factors that influence the flow of individuals between the different developmental stages. In order to mimic the natural lifecycle of the parasites, factors that determine the development and abundance of parasites in each state need to be incorporated into the model. Time taken to develop from one stage to the next is required to model the abundance of individuals in each stage of the lifecycle at any time. The effect of climatic conditions on the availability and on the survival of infective larvae on pasture, and the effect of previous exposure or worm burden of the animals on the establishment of the incoming infective larvae are also factors, which need to be taken into account. When the relevant above factors and parameters of importance are included in the simplified network diagram in Fig. 7.2, a new conceptual model for the dynamics of cyathostomins in equids has been produced as shown in Fig. 7.3 using Modelmaker format (see Appendix VII).

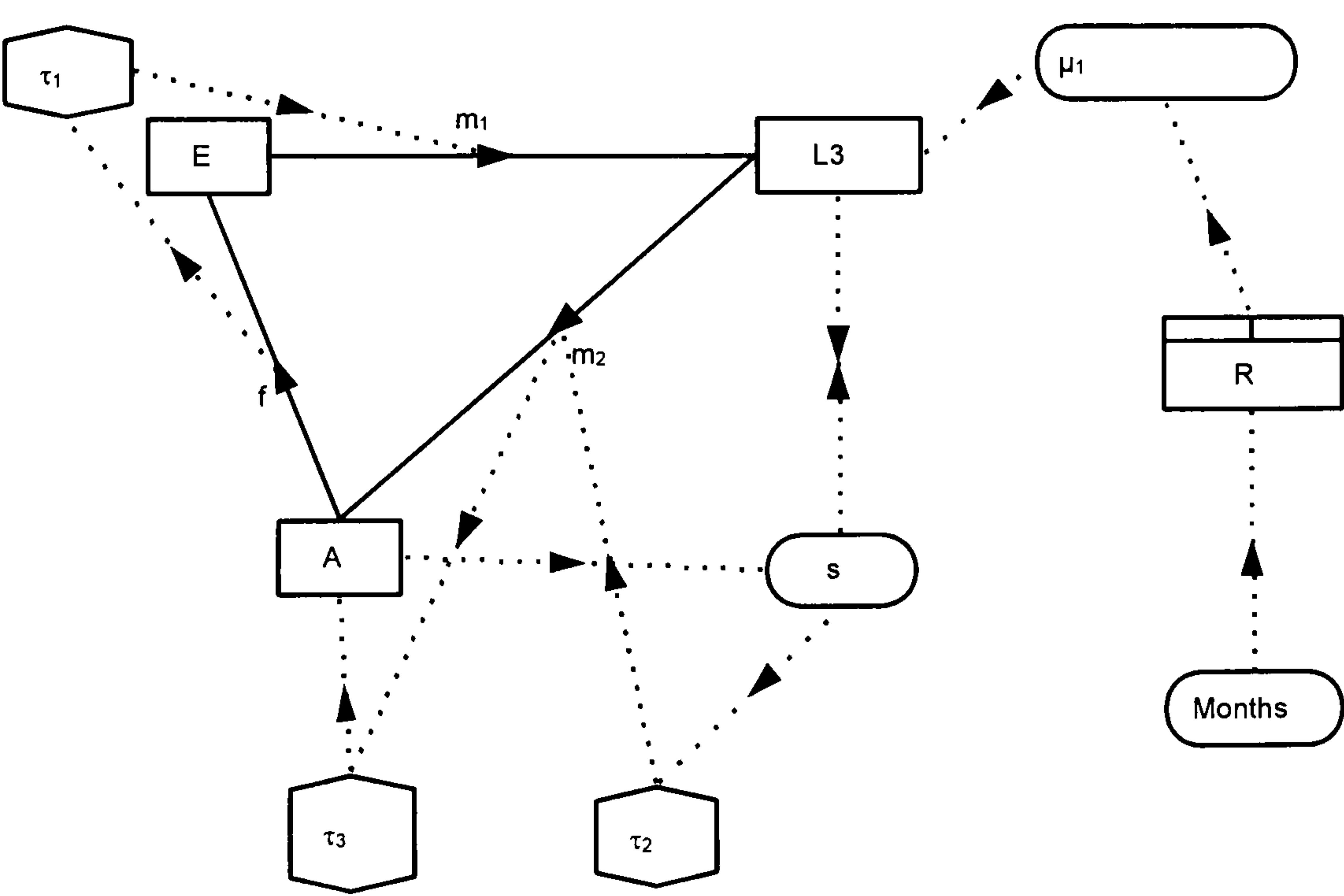


Fig.7.3. Network representation of the basic architecture of the simulation model constructed in ModelMaker.

As described in a simplified model, Fig. 7.2, the state variables, E, L3 and A are indicated in rectangular boxes and are defined in Table 7.3. f , s , μ_1 and R are all parameters as defined and described in Table 7.1 and subsections 7.2.1 to 7.2.8. m_1 and m_2 show flows of developments of eggs to L3 and L3 to adult, respectively.

Table 7.3. State variables used in the model.

Population variables	Definition
E	The number of eggs present on the pasture
L3	The number of infective larvae on pasture
A	The number of matured adult cyathostomins in the intestinal lumen

Explicit time delays in the life cycle are represented in the model by hexagonal boxes and are defined in Table 7.4. These include time delays to model the development of eggs to infective L3 stage (τ_1), ingested infective larvae to adult (τ_2), and delay to model the life span of adults in the gut (τ_3). The time delay implies that the model does not consider individuals within a particular developmental stage until they finish this developmental time. All parasites in a particular stage that have finished their development time move on to the next stage with a specified survival rate during the model run.

The variable ‘months’ in the model stores the calculated independent variable, i.e., time (day) converted to calendar months as described in subsection 7.4.3. The network diagram and series of differential equations are produced in a computer-based modelling software called ModelMaker. ModelMaker and its operation are described in appendix VII.

Table 7.4. Development time delays and their definitions

Symbols	Definitions	estimated values
τ_1	Time for maturation of eggs to L3	7 days
τ_2	Time for maturation of L3 to adults	60 days*
τ_3	Life span of adults in the gut	180 days*

* Initial estimated values.

7.4.1 Differential equations used to describe the rates of change with time in the constructed model

In a differential equation model a set of equations will be used in which the flow of individuals between groups depends on the number of individuals in the group at that instant. When modelling a system it is useful to look at the flows that occur between the groups, and to determine how the flow between the groups depends on external influences. If we consider one of the groups, then the rate of change of the number of individuals within that group or developmental stage will be the sum of the flows into that group minus the sum of the flows out of that group. It is a relatively simple task to represent the rate of change of a value with some function.

The first stage in formulating a differential equation model is to decide which groups of animals or in our case stages of development of the life cycle, are to be used as state variables, and to decide the rate of flow into and out of these groups considering the different factors involved. As described in the simplified network diagram, Fig. 7.2, in order to produce a reasonably simple model, only three variables, which are believed to represent the system under study, are considered as state variables. These are E, L3 and A as defined in Table 7.3.

The network shown in Fig. 7.3 can be interpreted as a series of differential equations mapped on to the lifecycle of cyathostomins defining the rate of changes with time of the abundance of these state variables. These are given by equations 7.3 to 7.5 as follows:

7.4.1.1 The rate of change of infective larvae on pasture (L3).

$$dL3(t)/dt = yfA(t - \tau_1) - (\mu_1 + \beta)L3(t) \quad 7.3$$

where, f , y , β and μ_1 are parameters representing fecundity, larval yield, larval ingestion rate and mortality rate of L3, respectively (Table 7.1). $fA(t)$ is the number of eggs (E) deposited on pasture at time t . The mortality rate of L3, μ_1 , is a function of rainfall defined by the equation $R/S_w + (1-R)/S_d$. Substituting this for μ_1 , equation 7.3 becomes

$$dL3(t)/dt = yfA(t - \tau_1) - [(R(t)/S_w + (1-R(t)/S_d) + \beta)]L3(t) \quad 7.3.1$$

where R represents availability of rainfall and takes the value of 1 or 0 in wet or dry seasons, respectively, as described in subsection 7.2.2. S_w and S_d are the survival times of L3 on pasture during wet and dry seasons, respectively, as described in subsection 7.2.3.

7.4.1.2 The rate of change of adult cyathostomins in the lumen of large intestine (A)

$$dA(t)/dt = \beta s L3(t - \tau_2) - \beta s L3(t - \tau_2 - \tau_3) \quad 7.4$$

where $\beta L3(t)$ is the number of L3 ingested at time t . This takes τ_2 time to develop to adults (A) and only a proportion s are able to establish in the gut. After a further τ_3 time units the adults will die. The parameter s is establishment rate of ingested L3 and is a function of adult worm burden as described in subsection 7.2.5. $s = 1$ when $A < P$, but $s = P/(A+1)$ when $A \geq P$. Substituting this for s , equation 7.4 becomes

$$dA(t)/dt = \beta s L3(t - \tau_2) - \beta s L3(t - \tau_2 - \tau_3) \text{ when } A < P \text{ and} \\ dA(t)/dt = \beta L3[P/(A+1)](t - \tau_2) - \beta L3[P/(A+1)](t - \tau_2 - \tau_3) \text{ when } A \geq P \quad 7.4.1$$

where P is the estimated number of adult parasites up to which all infective larvae ingested are able to develop into adults as described in subsection 7.2.5.

7.4.1.3 The rate of change of egg production (E)

$$dE(t)/dt = fA(t) - fA(t - \tau_1) \quad 7.5$$

Where f is the rate of egg production as described in subsections 7.2.8.

7.4.2 Initial conditions and sequence of events in the model

From studies by Eysker and Pandey, (1989), Getachew (1999) and Matthee et al.(2000) an average worm burden of 48,211 cyathostomins per donkey can be calculated. Assuming a 1:1 ratio of female and male worms, a single donkey with an estimated initial female adult worm burden of 24,000 was assumed to remain on pasture starting in January for the 10-year simulation period. The number of eggs and infective larvae (L3) on pasture were assumed to be zero initially as January and subsequent months are dry and hot, during which herbage coverage is scarce and survival of free-living and infective stages are negligible. Pasture larval contamination in the subsequent wet season is therefore due to the adult worms in the donkeys infected the previous year.

7.4.3 Model run

A computer-based modelling software called ModelMaker, v.4, was used to model the lifecycle of the cyathostomins. Detailed description of how ModelMaker works is given in Appendix VII. It uses differential equations to calculate daily development and mortality rates of the free-living and parasitic stages. By default ModelMaker's independent variable is time, t . All equations are written with respect to t and solved over a range of values for t . The model considers the first day to be January 1st. It is constructed using daily rates of change. To simulate the month of interest the following formula was used: $[\text{trunc}(t/30.4)\text{mod}12]+1$. Where t is time (days) and takes values of 1 to 365 or more days; mod12 is a modulo system, which forces the model to switch between values from 0 and 11 corresponding to months from January to December, respectively. The model calculates and truncates (trunc) so that each day corresponds to a single month. This was stored as the variable 'Months' as indicated in the basic architecture of the model (Fig.7.3).

The model was run for 3665 days (10 years) and results were recorded for the mid-day of each month for 120 months starting from January. This enabled us to see whether the changes in the values of the model state variables follow regular patterns of distribution from year to year. Since the initial values of the state variables of the model are all estimates we are not trying to predict the actual values but the pattern or shape from the model output.

7.4.4 Standardisation of output data values and model validation

Both the observed and predicted data values of faecal egg counts were standardised to compare the pattern of seasonal distribution between the two on a similar scale using the following formulae:

$$\bar{O}_i = O_i / \hat{O} \quad 7.6$$

where $i = 1, 2, 3, \dots, 12$ corresponding to months of January to December, respectively, O_i is observed faecal egg count on month i , \hat{O} mean faecal egg count of O_i s.

$$\bar{P}_i = P_i / \hat{P}, \quad 7.7$$

where i is as explained above, P_i is predicted faecal egg counts on month i , \hat{P} is the predicted mean of P_i s. It is then possible to compare \bar{O}_i and \bar{P}_i .

The model was finally validated against the observed faecal worm egg count data. The effects of varying the different parameter values corresponding to different developmental and mortality rates, as well as to initial values of model variables, were fully explored and used in fitting the model to the observed data. How well the different models fit the observed data was measured using sum of squares of the residuals.

7.5. Simulation of anthelmintic control strategy

The developed model was used to simulate the effect of timing and frequency of anthelmintic treatment in controlling strongyles in the donkey population under the Ethiopian mid-lowland climatic condition. The effect of giving an anthelmintic drug to animals is to reduce the adult worm burden and the number of intra-host larvae. This has an immediate beneficial effect on the animal and will also reduce future pasture contamination. Hence, the action of the administered drug was simulated in the model by reducing the adult worm burden and developing larval stages. The effect was explored in each treatment strategy over a 10-year simulation. The administration of ivermectin, a macrocyclic lactone, of avermectin group (Lynn, 1995) is more than 99% effective on luminal stages of cyathostomins and non-inhibited larval stages developing within the mucosa (Love et al. 1995). For the present simulation study the efficacy of the drug is assumed to be 99%. It was also assumed no inhibited early third stage mucosal larvae in donkeys (Eysker, 1987). The treatment protocols explored were:

1. Dosing the animals once at the end of dry season (May)
2. Dosing the animals once at the end of main rainy season (October)
3. Both at 1 and 2
4. Annual dosing of donkeys in May
5. Biennial dosing of donkeys in May
6. Dosing every four years in May

7.6. Results and model tuning

The observed and predicted seasonal patterns in faecal worm egg count based on the estimated parameter values for the development and mortality rates of the free-living and parasitic stages of cyathostomins before any adjustments were made ('original model') is

shown in Fig. 7.4. As can be seen from the figure, the predicted seasonal faecal worm egg count did not fit the observed data particularly well. The predicted seasonal availability of infective larvae on pasture revealed two peaks: small peak during the short rainy season of March and April and high peak during the long rainy season of June to September as expected (Fig.7.5).

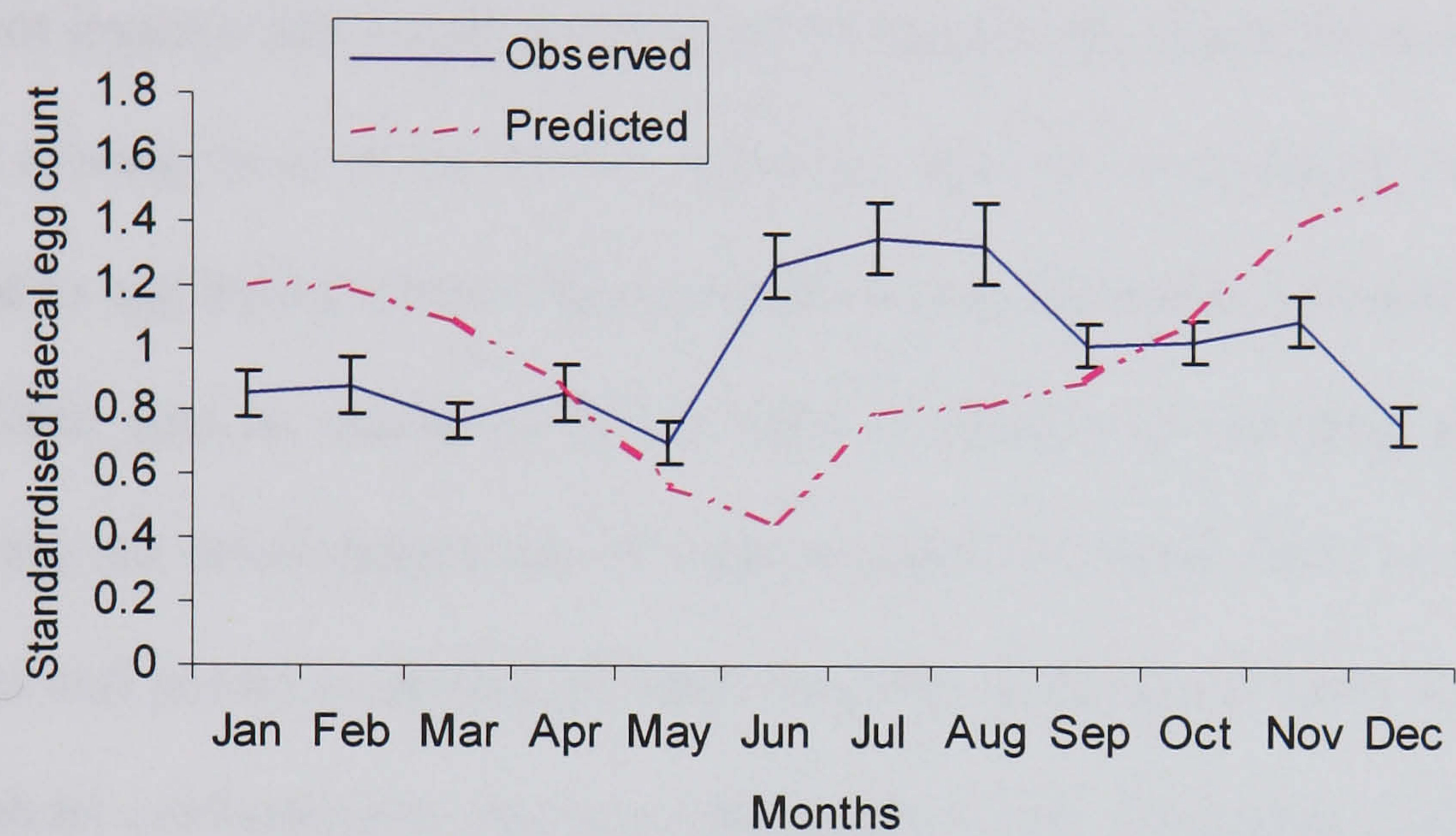


Fig. 7.4 Observed and predicted average monthly faecal worm egg counts of cyathostomins of donkeys in the mid-lowland regions, Ada and Akaki, Ethiopia. The sum of squares of residuals is 32.9. Error bars indicate the 95% confidence interval.

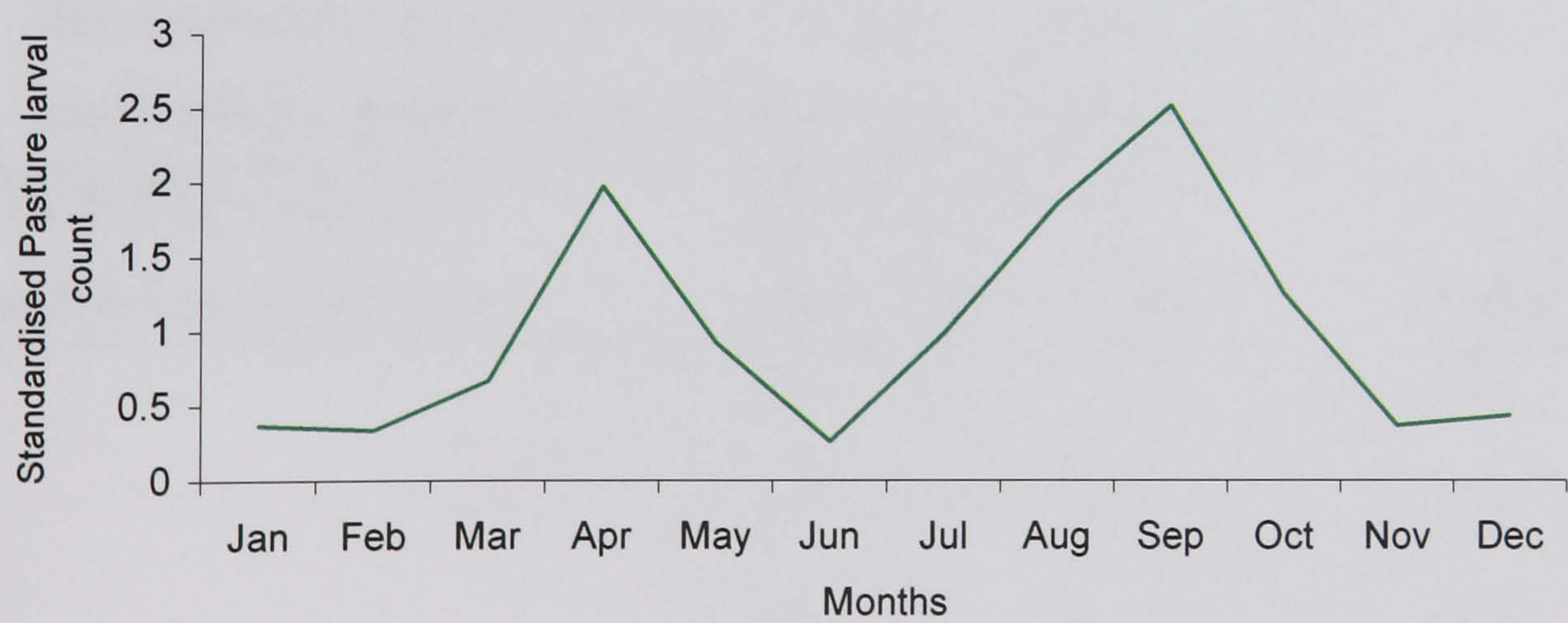


Fig. 7.5 Predicted peaks of infective larvae on pasture before adjustment was made for development time of ingested infective larvae to adult and survival time of adults.

7.6.1 Effect of varying some model parameter values on the prediction of peak faecal egg counts and pasture larval contamination.

Varying the development and mortality rates of free-living stages, larval yield, proportions of L3 dispersed to pasture, ingestion and establishment rates, fecundity and initial values of the model variables did not improve the prediction of seasonal faecal worm egg counts. The increase or decrease in survival time of adults alone from the previously estimated 6 months did not improve the model fit although it changed the shape towards the observed data. The main driving force of the model, however, was the development time of ingested infective larvae to egg laying adults. The result of varying the values of these parameters in fitting the predicted data as measured by the sum of squares of residuals are shown in Table 7.5. Varying the development rate of ingested infective larvae from an estimated 5-18 weeks in horses and ponies to around 36 weeks together with an estimated survival time of 37 weeks for adults cyathostomins produced the smallest sum of squares of the residuals dramatically improving the model fit, particularly, in predicting the peak faecal worm egg counts during the wet seasons and the decline towards the dry season (Fig.7.6).

Table 7.5 Effect of varying the values of parameters, ingested infective larval development rate and the survival rate of adults, as measured by sum of squares of residuals, in fitting model prediction to the observed data.

Estimated development time of L3 to adult (wks)	Estimated survival time of adults (wks)	Sum of squares of residuals
9	26	32.9
9	37	35.8
35	26	9.6
35	37	5.8
36	37	5.2

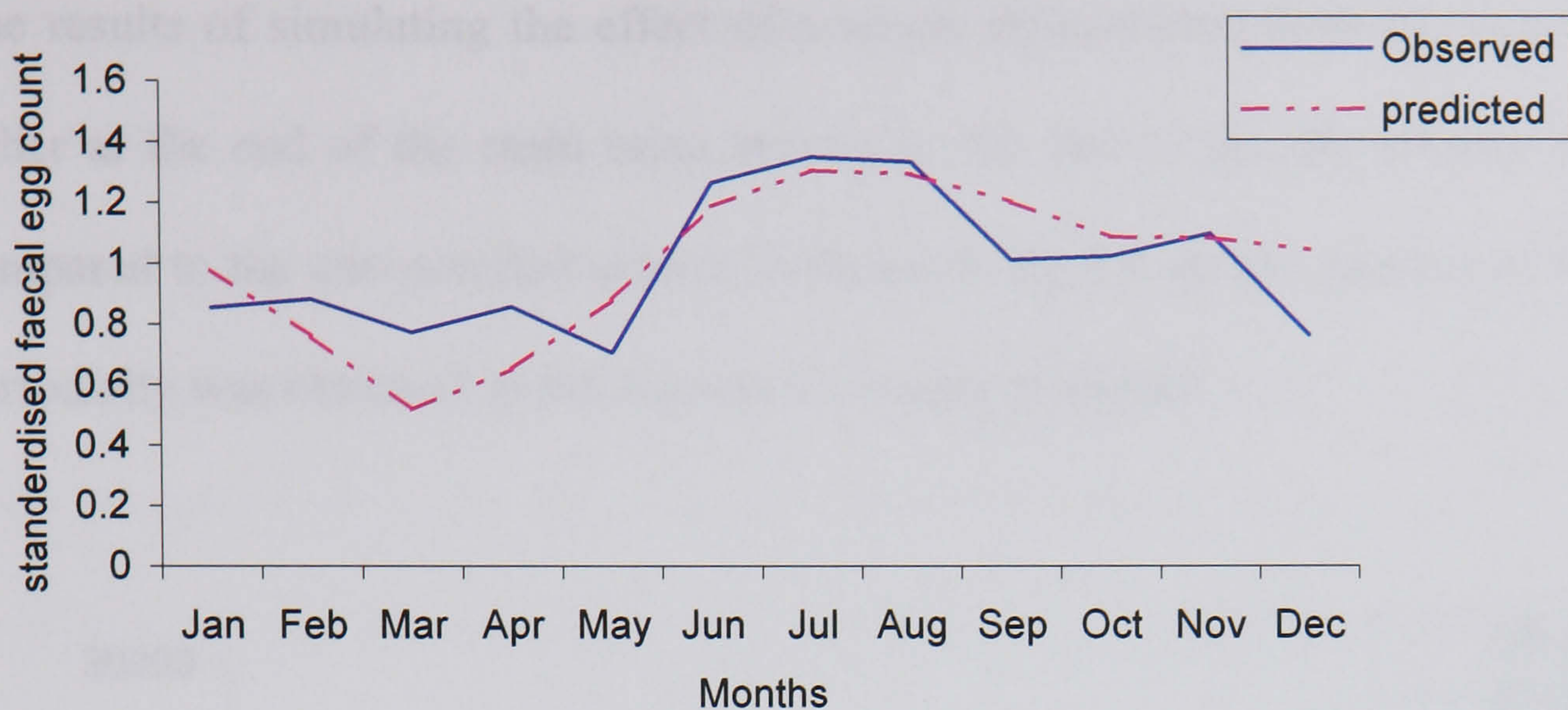


Fig. 7.6 Observed and predicted average monthly faecal worm egg counts of cyathostomins of donkeys in the mid-lowland regions, Ada and Akaki, Ethiopia, after the development rate of ingested infective larvae and survival rate of adults were adjusted. The sum of squares of residuals is =5.2.

Although these changes to the model parameters dramatically improved the predicted seasonal pattern of faecal egg count, it did not greatly affect the prediction of peak pasture larval availability (Fig.7.7).

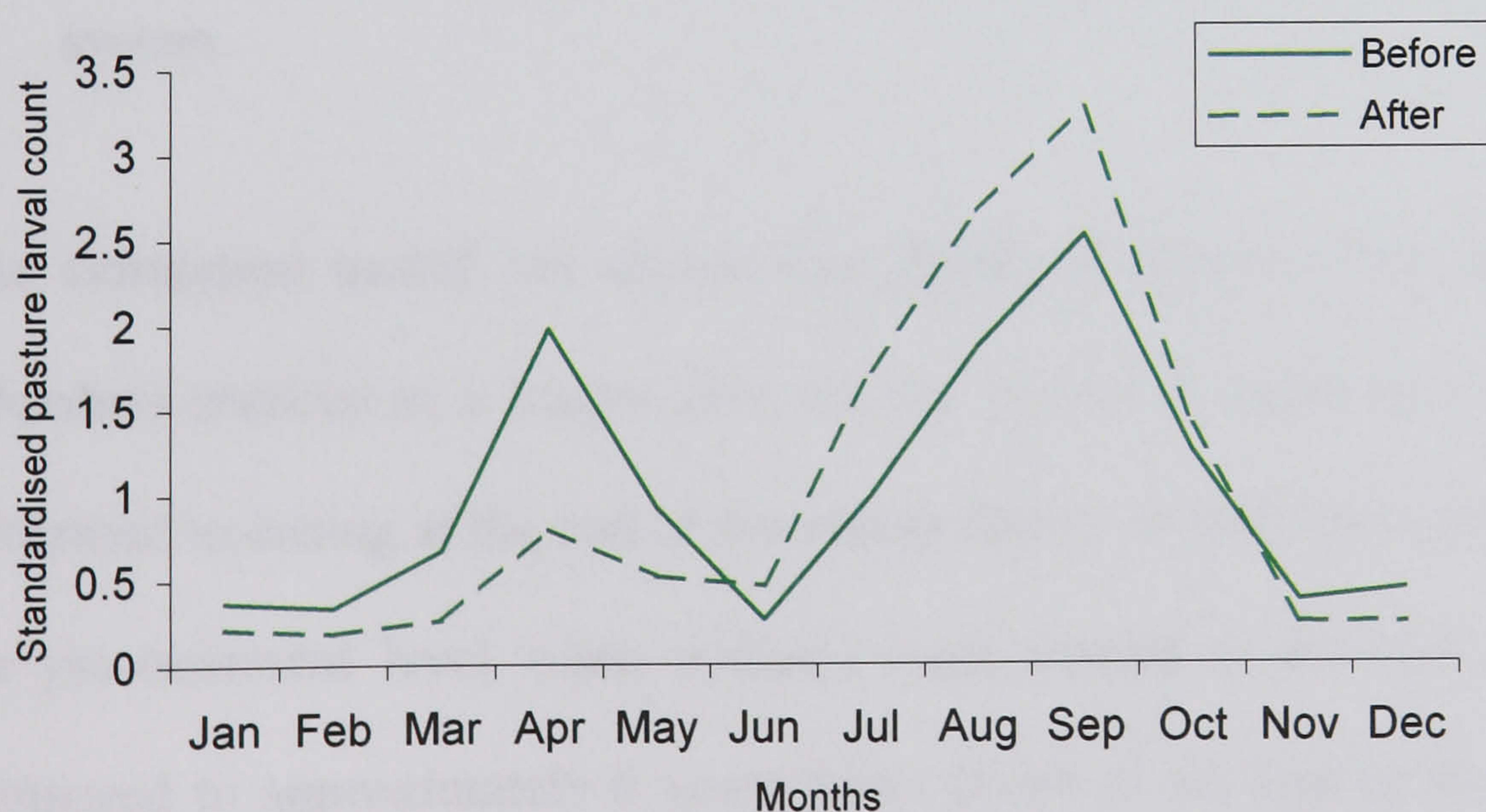


Fig.7.7 Peak predicted infective larvae on pasture before and after adjustment was made for development time of ingested infective larvae to adult and survival time of adults.

7.6.2 Simulated effect of anthelmintic control strategy

The results of simulating the effect of a single anthelmintic dose on worm burden treated either at the end of the main rainy season or the end of the dry season, or at both times compared to the uncontrolled system is shown in fig.7.8. In the absence of control a regular periodicity was observed in the number of worms predicted.

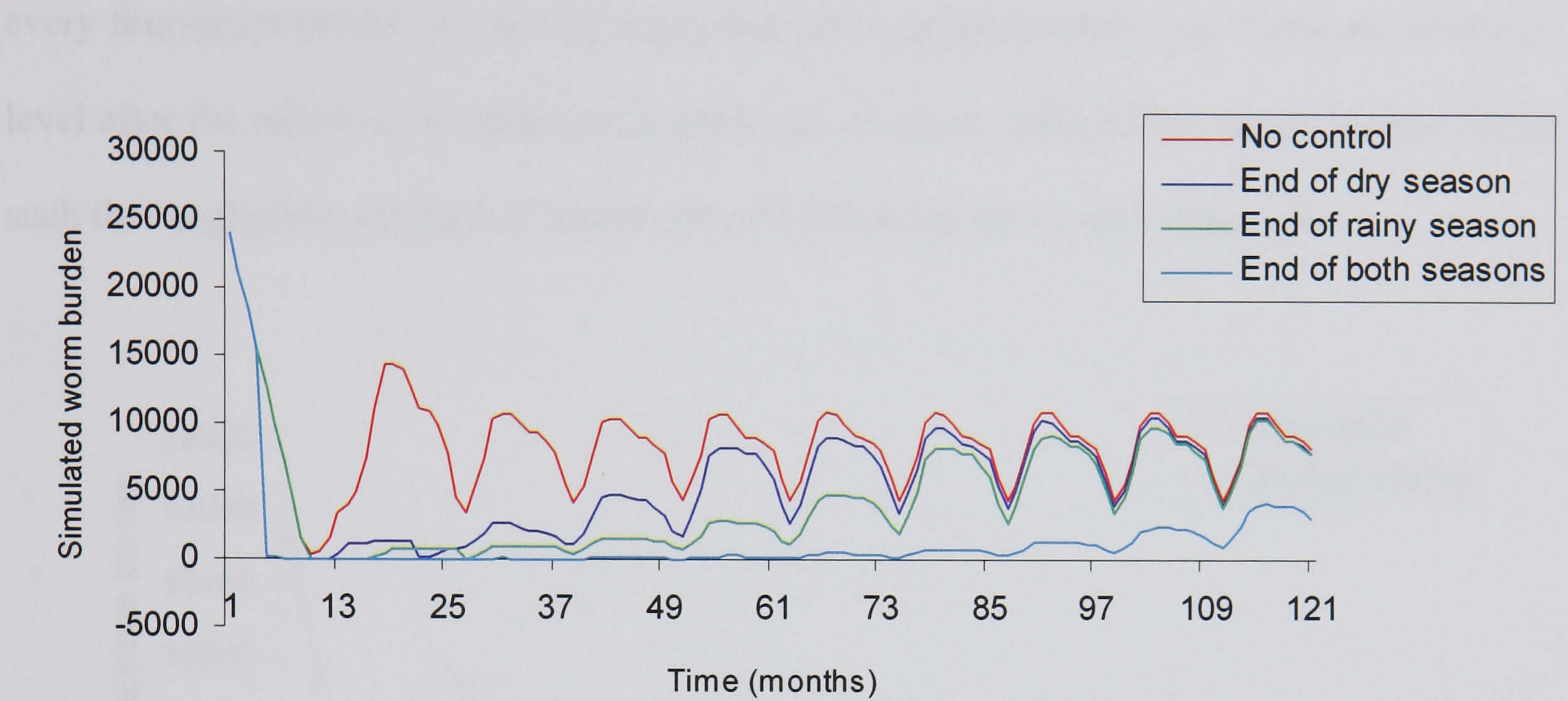


Fig. 7.8. Effect of a single anthelmintic dose on the level of worm burden administered at the end of dry season, main rainy season and at both times compared to the uncontrolled system.

The simulation model has shown that dosing donkeys at the end of main rainy season (October) resulted in a longer time for the worms to come up to their pre-treatment level compared to dosing at the end of dry season (May). It took approximately 9 years to return to the pre-treatment level when donkeys were treated at the end of main rainy season as compared to approximately 6 years when dosed at the end of dry season. However, when treatment was carried out at the end of both seasons, worm number did not return to pre-treatment levels even after 10 years of simulation, although by 10 years the worm burden had returned to approximately half the normal level.

Figures. 7.9 to 7.11 show the effect of dosing donkeys annually, biennially and every four-years compared to the uncontrolled system. The rise of worm burden following the first dose both in annual and biennial treatment regimens was far below the pre-treatment levels. Moreover, following second doses, the level of worm burden was reduced to a negligible level throughout the simulation period in both treatment regimens. Simulation of treatment every four-years results in a rise of worm burden to approximately half of the pre-treatment level after the initial dose. Subsequent treatment however, reduced the worm burden further such that negligible numbers of worms remain following the second treatment.

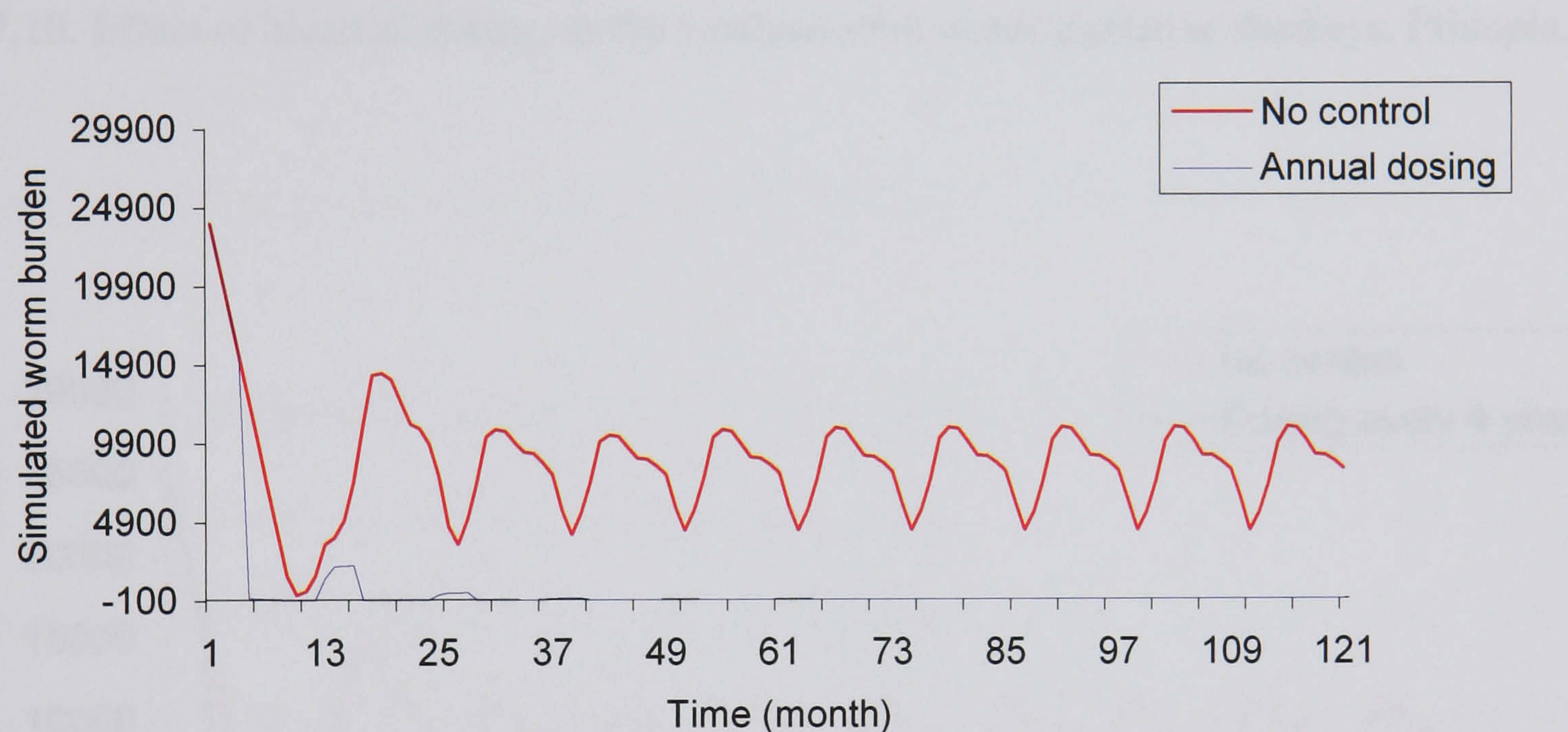


Fig. 7.9 Effect of annual dosing on cyathostomin worm burden in donkeys, Ethiopia.

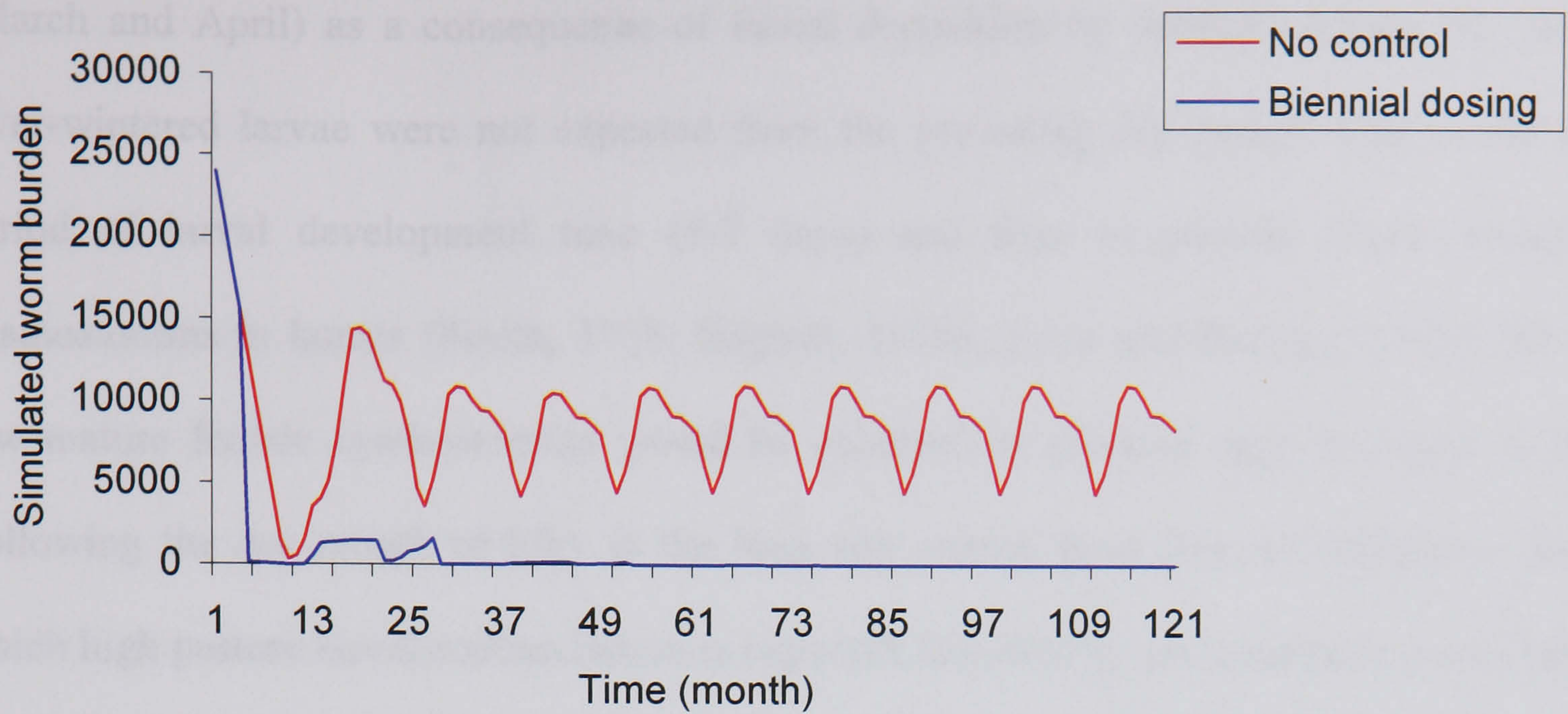


Fig. 7.10. Effect of biennial dosing on the cyathostomin worm burden in donkeys, Ethiopia.

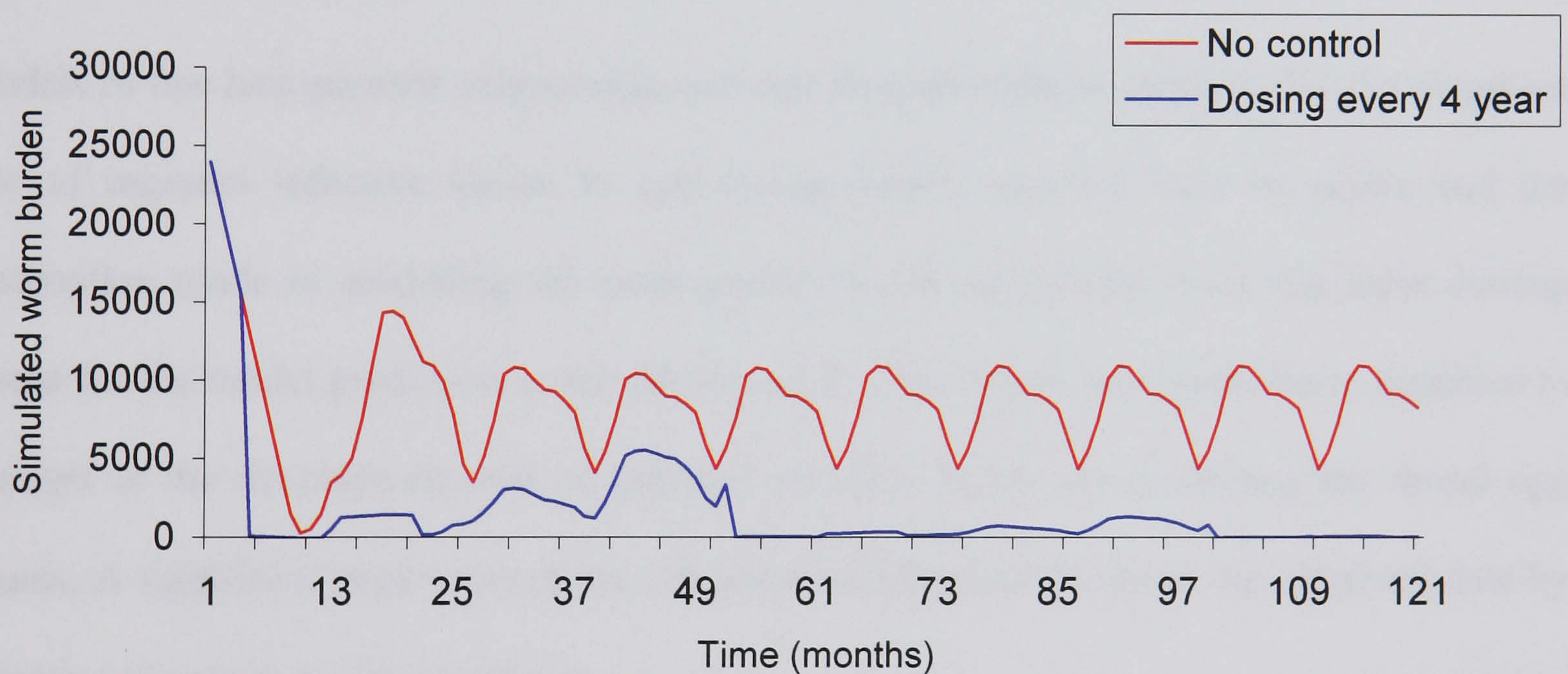


Fig 7.11. Effect of dosing every four-year on the cyathostomin worm burden in donkeys, Ethiopia.

7.7. Discussion

7.7.1 Predicted seasonal pattern of faecal egg count and infective larvae on pasture.

Donkeys have a very small chance of acquiring larval infection during the dry months of the year. Relatively low levels of infection with larvae are expected during the short rainy season

(March and April) as a consequence of faecal deposition by donkeys during this period. Over-wintered larvae were not expected from the preceding dry period. Due to the short period of larval development time (5-7 days) and time to patency (5-18 weeks) of cyathostomins in horses (Smith, 1978; English, 1979a; Love and Duncan, 1992), the first few mature female cyathostomins would be expected to produce eggs by April or May. Following the dry month of May is the long wet season from June to September during which high pasture larval contamination is expected followed by an increase in worm burden and high faecal worm egg counts. The observed data showed peak faecal worm egg count during this time of the year but the 'original model', the model constructed without any parameter adjustment, did not predict this phenomenon. However, peak pasture larval availability were predicted both during the small and long rainy seasons as expected.

Models of one host-parasite relationship may not be applicable to another. The development rate of ingested infective larvae to egg laying adults, survival time of adults and the assumption made in modelling the peak pasture larval availability were the main driving forces for the model prediction in the present study. The model was particularly sensitive to changes in the development rate of ingested infective larvae for predicting the faecal egg counts. A significant improvement was obtained in fitting the model to the observed data by adjusting the value of this parameter.

The peak in numbers of infective larvae on pasture had a similar pattern of seasonal distribution both before and after parameter adjustment. A study by Mfitilodze and Hutchinson (1988) has shown that cyathostomin infective larvae reached a peak on pasture at approximately the same time as peak faecal worm egg count. This is mainly because of the high mortality rate of eggs and larvae on pasture due to the adverse tropical weather condition, so that the cumulative effect of egg production does not play a significant role.

Moreover, varying the development time of the free-living stage did not seem to have any effect on the result of the model. After adjusting the development rate of ingested infective larvae and survival rate of adults, the model was able to predict a similar seasonal pattern of faecal egg counts to the observed data at approximately the same time it predicted peak pasture infective larvae.

There are two main possible explanations for this. First, the ingested infective larvae during the wet seasons may stay encysted longer and undergo a prolonged development period; more than that was estimated in horses or ponies (Smith, 1978; Love and Duncan, 1992) before they emerge as adult. This may represent a strategy to avoid the adverse environmental condition found in the tropical dry season. This is in contrast to data derived from horses and ponies under temperate conditions (Ogbourne, 1972). Alternatively this may represent their normal developmental life-cycle. Second, our assumption that larvae are only available during the wet season might be wrong, which is most unlikely as many studies (English, 1979a; Hutchinson, et al., 1989; Mfitilodze and Hutchinson, 1988; Onyali, et al., 1990; Waruiru, et al., 1998, 2002) and our observations have shown that gastrointestinal nematode larval availability during the dry hot period of tropical weather conditions is negligible. It seems, therefore, that ingested larvae not only take several months to develop to adult but also have a prolonged life span in donkeys supporting the suggestions made by Eysker and Pandey (1989) and Reinemeyer, et al. (1986). Moreover, the model further indicated that either the ingestion of a high number of larvae during the main rainy season and/or the expulsion of old adult parasites from the lumen due to their age or via a host protective mechanism (Colglazier, 1979) might have triggered the emergence of a high number of developed adults from previous infections, which is followed by high faecal egg counts.

The apparent good fit of the model predictions to the field data obtained after parameter adjustment may generally indicate some major differences between donkeys and horses in their reaction to the parasite and/or between cyathostomins of donkeys and horses. The parasitic development of cyathostomins is likely to be complex since a host may be parasitised by multiple species and it is not known if all cyathostomin species have a similar rate of development. Parasite populations change constantly and even though some studies indicate similar cyathostomin species are found in horses and donkeys, others have shown that there are at least 10 different species of cyathostomins, which are found only in donkeys and zebras but not in horses (Lichtenfels, et al., 1998), and hence may follow a different biological development.

Harsh winter weather conditions in temperate regions produce an unfavourable environment, which is believed to be one of the major factor that stimulate arrested development (Ogbourne, 1972). In contrast in the tropics hot dry weather is the typical unfavourable environment (Hutchinson, et al., 1989; Mfitilodze and Hutchinson, 1988; Onyali, et al., 1990; Waruiru, et al., 1998, 2002). Whether such difference in unfavourable environmental condition forces the larvae to undergo different strategies of arrested development or whether they do not undergo arrested development in donkeys as suggested by Eysker (1987) but follow prolonged development is unknown.

A mathematical modelling approach was used to describe the epidemiology of donkey cyathostomins, particularly the seasonal variation of faecal egg outputs and pasture infective larvae, using the limited available body of information. The model has been shown to fit the observed data set reasonably well with some adjusted parameters. Actually it is surprising to obtain such a good fit, with such limited information. While it may not be true in all situations, it indicates that a simple model with few parameters and many reasonable

assumptions is capable of describing the behaviour of the real system. This synthesis is by far incomplete. Indeed a major strength of the model was that it has highlighted the gaps in information of our knowledge of equine strongylosis in general, and donkeys in particular. Although it is not necessary to know everything about a system in order to write a useful model nor is it necessary to include all we know about a system (Smith and Guerrero, 1993), data on important parameters on which the model depends is very important. Thus emphasis should be given as to whether the same species of cyathostomins infect donkeys and horses but follow different pathways of biological development or different species of cyathostomins with different biological dynamics infect the different equidae.

As faecal egg counts are extremely poor indices of parasite burden, field data on the pasture larval contamination would be an appropriate approach in validating such a model and should be the main focus for future study. On the other hand, the assumption that the model involves only cyathostomins is also one area to be viewed with caution. Although studies have shown that the largest proportion of faecal egg and pasture larval counts are made up of cyathostomins (Mfitilodze and Hutchinson, 1988; Wells, et al., 1998, Getachew, 1999), one of the main model assumptions, there is still some contribution from large strongyles, which have a much longer prepatent period compared to cyathostomins (Ogbourne and Duncan, 1985). Providing explicit solutions of the model is a good way of investigating the interrelationship of the various parameters in a single-host, single-parasite system (Grenfell, et al., 1987a). Natural infection involves several species of parasites. Therefore, future modelling approaches should be focused on using only cyathostomin-infected equids, and if possible each cyathostomin species. A model is not reality, however, as the properties of the model are dependent upon its formulation and assumptions, conclusions, based on models, about biological systems are derived by analogy and as such, must be accepted with caution.

The constructed model can be useful as a base for further detailed studies in modelling the complete biological dynamics of cyathostomins both in donkeys and horses. It can also provide a theoretical framework within which ideas concerning the epidemiology of equine cyathostominosis can be developed and checked, and in determining which of many possible control measures can be applied.

7.7.2 Simulation of anthelmintic control strategy

The effect of various protocols for the timing and frequency of anthelmintic treatment on the adult cyathostomin worm burden in donkeys have been simulated. The results from each simulation have been compared to the uncontrolled system. When the host is treated with anthelmintic, the parasitic stages are killed, and the age structure and overall abundance of the population is changed. Moreover, a given treatment affects only a fraction of the entire parasite population; the remainder being on pasture as free-living or infective stages (Smith, 1990; Barnes, et al., 1995). Therefore, if no further treatments are given, the parasite population abundance eventually returns to its pre-treatment condition. The main aim of anthelmintic treatment is not total eradication of the parasite but to maintain a level that the animal can tolerate so that the parasites do not induce disease; and also to reduce pasture larval contamination.

The simulation model has shown that the parasite population returned to its pre-treatment level quicker when donkeys were treated at the end of the dry season as compared to treating at the end of the main rainy season. This is because the post-treatment recruitment of new parasites from the large pool of infective larvae on pasture during the subsequent wet season is higher than that when donkeys are exposed to dry pasture immediately following treatment. Therefore, the establishment of worm burden to pre-treatment levels takes longer following treatment at the end of the wet season compared to at the end of the dry season.

However, the major disadvantage of this treatment regimen is that treatment is given after the parasites have induced damage to the host following high levels of infection during the wet season.

Simulating the frequency of anthelmintic treatment has shown that parasite levels fall rapidly and remain relatively low if donkeys are dosed either annually or biennially. Although there were rises following the first dose, the worm burden is far below the pre-treatment level in both treatment regimens and the re-establishment of the parasites to their pre-treatment level was never achieved throughout the simulation period following subsequent treatments. On the other hand, simulating the use of anthelmintic every four years showed that there was a rise of worm burden to approximately half of the pre-treatment level within the four years after the initial dose. The two subsequent treatments substantially reduced the parasitic burden compared to the uncontrolled system. However, the health effect of the rise of worm burden during the first four year period following the first dose, and whether such a control strategy would be cost effective would need further investigation before definitive advice could be given.

The parasites lost as a result of treatment are quickly replaced by ingestion of infective larvae in temperate regions (Smith, 1990). However, the dry hot period of the tropics in the mid-lowlands of Ethiopia restricts the acquisition of infective larvae from pasture. Moreover, no residual larvae can survive over the dry hot period. This time of year therefore, serves as a natural control of parasitic infection. It would be appropriate to treat equids during the hot dry period immediately prior to the wet season when the herbage coverage is very scarce and helminthologically 'sterile'.

Based on the results of the simulation model, taking into consideration the natural parasite control afforded by environmental conditions, the poor local economic climate and the

possibility of drug resistance developing due to anthelmintic overdose, the biennial treatment regimen seems to be ideal for the control of worm burden under mid-lowland tropical conditions in Ethiopia. However, the combination of treatment protocols such as treating annually followed by biennial or biennial followed by dosing every four years can be applied.

CHAPTER EIGHT

GENERAL DISCUSSION

The studies outlined in this thesis describe the different helminth parasites and arthropods of working donkeys, with particular emphasis on the cyathostomins, cestodes, ascarids, trematodes, and gasterophilus larvae, studied using different epidemiological techniques: coprology, post-mortem, immuno-diagnosis and mathematical modelling. Most of the previous reports of these parasites, particularly the non-strongyle intestinal helminths, are from a small number of donkeys and the information is rather disparate and fragmentary (Pandey, 1994). The current study made on relatively large numbers of donkeys is not only first of its kind, but is important for two reasons. Firstly, the poor body condition of the animals and the high strongyle faecal egg count obtained in all of the animals examined (data not shown), means it is very unlikely that donkeys have ever received anthelmintic treatment. Secondly, they were allowed to roam free in herds, and not managed or grouped in any sort except when they were working. Therefore, this study represents not only natural infection but also the prevalence of all species of helminths and faecal egg counts represent the natural levels in the absence of helminth control. However, data on post-mortem study should be interpreted cautiously as the donkeys examined are those admitted to the hospital and may not represent the true population.

The cross-sectional coprological survey and post-mortem findings showed not only high infection prevalences but also the potential infection of donkeys with multiple helminths demonstrating the case for polyparasitism in the studied donkey population. The presence of polyparasitism with high level of infection is an indication that favourable environmental conditions for infection, survival and perpetuation of the parasites and/or the absence of anthelmintic therapy exist in the working donkeys of Ethiopia. However, the variation in

infection prevalence between the different regions further indicates that environmental conditions in some areas are more favourable to some parasites than others. This was particularly noticeable in cestode and *Fasciola* infection in which the prevalences of both were significantly higher in the highland region of Bereh.

The high infection prevalence and infection intensity irrespective of the age of donkeys was an interesting finding. For the parasites studied, faecal egg outputs and worm burden recovered on post-mortem examination were not significantly different between the different age groups. There has been no study to investigate the effect of age of donkey on infection prevalence, egg output or worm burden. The present study is the first to report such an association. Ascarid infection was one of the parasites, which took our attention. *P. equorum* is a highly immunogenic parasite of young horses and ponies (Clayton and Duncan, 1977b). But the present study has shown not only high faecal egg count, but also high worm burden in adult working donkeys. The similarity of our result with studies made by Vercruysse, et al. (1986) and Wells, et al. (1998) suggests that unlike in horses (Clayton and Duncan, 1977b; Clayton, 1986) age-dependent immunity may not develop in donkeys; or else they might have been immuno-compromised. Although it is difficult to associate faecal egg counts with ascarid worm burden, the high faecal egg count obtained in adult working horses may substantiate the above explanation and it is not uncommon to find adult working equids infected with ascarids under tropical conditions in Ethiopia.

It is noteworthy that there are greater differences between populations of the same species of parasite in different environments than between different species in the same environment (Michel, 1982). Moreover, animals may react to the same parasitic infection differently in different climatic regions. For example, in the more temperate regions calves rapidly acquire natural immunity to become refractory to *H. contortus* infection by 12 months of age

(Southcott and Barger, 1975). However, a study made in the tropics (Benitez-Usher, et al., 1984) revealed no indication that cattle had acquired significant immunity to *H. contortus* infection after 2 years of grazing. Whether such phenomena hold true for helminths of equids in general and donkeys in particular in the tropics would need further investigation. The present finding, however, indicates that even if we assume that adult equids are not suffering much from ascarid infection, they could be a potential source of contamination, putting young animals at a high risk of infection. Therefore, adult working equids in general and donkeys, in particular, should be considered in any treatment programme. The findings further indicate the need for a thorough experimental study to understand the immune status of working equids to helminth infection in general and parascaris, in particular, under tropical climatic conditions.

The information on the extent of the non-strongyle intestinal helminths and their pathogenic effect are limited. It is only in extrapolation of data from horses that strongyles are believed to be the most pathogenic parasite of donkeys. However, the pathogenicity of each and every parasite infecting donkeys has not been fully studied. Although it was not the aim of this study, the observed gross pathological lesions believed to be caused by trematodes, ascarids and cestodes indicate their potential to be pathogenic and the effect they might have on the animals' working performance and reproduction. This is one of the areas of donkey parasitology, which could benefit from thorough investigation.

The post-mortem study also revealed that parasites have an aggregate or clumped distribution, in which many donkeys are infected with a low burden of parasites and few donkeys are infected with a high worm burden. This was particularly pronounced in *A. perfoliata*, *P. equorum*, *Gastrodiscus aegyptiacus* and *Gasterophilus* larvae. This situation is documented for many of the parasites infecting both humans and also domestic ruminants

(Guyatt, et al., 1990; Wilson and Grenfell, 1997; Stear, et al., 1998). This is the first report in donkeys. However, the frequency distribution of donkey helminths is not generally known and should be further studied using appropriate statistical packages, such as the negative binomial (Smith and Guerrero, 1993), to quantify how over-dispersed they are. One of the advantages of knowing such parasitic frequency distributions is that it helps to decide the type of approach in anthelmintic therapy.

The high prevalence of fasciolosis in donkeys, the evidence that they can be clinically affected possibly leading to death (Collin, 1961; Pankhurst, 1963; Green et al., 1968), the gross pathological lesions believed to be caused by *Fasciola* spp in the present study and histopathological findings by Collin (1961) and Fahmy El-Attar (1990) all lead us to believe that these animals can potentially be affected by fasciolosis. However, it may not be as severe as it is in domestic ruminants. Boray's (1969) classification of donkeys in the delayed resistance group together with cattle, horses and humans, in which the disease is self-limiting but may cause severe pathogenic lesions, substantiate these findings.

Generally, most workers have reported the presence of flukes through faecal examinations when they found donkeys losing weight with poor performance and/or post-mortem fluke recovery in fasciola-endemic areas. However, with few exceptions, there have been no attempts made to reveal either the gross or histopathologic lesions in the liver of donkeys affected by *Fasciola* spp. More importantly, no experimental studies have been conducted to determine the resistance status of donkeys and the extent of the pathogenicity of the fluke. Maybe because of this, the absence of obvious clinical signs in most cases, the masking of clinical signs by other pathogenic parasites, and the difficulty in detecting fluke eggs by routine faecal examination, it is likely that infections might have been overlooked. This

might have contributed to the belief that donkeys are resistant to fasciolosis and led to underestimation of its effect on their general health.

Fasciolosis is an important trematode pathogen of livestock worldwide with severe economic losses in domestic ruminants. More recently it has been found to have increasing importance as an infection of humans, generally in rural areas associated with poverty and poor water distribution. Recent estimates suggest that up to 17 million people are infected worldwide (WHO, 1999). Although fasciolosis is not a major human problem in Ethiopia, it is one of the major causes of economic losses in ruminant livestock production (Ngategize, et al., 1993; Yilma and Malone, 1998). However, there is a high risk of infection in people, particularly those living by irrigation canals and rivers, because they drink water from these sources or use it to wash vegetables. Water has been incriminated as the source of human infection in endemic areas, directly or indirectly, by contaminating vegetables or kitchen utensils (Mas-Coma et al., 1999; Bargues, et al. 1996). The close association of donkeys with poor people may play an important role in this regard as a potential source of infection.

The importance of fasciolosis in equids is not generally realised both from their welfare point of view, and the role they may play in the epidemiology of the disease. Studies made in Bolivia and Egypt (Mas-Coma et al., 1997; Mas-Coma et al, 1999; Haridy et al., 2002) showed that, next to cattle and sheep, donkeys are the second most important definitive hosts of *F. hepatica* and *F. gigantica*. Moreover, it has been demonstrated that eggs shed by donkeys are viable and able to infect lymnaeid snail, and that the metacercariae subsequently produced are infective for other definitive hosts (Mas-Coma et al, 1999). This clearly indicates the important role of donkeys in the epidemiology of fasciolosis. Donkeys can be infected sub-clinically or chronically and can contribute to the continuous contamination of

pasture. The residual infection derived from this contamination could be sufficient to produce infection in domestic ruminants and humans, particularly in endemic areas.

The control of fasciolosis depends on the application of curative and preventive measures, integrated with management practices. The strategic application of these methods should be based on information on the definitive or reservoir hosts, and seasonal variation in the occurrence of fasciolosis. This may be derived from the knowledge of the ecology of the intermediate hosts, the free-living and parasitic stages of the fluke species in any particular area. The high infection prevalence obtained in the present study and the worldwide distribution of fasciola infection in donkeys shows the need for these animals to be considered in any epidemiological study and in any control strategy for the prevention of both livestock and human fasciolosis.

The other important trematode found with high infection prevalence was the paramphistomatid *Gastrodiscus aegyptiacus*. Although this parasite is normally considered non-pathogenic (Soulsby, 1982), the present finding of severe haemorrhagic and oedematous colitis in a 14 year-old donkey and the similar pathologic condition reported by Azzie (1975) and Bracegirdle (1973) in horses shows its pathogenic potential. The parasite is not well studied and its occurrence, biology, pathology and clinical symptoms are not well known in equids in general and donkeys in particular.

The *Gasterophilus* species, like their hosts, equidae, were originally restricted to the Palearctic and Ethiopian regions before they reached most parts of the world (Zumpt, 1965). This is the first extensive epidemiological study of *Gasterophilus* spp undertaken in donkeys in Ethiopia. The findings of only two species of bots: *G. intestinalis* and *G. nasalis* may indicate that the climatic condition in the study region is not suitable for the

development and perpetuation of the other species. Eight species are known to infect equids worldwide (Zumpt, 1965). Lyons et al (2000) reported the decline of the population of bot larvae in many parts of the world over the last 25 years, which they attributed to the use of organophosphates in early years and, more recently, extensive treatment of equids with ivermectin and moxidectin. The current high infection prevalence in donkeys may therefore indicate the absence of treatment and/or the favourable climatic condition for the development and survival of the different stages of the two species of bot in Ethiopia.

The veterinary importance of these larvae has been discussed by many authors, and has generally been regarded as minimal. However, the gross pathologic lesions: gastric erosions, ulcers, abscesses, stomach rupture and peritonitis reported in horses (Rooney, 1964; Waddell, 1972; Pandey et al., 1980; Dart et al., 1987) associated with these gasterophilus larvae indicated their potential pathogenicity. The findings of the present study on *Gasterophilus* spp in Ethiopian donkeys further substantiate their pathogenic potential.

There is no experimental evidence of the pathogenicity of bots in donkeys. However, ulcerative lesions, particularly at the junction of the squamous and glandular region (margo plicatus), may lead to weakening of the gastrointestinal wall, which is an anatomically inherently weak locus (Rooney, 1964). The clusters of *G. nasalis* larvae found at the pylorus may lead to stricture, and the tissue reaction to these larvae may have an important pathologic effect. Moreover, although the majority of larvae are found attached to the non-glandular region of the stomach, the few larvae at the glandular region may have a different pathological effect. The process of ulceration could also be enhanced by the invasion into damaged mucosae by bacteria such as *E. coli* (Davies, 1971) and *Streptococcus zooepidemicus* (Waddell, 1972) associated with abscess formation. On the other hand, migrating first and second stage larvae were found causing lesions of different degrees of

severity in the mouth of horses (Cogley, 1989; Wall and Shearer, 1991) but no studies have been made in donkeys to investigate this. With a high infection prevalence of bots in donkeys, the possible pathogenic effects warrant further investigation of the infection of donkeys with bots.

The other important observation regarding gasterophilus larvae in the present study was that they have been found as a major cause of rectal prolapse in working donkeys. This condition is highly prevalent and a serious problem sometimes necessitating surgical intervention. Although there is information as to the temporarily attachment of some of the larvae after passage through the digestive tract causing irritation (Soulsby, 1982) there have been no previous reports of rectal prolapse associated with them.

The sero-epidemiological study presented in Chapter Five demonstrates substantial serological evidence that donkeys can be infected with *A. perfoliata*. The consistently high infection prevalence obtained by ELISA, coprological assay and by post-mortem examination clearly indicates that cestodosis is one of the major problems in the donkey population of Ethiopia, in addition to other helminthosis. The significant variation in infection prevalence and infection intensity, as determined by ELISA OD, between the mid-lowland and highland regions indicates that general agro-ecological differences exist which are important for the development and survival of the oribatid mites and free-living stage of the cestodes, and for the transmission of infection. Both the serology and the coprological studies have shown that areas with pastures that are low-lying and wet, and areas regularly practicing permanent pasture management suffer most from the infection.

Cestodes are increasingly incriminated with many digestive disorders including colic (Williamson et al., 1997; Proudman and Edwards, 1993; Proudman and Holdstock, 2000),

particularly ileal impaction colic and ileocaecal intussusceptions (Proudman et al., 1998) in horses and ponies. Donkeys are thought to have a higher pain-threshold than horses and the classic symptoms of colic in horses are rarely seen and then only once the disease condition is severe and well advanced (Crane, 1997). Therefore, from the high infection prevalence of *A. perfoliata* coupled with this unique behaviour, donkeys could suffer much longer with intestinal disease without showing symptoms of colic. On the other hand, horses and donkeys share the same pasture in Ethiopia, as in most developing countries, and the failure to treat donkeys for cestode infection put the horses at higher risk of infection with tapeworm than if they are managed separately.

Although some studies have indicated that the probability of detecting eggs by the centrifugation/flotation technique is high with high infection intensity (Proudman and Edwards, 1992; Williamson et al., 1998), generally it is of low sensitivity, difficult and time consuming. In serological diagnosis a relatively small serum sample is all that is necessary provided assay facilities and expertise are readily available, and it is economically affordable. Therefore, ELISA would be a useful tool in monitoring the epidemiology of *A. perfoliata* on a herd level, particularly if we wish to determine the infection intensity rather than just the prevalence of the disease. According to Anderson and May (1991), the determinants of disease in intestinal helminth infections are not merely the presence or absence of the parasite, but the infection intensity. Therefore the application of conventional sensitivity analysis as used for microparasitic infection, is not relevant to all macroparasitic infections. It is those animals with high infection intensities that must be identified for treatment as they are at risk of disease and also represent a potential source of infection. Although the strength of association between anti-12/13 kDa IgG(T) and the number of tapeworms is not sufficient for us to accurately predict the number of parasites present, the

high degree of correlation between anti-12/13 kDa IgG(T) and infection intensity in horses makes the assay a potentially useful diagnostic and epidemiological tool.

The main drawback of the current assay, however, is its low sensitivity to detect antibody in animals with low infection intensity, and its inability to distinguish current from past infections. Craig and Nelson (1984) described the detection of circulating immune complexes in the serum of false-negative human hydatidosis patients, the complexed antibody being undetected by antibody-capture ELISA. The possibility of antibody-antigen complexes reducing the sensitivity of the 12/13 kDa antigen, especially in animals with low infection intensity needs further investigation. Improving the current assay or developing another, such as the use of coproantigen (Kania and Reinemeyer, 2004) so as to detect antibodies in animals with low infection intensity and to be able to distinguish current from past infection, is important.

To our knowledge this is the first sero-epidemiological study to be conducted in donkeys. However, although the present ELISA was able to detect antibody against *A. perfoliata* in donkey sera, the development of an ELISA specific to tapeworm antigen isolated from donkeys would be expected to improve the results obtained. This may allow the full range of antibody responses to be explored and evaluated, and to explain some of the differences between horses and donkeys in their responses to cestode infections. Moreover, knowledge about the population biology and dynamics of *A. perfoliata* and their intermediate hosts, the oribatid mites, including prepatent period, turnover of the worms and half-life of the equine IgG(T) in serum are all unknown. Filling these gaps is required not only from the sero-epidemiological point of view but also for the design of rational control programmes against equine cestodosis in general.

Equine intestinal helminth control programmes have traditionally focused upon the strongyles, large and small. The present parasitological findings suggest that for optimum equine health in general, and donkeys, in particular, the non-strongyle intestinal helminths should also be considered. Because tapeworm infections are now considered a significant risk factor for colic (Proudman, et al., 1998), it has now become common practice to treat tapeworm infection in horses and ponies in the developed world. Chapter Six of this thesis demonstrates the high efficacy of praziquantel (Equitape, Fort Dodge) against cestode infections of donkeys in reducing cestode faecal egg counts and serum antibody levels. This is the first study to evaluate the efficacy of a cestocidal drug in donkeys. The low sensitivity of centrifugation/flotation techniques in detecting cestode eggs was observed in that there were individuals, which were positive at one sampling date and negative at others within the control (untreated) group. However, the evidence that no cestode eggs were recovered and the continuous decline of serum antibody level in the treated group within the treatment period indicated the efficacy of the drug. A critical test, which depends on the recovery of worms after treatment at necropsy, is recommended to substantiate the efficacy of the drug.

In previous years treatment of equine tapeworm infection has not been investigated extensively and no commercial cestocidals were licensed. A specific combination of ivermectin and praziquantel or praziquantel and moxidectin, which have a wide spectrum of activity against gastrointestinal parasites of equids are nowadays available in the market. This is particularly important in countries such as Ethiopia, where polyparasitism is a common health problem of equids.

Because of the lack of specific anthelmintics for the treatment of donkeys, it is the dose rate of the horse that is mostly used. Although most anthelmintics licensed for horses are effective against donkey gastrointestinal parasites, there is a need to establish optimum or

sufficient anthelmintic dosage rates for donkeys independently. This is important as the difference in anthelmintic metabolism between the different species of equids or route of administration may result in under or over dosing. Such establishment of dosage specific to donkeys would contribute to delaying anthelmintic resistance, which could be caused due to under dosing or inappropriate route of administration.

The mathematical modelling of seasonal variation of cyathostomin faecal egg output and infective larvae on pasture is demonstrated in Chapter Seven. The model's ability to predict a similar seasonal pattern of faecal egg output to the observed data after model tuning shows that simple models which ignore many of the more complex aspects of the parasite's life-cycle are invaluable in understanding many aspects of the parasite and host-parasite interactions which are not always intuitively obvious. On the other hand, its inability to predict the observed pattern of faecal egg output before parameter adjustment was not surprising, because most of the parameters used by the model were based upon work done specifically in horses or ponies and not in donkeys. The model has highlighted areas where our knowledge of the biology of cyathostomins is not complete. In particular, it has highlighted the paucity of information related to the parameters associated with the development and mortality rates of the parasitic stages in the lifecycle of the cyathostomins. Field and experimental studies are mandatory to fill such information gaps.

The model further indicated the existence of some differences between donkeys and horses in their reaction to the cyathostomins and/or between cyathostomins of donkeys and horses. Thus emphasis should be given in the future to explore the gaps observed, particularly in the parameters associated with the population biology of cyathostomins both in donkeys and horses in order to see if they are different, and so improve the model's predictive power. The effect of host age or host immunity on larval establishment or fecundity, density dependent effect on fecundity, survival rate of adults and the proportion of larvae undergoing arrested

development are all important factors in the regulation of parasite population. Simulations of such biological phenomena are possible with complex models. However, estimating these parameters to incorporate in a model requires data both from field and experimental work. Although it is not necessary to know everything about a system in order to write a useful model nor is it necessary to include all we know about a system (Smith and Guerrero, 1993), data on parameters on which the model will be based is very important. It is, however, believed that the model produced here represents a useful interpretation of the biological dynamics within the lifecycle of the cyathostomins of donkeys. It can help by predicting seasonal peaks in faecal egg output and infective larvae on pasture, and in simulating plausible parasite control strategies.

The model produced was used to simulate the effect of various protocols for the timing and frequency of anthelmintic treatment on adult cyathostomin worm burden in donkeys. The results of the simulation model have shown that frequent anthelmintic treatment of donkeys is not necessary. Treating donkeys only once in a year or a combination of once in a year followed by every two or even four years can substantially reduce and maintain the parasite burden far below the pre-treatment level for many years. Parasite prevention strategies, which maintain parasitic burden at a low level for a long time period are essential. This is particularly important to reduce the development of drug resistance and also from an economic stand point. However, in any such prolonged treatment protocol, the infection level should be monitored regularly in case there is an unexpected rise in worm numbers from climatic change, parasite evolution, etc..

There are a number of reasons why one should want to model the dynamics of parasitic diseases. Among the most important are that models provide insight into the mechanisms underlying observed patterns and they enable the modeller to conduct thought experiments

concerning the efficacy of plausible parasite control strategies (Smith and Grenfell, 1994). Models do not replace real data from field experiments but, in these days of ever-decreasing resources and time, they play an important role in research into the control of parasitism (Barnes, et al., 1995). The present simulation model may offer the prospect of investigating the quantitative and qualitative consequences of anthelmintic strategies against strongyle species under similar climatic conditions with similar biological development. However, the true impact of the simulated therapeutic control strategies ought to be supported by field data to see if these confirm the theoretical predictions.

The application and use of mathematical models to the study of equine health problems in general and parasitic infection in particular, is not exploited as much as it is in human and other animal species. To maximise the benefit and use of these tools in the study of equine health problems, future generations of veterinarians will require at least a rudimentary understanding of these methods.

Generally this study is believed to have increased our understanding of many aspects of donkey parasites and further contributed to narrowing the existing information gap. It has advanced donkey parasitology, and provides a background to assess the extent of their adverse effects and economic importance.

It is estimated that over 50% of the energy required for agricultural production in the world is derived from animals and the donkey is a major contributor to this need. Parasites have been found as a major cause of early demise of donkeys in developing countries (Svendsen, 1997a). The health and welfare of such draught animals is the only wealth of a poor family in most developing countries. Despite the fact that donkeys play a significant role in the farming system and the livelihood of a large number of farmers and traders, research and

development into the different aspects of donkey parasitology has been lacking, an issue identified by several people writing or working on promoting the case of the donkeys (Svendsen, 1991; Starkey, 1994a).

Extensive work is underway with the donkey population, mostly with the help of the animal charity organisations such as the Donkey Sanctuary, there should be, therefore, ample opportunities in the future to examine parasitic problems of the donkey. There is still much to be done in the classical parasitology of the donkey. It is important to produce a definitive list of parasites of the donkey and study their biological dynamics, epidemiology, pathogenic effect and their economic significance. The new molecular techniques should be able to solve the problem of identification and pathogenesis, and determine whether they are shared by the horse or vary from them. The immunological status of donkeys against parasitic infection is totally unknown and is one area of interest. It is also of interest whether equids in the tropics react to parasitic infections differently from their counterpart in temperate zones. Unraveling many of the aspects of donkey parasitology may benefit not only the donkey but also other equids.

In the past, the lines of G. K. Chesterton:

“With monstrous head and sickening cry

And ears like errant wings,

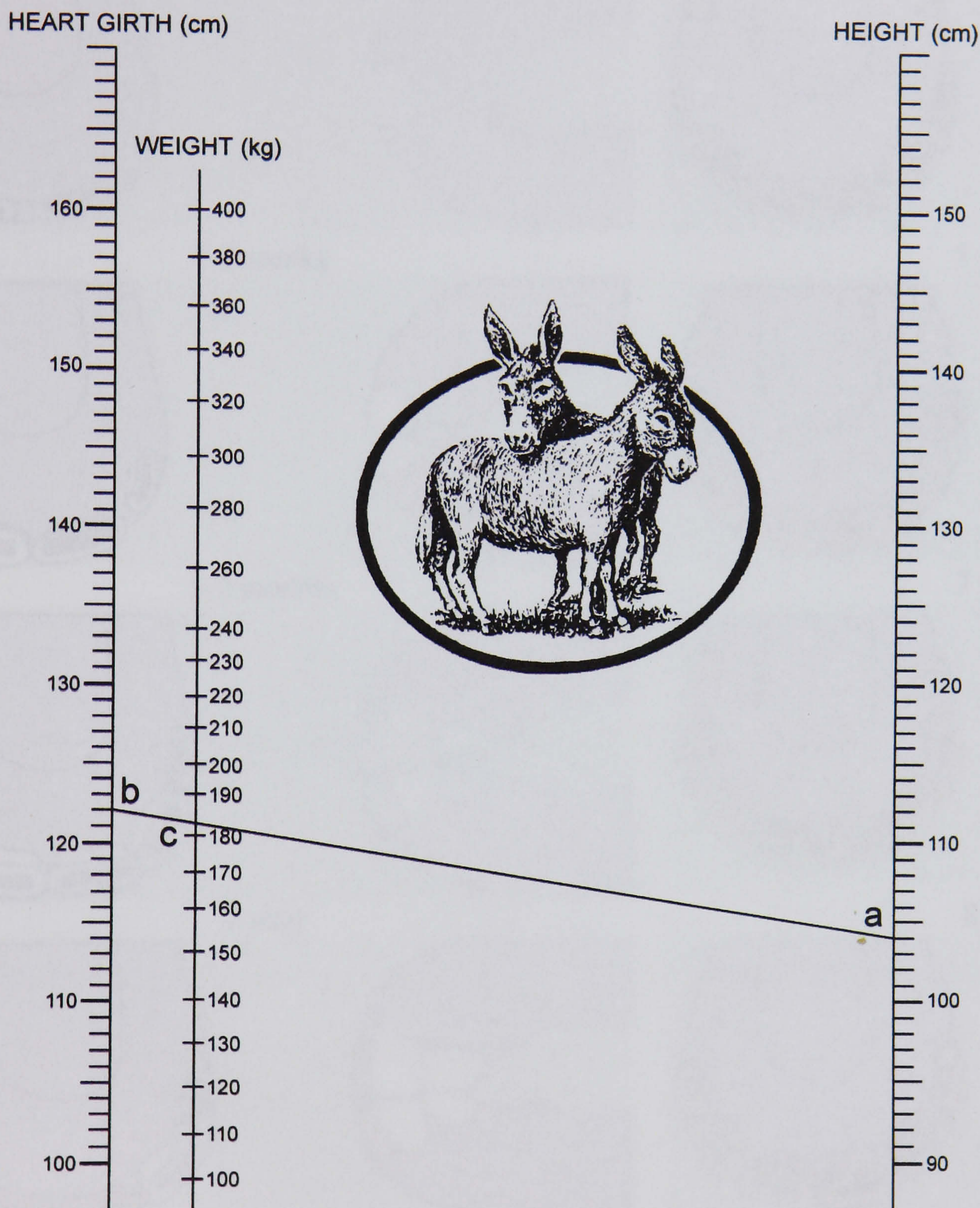
The devil’s walking parody

On all four footed things”,

may well have epitomized our ignorance and misunderstanding of the many aspects of the donkey; not so now!

APPENDICES

Appendix I: Heart girth nomogram for the estimation of body weight of donkeys.



A donkey 104cm tall (a) and with a heart girth 122cm (b) should weigh 181kg (c).
The nomogram is accurate to within 10 kilograms.

Weight table for donkeys under 2 years:

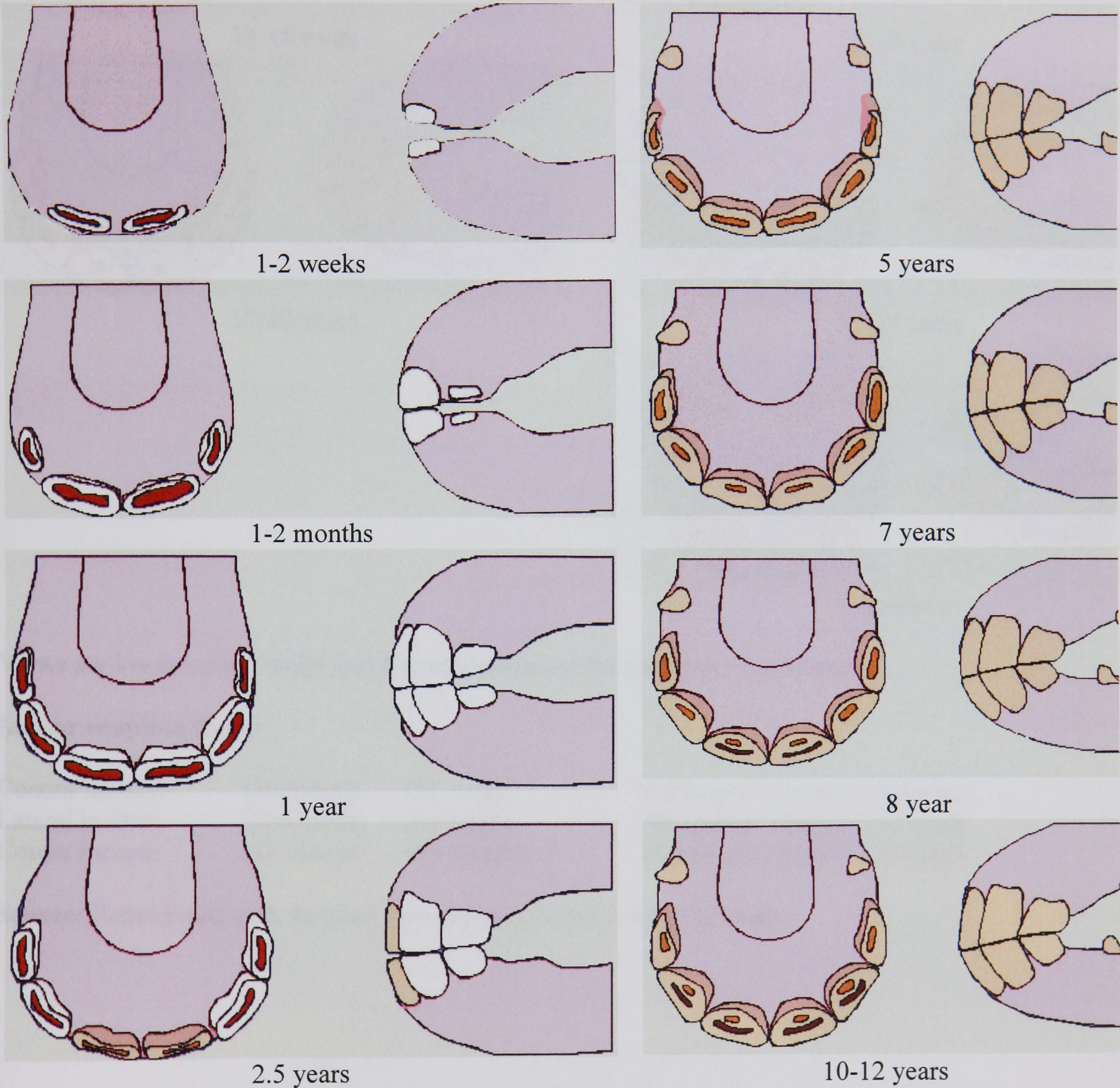
Heart Girth cm	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100
Weight kg	46	47	49	51	53	55	57	59	61	63	65	67	69	71	74	76	78	81	83	86	88	91	94	96	99	102

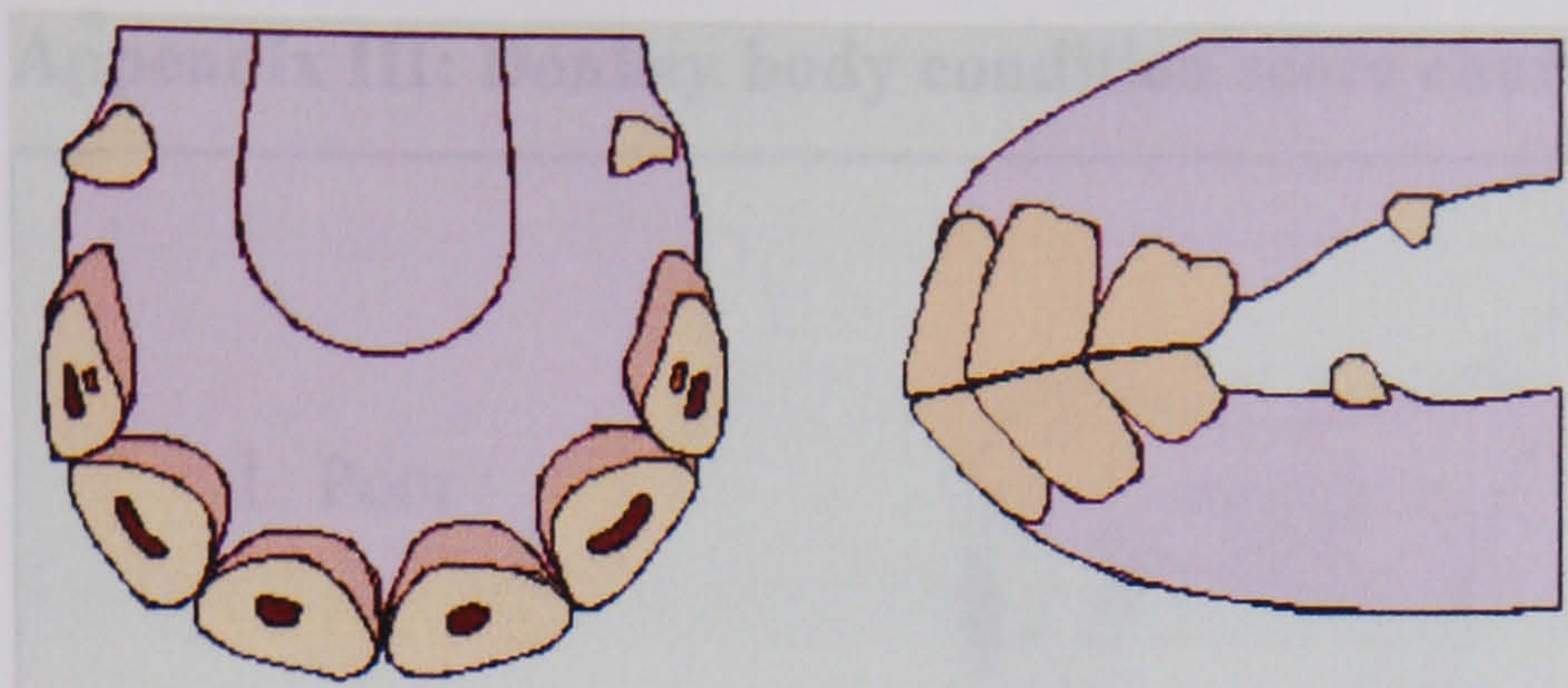
For adult donkey; weight = $0.000252 \times \text{height}^{0.240} \times \text{heart girth}^{2.575}$
For donkey under 2 years; weight = $0.000283 \times \text{heart girth}^{2.778}$

Source: Adapted from Svendsen, (1997).

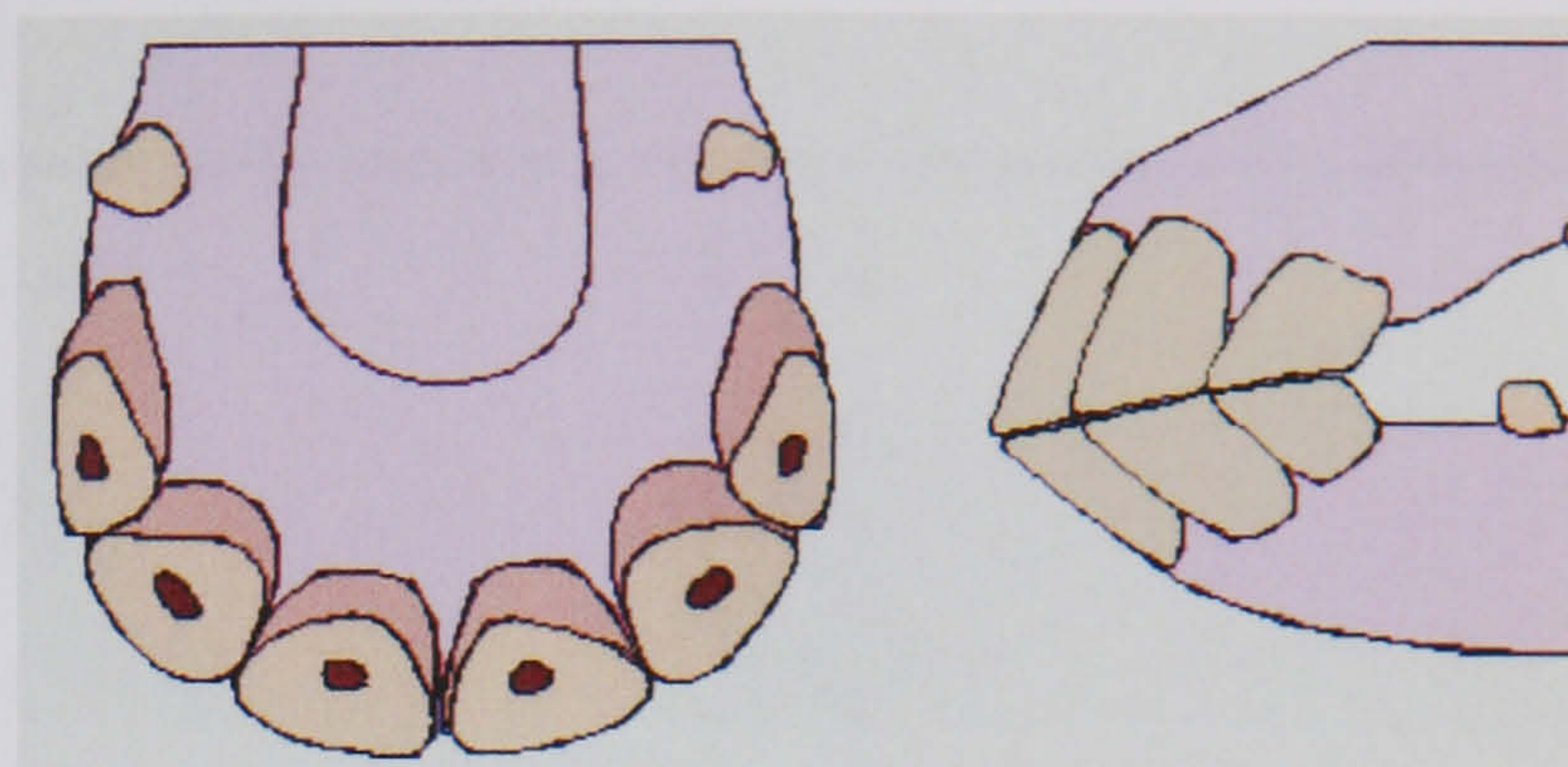
Appendix II: Guide to the ageing of donkeys using incisor eruption times and wear.

All ages are approximate, however up to 8 years reasonable accuracy can be assumed, above this age factors such as mal-occlusion, stable vices and quality of feed can have a large effect on incisor rate of wear and therefore decreasing accuracy of age estimation.

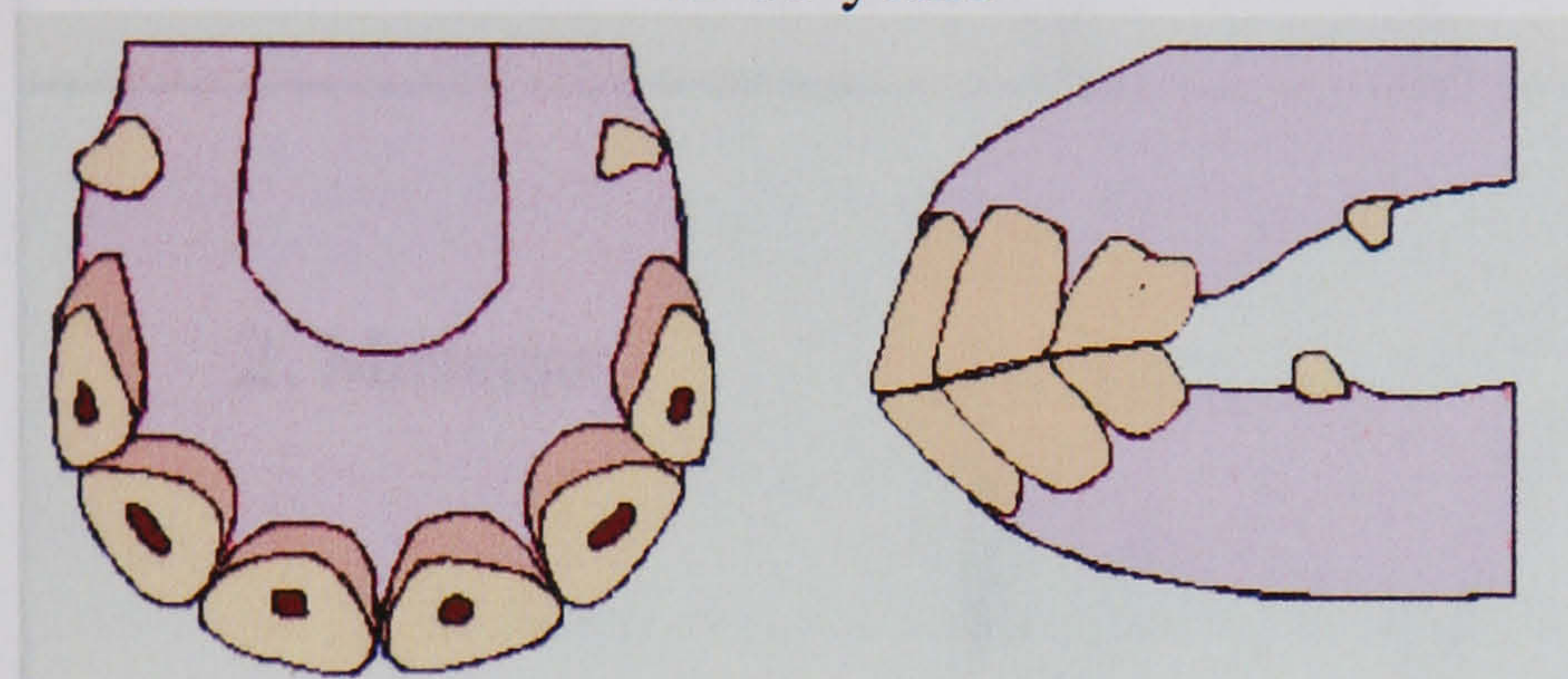




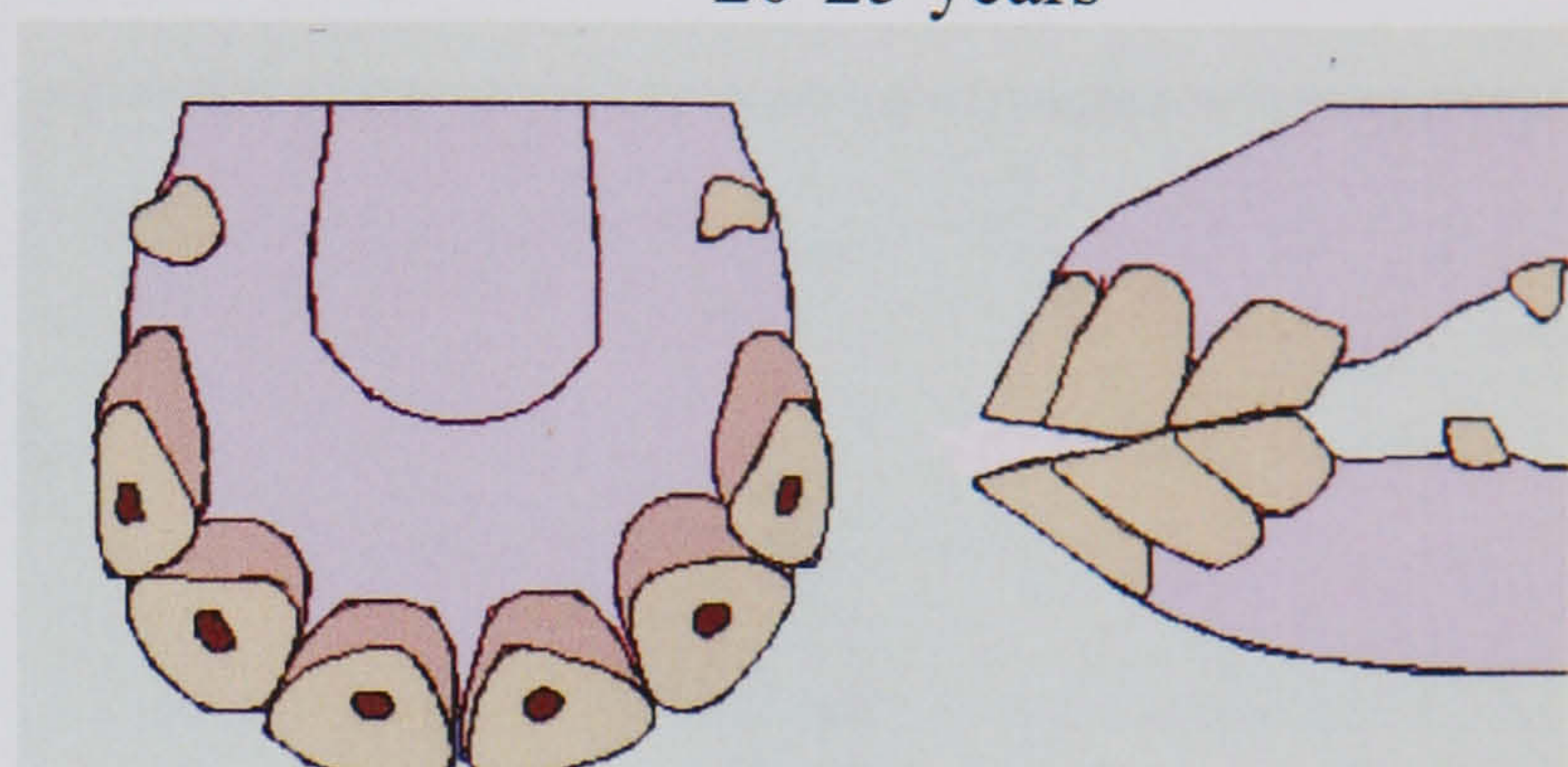
12-15 years



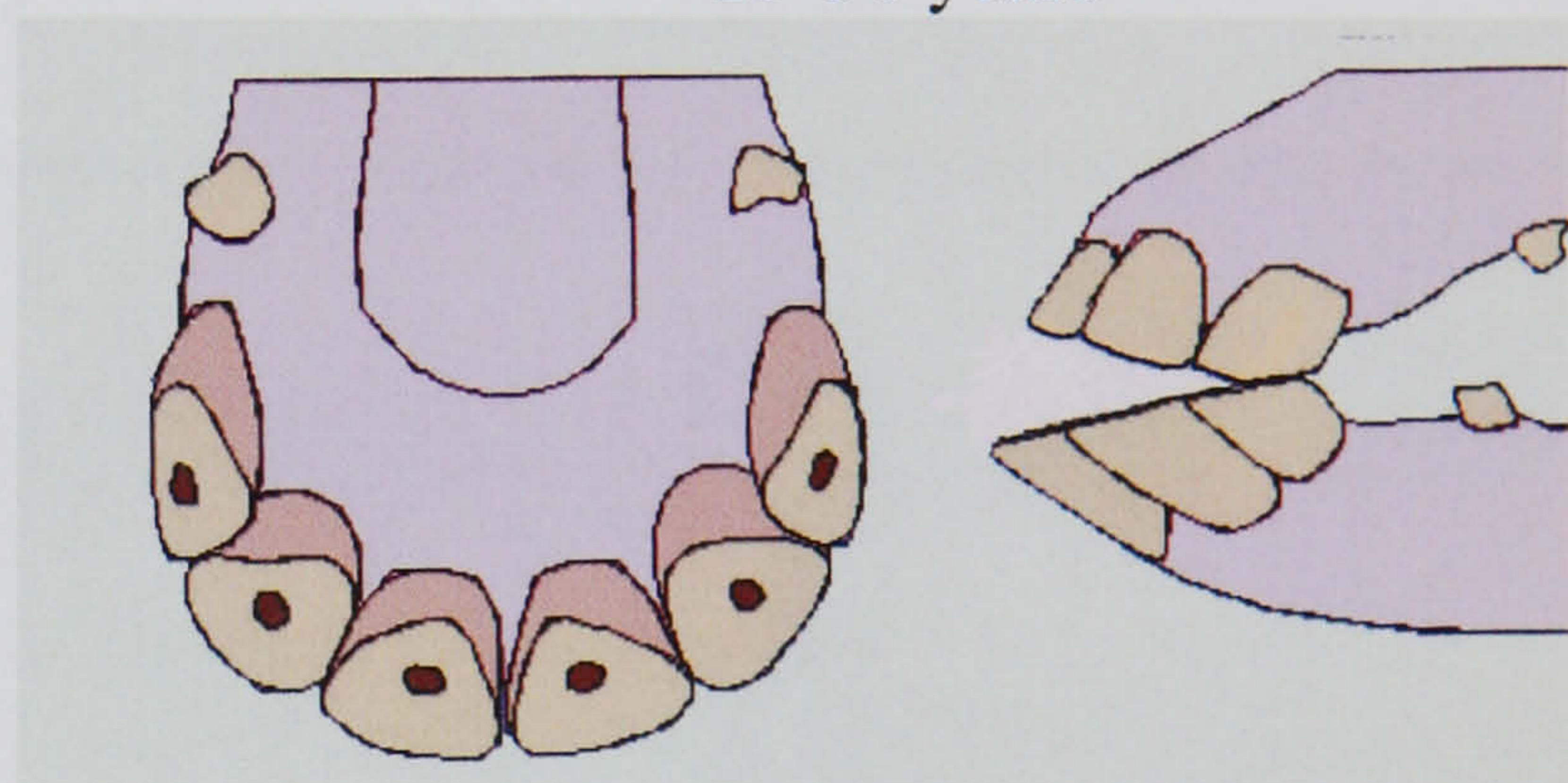
20-25 years



15-20 years



25-30 years



30 years +

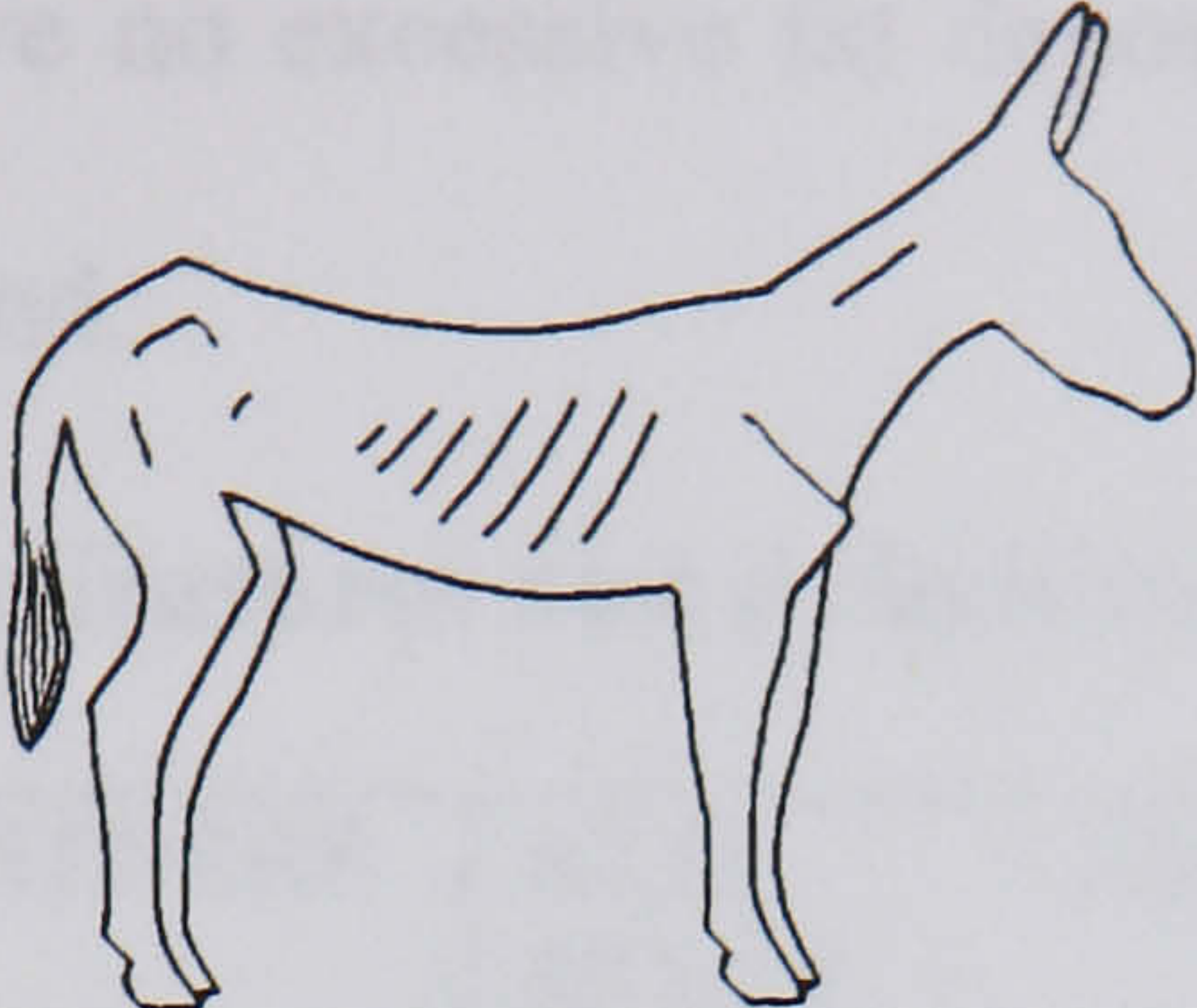

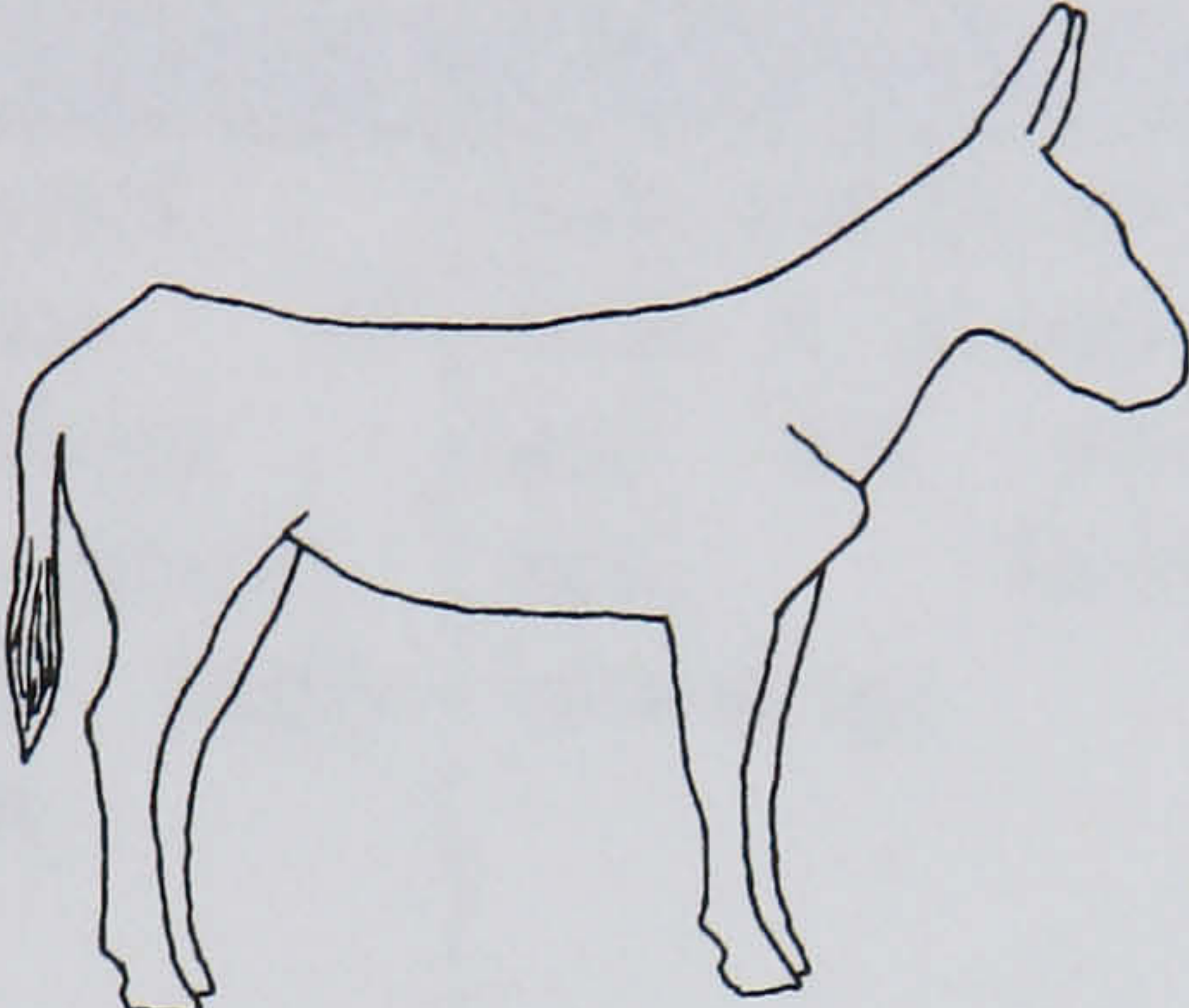

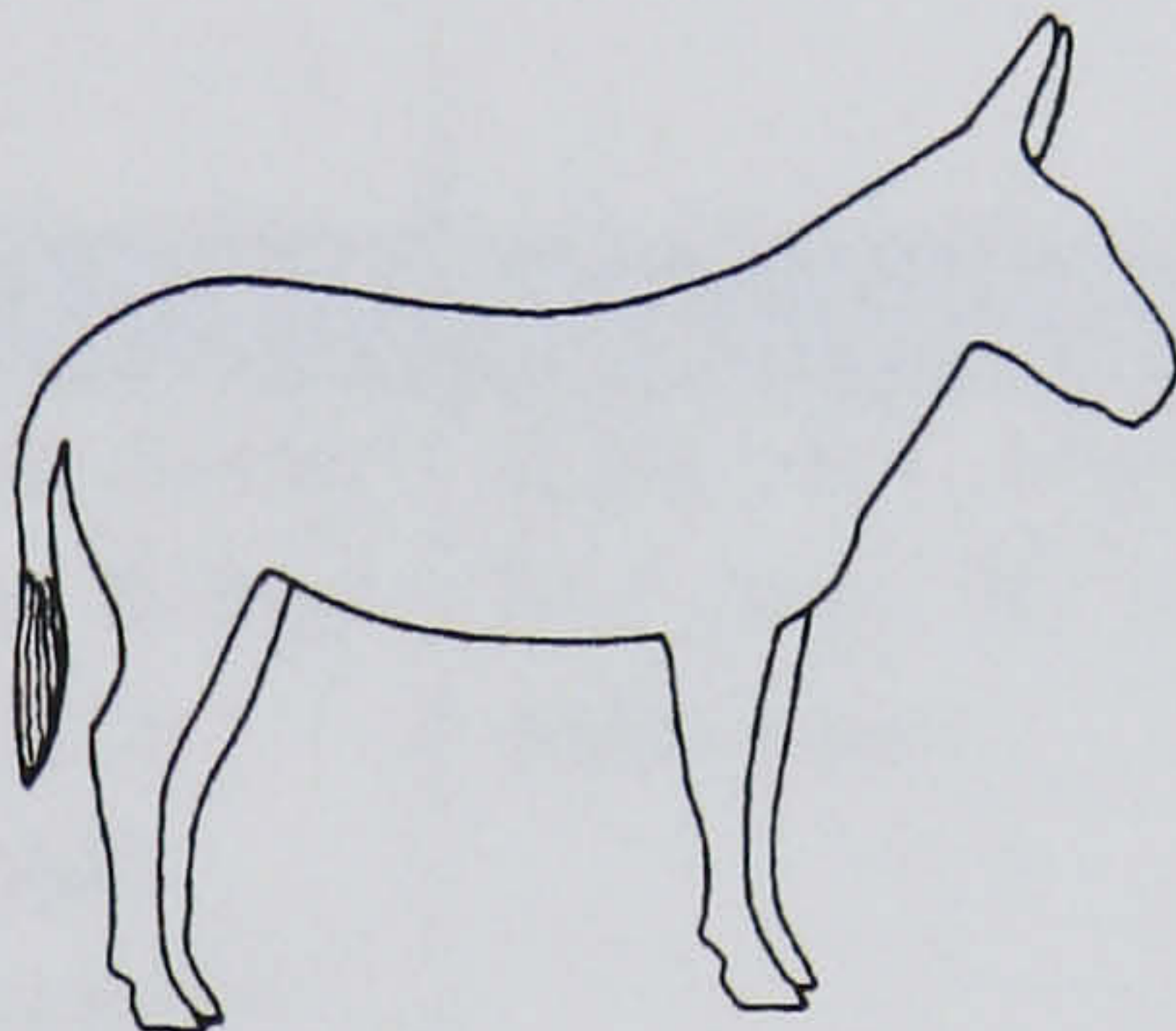

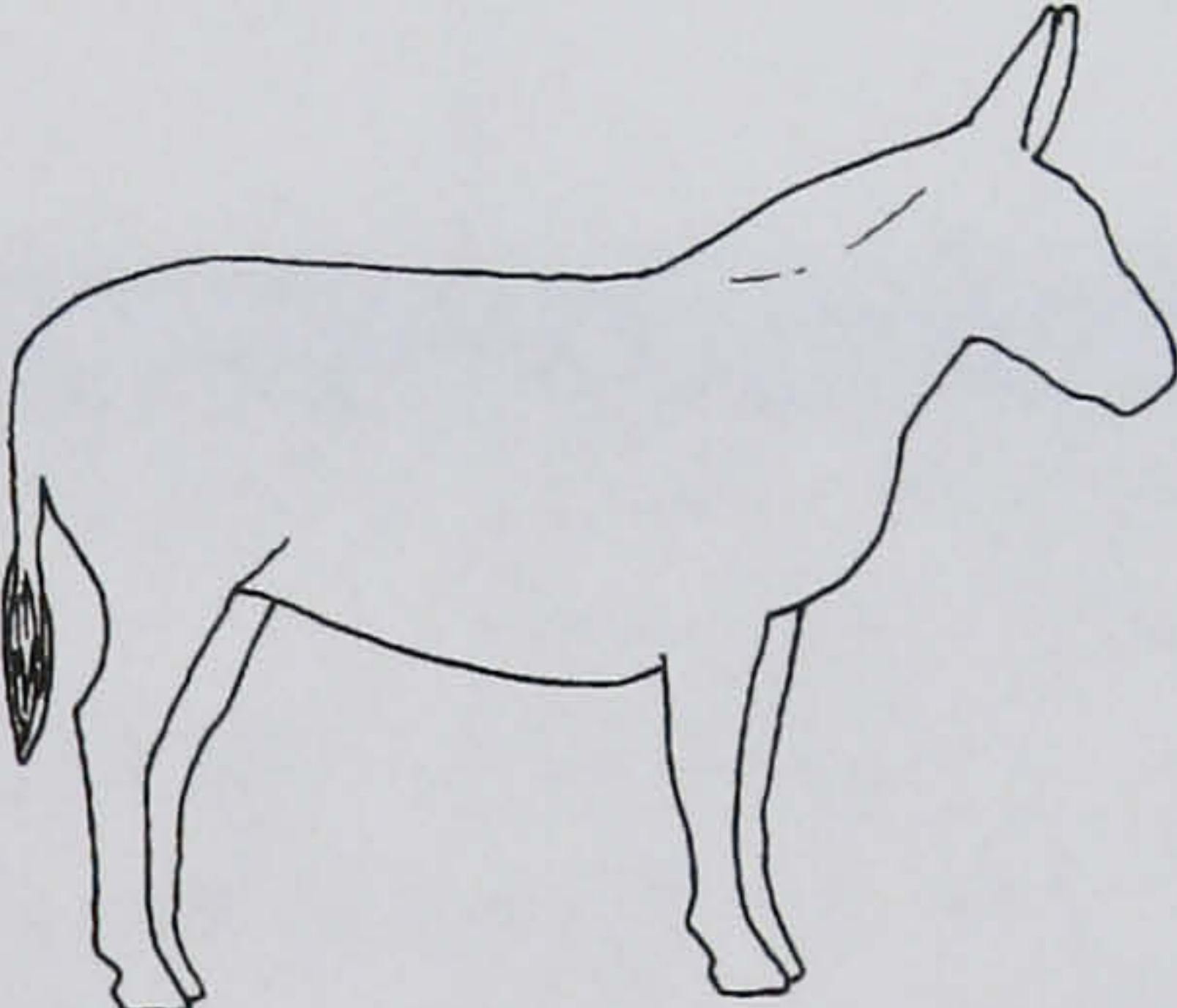
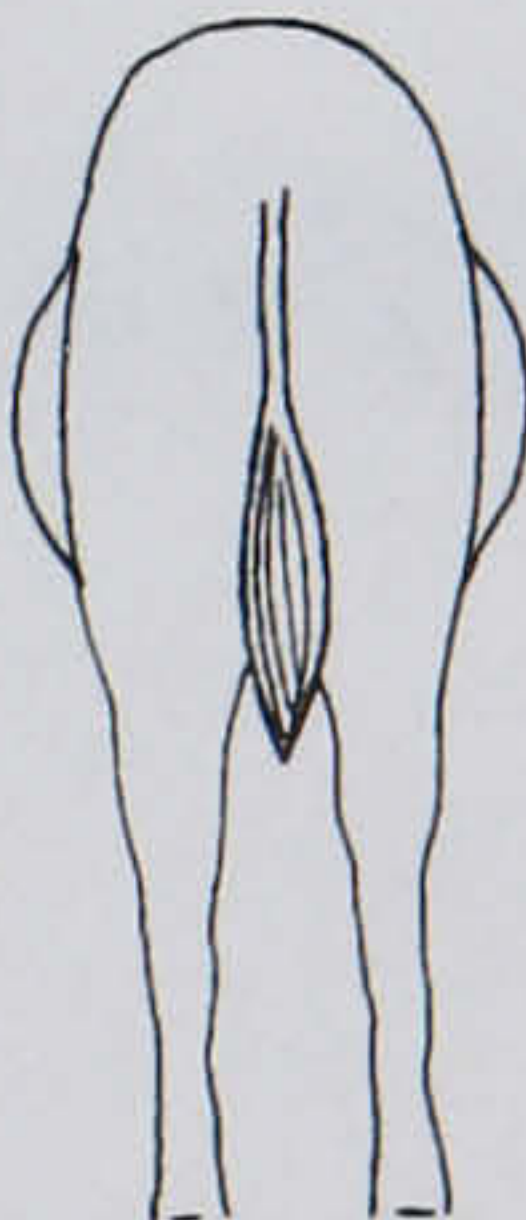
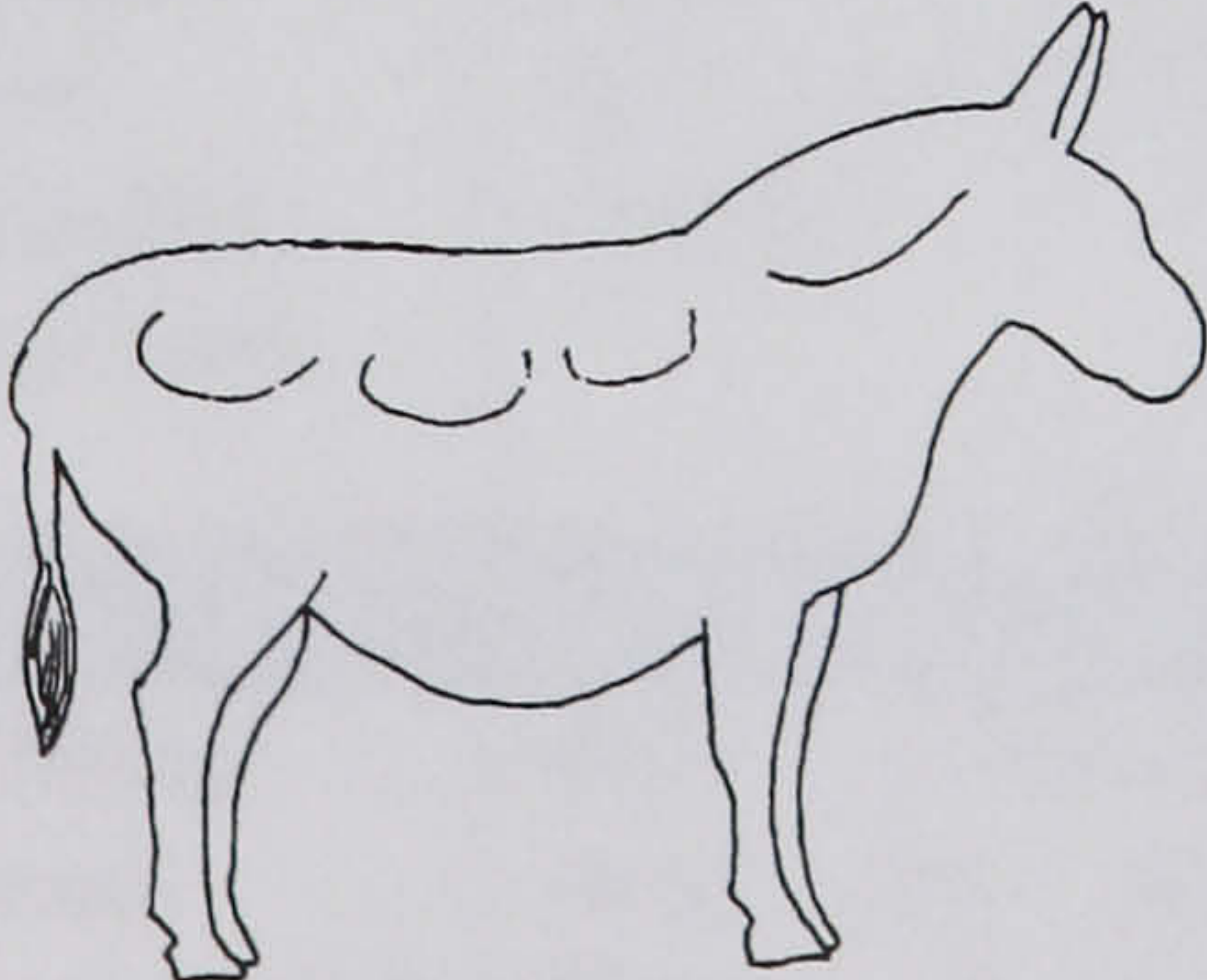
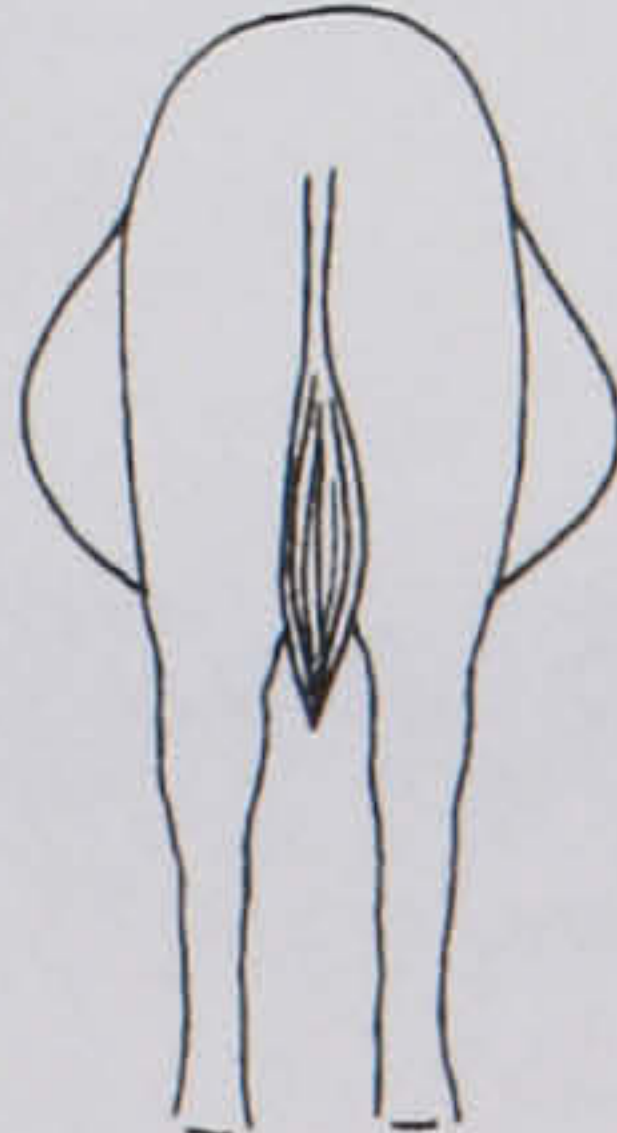
Views are lower incisor tables and lateral. Permanent teeth are shown coloured.

Incisor eruption times:

Central incisor:	Deciduous:	0-1 week	Permanent: Approx. 2.5 years
Lateral incisor:	Deciduous:	2-4 weeks	Permanent: Approx. 3.5 years
Corner Incisor:	Deciduous:	7-9 months	Permanent: Approx. 4.5 years

Source: Reproduced with the kind permission of The Donkey Sanctuary.

Appendix III: Donkey body condition score chart.

1. Poor		
2. Moderate		
3. Ideal		
4. Fat		
5. Obese		

Fat deposits may be unevenly distributed especially over the neck and hindquarters. Some resistant fat deposits may be retained in the event of weight loss and/or may calcify (harden).

Careful assessment of all areas should be made and combined to give an overall score. Most of the donkeys in Ethiopia have no excessive fat deposit and donkeys with body condition score more than 3 were not found.

Table III.1. The characteristic features and definition of body condition scores

CONDITION SCORE	NECK AND SHOULDERS	WITHERS	RIBS AND BELLY	BACK AND LOINS	HINDQUARTERS
1. POOR	Neck thin, all bones easily felt. Neck meets shoulder abruptly, shoulder bones felt easily, angular.	Dorsal spine of withers prominent and easily felt.	Ribs can be seen from a distance and felt with ease. Belly tucked up.	Backbone prominent, can feel dorsal and transverse processes easily.	Hip bones visible and felt easily (hock and pin bones). Little muscle cover. May be cavity under tail.
2. MODERATE	Some muscle development overlying bones. Slight step where neck meets shoulders.	Some cover over dorsal withers, spinous processes felt but not prominent.	Ribs not visible but can be felt with ease.	Dorsal and transverse processes felt with light pressure. Poor muscle development either side of midline.	Poor muscle cover on hindquarters, hip bones felt with ease.
3. IDEAL	Good muscle development, bones felt under light cover of muscle/fat. Neck flows smoothly into shoulder, which is rounded.	Good cover of muscle/fat over dorsal spinous processes withers flow smoothly into back.	Ribs just covered by light layer of fat/muscle, ribs can be felt with light pressure. Belly firm with good muscle tone and flattish outline.	Cannot feel individual spinous or transverse processes. Muscle development either side of midline is good.	Good muscle cover in hindquarters, hip bones rounded in appearance, can be felt with light pressure.
4. FAT	Neck thick, crest hard, shoulder covered in even fat layer.	Withers broad, bones felt with firm pressure.	Ribs dorsally only felt with firm pressure, ventral ribs may be felt more easily. Belly overdeveloped.	Can only feel dorsal and transverse processes with firm pressure. Slight crease along midline.	Hindquarters rounded, bones felt only with firm pressure. Fat deposits evenly placed.

5. OBESE	Neck thick, crest bulging with fat and may fall to one side. Shoulder rounded and bulging with fat.	Withers broad, unable to feel bones.	Large, often uneven fat deposits covering dorsal and possibly ventral aspect of ribs. Ribs not palpable. Belly pendulous in depth and width.	Back broad, unable to feel spinous or transverse processes. Deep crease along midline bulging fat either side.	Cannot feel hip bones, fat may overhang either side of tail head, fat often uneven and bulging.

Half scores can be assigned where donkeys fall between scores. Aged donkeys can be hard to condition score due to lack of muscle bulk and tone giving thin appearance dorsally with dropped belly ventrally, while overall condition may be reasonable. Such body condition, however, is very rare in working donkeys and is not difficult to condition score.

Source: Reproduced with kind permission of The Donkey Sanctuary and adapted to local donkeys.

Appendix IV: Egg measurement of non-strongyle intestinal helminths of donkeys

Materials and methods

Eggs of parasitic cestodes, trematodes and parascaris recovered from matured adult parasites and faecal samples from donkeys were measured using an ocular micrometer in a calibrated microscope (Todd, 1979). Both the width and length (depending on the shape of eggs) were measured. Depending on the number of eggs recovered, 1-50 and 100 eggs were measured from positive faecal samples and matured adult female parasites, respectively.

Results and discussion

The number of eggs measured for each parasite and their respective measurements are shown in Table IV.1. The objective of the study was to fill the information gaps concerning the different aspects of donkey parasites. Egg measurement data are not available for donkey parasites and it is that of the horse which is used by extrapolation. Data published for horses on respective parasite egg measurements is given for comparison in Table IV. 2. Although there is variation, the result has shown that most of the average measurements of the different parasite eggs recovered from donkeys are within the range documented for horses. However, the ranges of measurement for *Gastrodiscus* eggs are quite notably larger than that of horses. The data will be useful for future references. Egg measurement of other parasites infecting donkeys is also under study and will be available soon.

Table IV.1. Measurements of eggs recovered from fresh specimens of matured adult parasites of donkeys and faecal samples.

Helminths	Number of eggs measured	Measurements (µm)			
		Length		Width	
		Average	Range	Average	Range
<i>A. perfoliata</i>	1224	-	-	73.8	64.3-100.8
<i>A. magna</i>	667	-	-	52.1	44.1-61.70
<i>P. equorum</i>	1064	-	-	89.7	73.1-107.1
<i>Fasciola</i> spp.	1033	142.1	88.2-163.8	78.5	63-100.8
<i>G. aegyptiacus</i>	1032	174.1	132.3-207.9	99.7	63-115.9

Table IV.2. Reported egg measurements of cestodes, trematodes and parascaris from horses

Helminths	Measurements (µm)		
	Length	Width	Sources
<i>A. perfoliata</i>	-	65-80	Foreyt, 2001; Soulsby, 1986
<i>A. magna</i>	-	50-60	Soulsby, 1986
<i>P. equorum</i>	-	90-100	Foreyt, 2001; Soulsby, 1986
<i>Fasciola hepatica</i>	130-150	63-90	Soulsby, 1986;Shah-Fischer 1988
<i>Fasciola gigantica</i>	156-197	90-100	Soulsby, 1986; Angus, 1978
<i>G. aegyptiacus</i>	131-139	78-90	Soulsby, 1986

Appendix V: Evaluation of the 12/13 kDa antigen in detecting anti-12/13 KDa IgG(T) in donkey sera

Materials and methods.

Prior to using the ELISA kit developed for the detection of *A. perfoliata* antibody in horses' sera (anti-12/13kDa IgG(T)) a test was made whether this antigen can detect *A. perfoliata* antibody in donkey sera. For this purpose sera from 20 donkeys known to be coprologically positive for cestode were obtained from the Donkey Sanctuary in Devon, England. Sera from 6 and 2 donkeys from Ethiopia known to be negative and positive for *A. perfoliata*, respectively, at necropsy were also validated. Assay protocol is as described in Chapters Two and Five.

Results.

The results of the evaluation are shown in Table V.1. In this assay 3(15%), 8(40%) and 9(45%) of the donkeys were identified with high, medium and low infection intensity, respectively.

Sera from 6 donkeys negative for *A. perfoliata* on post-mortem examination produced ELISA OD value of 0.196 ± 0.01 (mean \pm sd). This was considered as the negative cut-off value. ELISA OD of 0.174 was obtained from serum of a donkey negative to *A. perfoliata* but positive for *A. magna* (5 mature and 3 immature worms), which is below the cut-off values calculated for *A. perfoliata* negative ponies (0.20). Sera from two donkeys with worm counts of 2 and 23 *A. perfoliata* on post-mortem examination produced OD values of 0.129 and 0.306, respectively.

Table V.1. Results of the evaluation of the antigen 12/13 kDa in detecting anti-12/13 kDa IgG(T) in donkey sera coprologically positive for cestode eggs obtained from the donkey sanctuary, England.

Sample ID	OD	Result/intensity of infection
T1	0.733	High
T2	0.588	Medium
T3	0.503	medium
T4	0.040	Zero/Low
T5	0.200	Zero/Low
T6	0.721	High
T7	0.210	Zero/low
T8	-0.020	Zero/Low
T9	0.450	Medium
T10	0.046	Zero/Low
T11	0.220	Medium
T12	0.878	High
T13	0.276	Medium
T14	0.499	Medium
T15	0.156	Zero/Low
T16	0.441	Medium
T17	0.317	Medium
T18	0.050	Zero/Low
T19	0.179	Zero/Low
T20	0.141	Zero/Low

Discussion

Validation of the serological assay showed that the 12/13 kDa affinity purified E/S antigen of *A. perfoliata* was able to detect the anti-12/13 kDa IgG(T) in donkeys’ sera. Although all the donkeys used in validating the test were coprologically positive for cestode eggs, the practical difficulty in differentiating eggs of *A. perfoliata* from *A. magna* and the known inability of the immunological assay to recognize individuals with low infection intensity did

not allow us to tell whether the donkeys with low OD values (≤ 0.20) were those with very low worm burden or were, in fact, negative for *A. perfoliata*. The 55% sero-prevalence with OD values corresponding to either moderate or high infection intensity, however, showed the assay's ability to detect the antibody in donkey sera, and may also indicate that *A. perfoliata* was the dominant species.

The negative cut-off value of 0.196 OD determined from the 6 donkeys negative for *A. perfoliata* on post-mortem examination is close to the one obtained for helminth naïve ponies of 0.20 (Proudman and Trees, 1996b). Even though these 6 donkeys were found to be negative at sampling, their previous status was not known, so they may not have been helminth naïve. It is therefore difficult to rule out persistence of antibody from previous infections. However, they were not treated against cestodes, which reduces antibody level (Proudman and Trees, 1996b). Both from the immunological point of view and the limited number of samples, it may not be appropriate to use this as a definitive negative cut-off value in donkeys. A sufficiently large sample from helminth naïve donkeys should be used to come up with a valid cut-off value. Therefore, for all our data analysis the negative cut-off value (0.20) calculated for helminth naïve ponies (Proudman and Trees, 1996b) was used. Two donkeys found positive for *A. perfoliata* on post-mortem examination further validated the ability of the assay to detect IgG(T). The ELISA OD values 0.129 and 0.306 from these donkeys with 2 and 23 worms, respectively, indicate the assay's ability to categorise positive samples into low and medium infection intensity, although it is not possible to draw a definitive conclusion from such limited number of cases. Serological studies by Proudman and Trees (1996b) and Hoglund et al. (1995) showed no cross-reaction with other cestode species or other helminths. The OD value of 0.174, which is below the negative cut-off value, obtained from a donkey negative for *A. perfoliata* but positive for *A. magna* may support their findings although again a single case is not sufficient to draw such conclusion.

Appendix VI: Identification of *Fasciola* species recovered from donkeys in Ethiopia.

Introduction

The overall size and shape of *Fasciola hepatica* and *Fasciola gigantica* differ in that adults of the former are smaller and have a well developed shoulder distal to the oral sucker; the latter is more streamlined without a shoulder (Armour, et al., 1997). In mixed infections where both species are present there is an overlap in size and sometimes they are difficult to differentiate. Lee, et. al. (1993) also indicated that they are polymorphic and may vary morphologically depending upon the host being parasitised. Therefore a pilot study was conducted to identify the *Fasciola* species recovered from donkeys in Ethiopia.

Material and methods

Apart from morphological identification, a technique involving morphoanatomic differences (Graber and Perrotin, 1983; Lofty et al., 2002) was used to differentiate *Fasciola* species recovered from donkeys. For this purpose only adult flukes, identified by the presence of numerous eggs in the uterus, were used. 184 adult flukes were stained with alcoholic carmine hydrochloride to study the morphological details and branching patterns of intestine, ovaries and testes.

Stain and staining technique (Graber and Perrotin, 1983).

Alcoholic- carmine hydrochloric acid staining

1. 5 gm carmine was mixed in a mortar with 5 ml water and 5 ml HCL and left for an hour.
2. The mixture was transferred into a flask with flat bottom by washing the mortar with 200 ml absolute alcohol.

3. The flask was closed with stopper that has a hole and heated in a water bath until the carmine dissolves completely. Absolute alcohol is added to replace the evaporated alcohol until the volume reached 200 ml.

Techniques:

1. Flukes were washed in phosphate buffer solution (PBS) and then incubated in PBS at 37°C overnight to allow them expel their contents (Lee, et al., 1992).
2. Flukes were dipped into the stain for 12 hrs.
3. They were then immersed in alcoholic-chloride solution (100 ml absolute alcohol and 0.5 ml hydrochloric acid) and monitored until the parasites reach a rose colour (approximately 12-20 minutes). This procedure was repeated by changing the alcoholic chloride solution.
4. Finally the worms were immersed in toluene to lighten them and examined under a compound microscope for details of organs.

Results and discussion

Considering the general shape and size, some worms resembled *F. hepatica*, others *F. gigantica* and intermediate forms. The morphoanatomic study has revealed two groups of flukes. The first group of flukes were with few median intestinal (caeca) branches resembling pouches, smaller and club-shaped ovarian branches, and simple testicular branches, while the second group of flukes were with numerous secondary and tertiary median intestinal branches; larger, more numerous with a more complicated appearance of ovarian branches and extremely sinuous and tightly coiled testicular branches. According to Bergeon and Laurent (1970), Sahba, et al. (1972), Kimura, et al. (1984) and Lotfy, et. al. (2002), the first group should be identified as *F. hepatica* while the second group as *F. gigantica*. This pilot study therefore indicates the infection of donkeys with both *F. hepatica*

and *F. gigantica* in Ethiopia. Many studies made in Ethiopia (Yilma and Malone 1998) have shown the presence of both species in domestic ruminants although they were based only on morphological identifications.

Only *F. hepatica* has been reported from donkeys in most previous studies. However, Hasslinger and El-Seify (1996) and Burgu (1995) reported only *F. gigantica*. Such differences in fluke species and infection prevalences could be attributed to geographical and/or management variations. Most studies have reported the identification of fluke species based on their morphometric and/or morphological differences. The use of isoelectric focusing (IEF), which shows soluble protein bands unique to each fluke species is a powerful tool for species identification and is recommended by Lee and Zimmerman (1993) and Lotfy, et. al. (2002).

Appendix VII: ModelMaker: its components and operation

ModelMaker is a computer-based modelling software package produced by Cherwell Scientific Ltd, Oxford, UK. It was used to model changes in the abundance and mortality rates of the different developmental stages of cyathostomins using the differential equations described in section 7.4.1. ModelMaker has different types of components each represented by a unique diagram. It is based on a network representation of the different groups to be modelled and has its own standard notations. There are many components used for different purposes but the main components used in the present model are of two kinds: component boxes and component lines.

1. Component boxes: these include, compartments, variables, delay and lookup tables.

1.1 Compartments: these are rectangular boxes defined in terms of what is entering and leaving them similar to a network diagram. They stand for the state variables of the model whose initial values are defined in the component definition dialog box. They represent integrators in the model flow. The value of each compartment represents the quantity held within it and is calculated using a differential equation, which gives the rate of change of the value. In our model these compartments represent the three state variables: E, L3 and A.

1.2 Variables: these are component of the ModelMaker whose values are calculated as the model runs by the defined function or formula of components defined in the model. They are represented by elliptical boxes. For example: in the basic architecture of the model (Fig. 7.3) the ingestion rate (s) and mortality rate (μ_1) of the infective larvae on pasture are variables whose values are calculated as the model runs using the defined functions.

1.3 Lookup tables: These are tables, which provide a mechanism for linking models with external numerical data. Lookup tables hold their data in a lookup view, which allows

direct entry of data into the model. For example, in our model structure (Fig. 7.3) '*R*' represents a lookup table, which contains the rainfall data used by the model to calculate the mortality rate of infective larvae.

1.4 Delays: The consequences of certain occurrences may not be felt by the model until some period of time has passed. To accommodate this, ModelMaker provides a delay component, which takes the value of another model component and delays it for a defined period of time before feeding it back into a model. A delay is defined using the delay definition dialog box in terms of the component whose value is to be delayed, a value or mathematical expression defining the duration of the delay and an initial value. Hexagonal boxes in the ModelMaker represent delays. For example, in our model structure τ_1 , τ_2 and τ_3 represent time delays to model the development of eggs to infective L3 stage, ingested infective larvae to adult, and delay to model the survival of adults in the gut, respectively.

2. Component Lines: These are another important ModelMaker component. They include flows and influences represented by solid arrows and dashed arrows, respectively. Flows are used to form a network diagram between the state variables or compartments. They represent the flow of individuals from one state variable to another, and are used in conjunction with the state variables to build up a system of differential equations. The influences on the other hand, show where the value of one component affects the value of another. For example, they influence the value of one component to be used in calculating the value of another using some defined function.

3. Parameters: ModelMaker uses parameters to store constant values. Parameters do not have a diagram representation but are defined in the parameters definition dialog box. All

parameters are added and manipulated in the parameter view of the ModelMaker. Their values can easily be linked to the model components for calculations.

4. ModelMaker run: Once a model has been defined by inserting and configuring all the necessary model components and parameter values, it can be run. This is the process of solving all the model component equations using the selected integration method, over the desired range of the model's independent variable. By default ModelMaker's independent variable is time, t . All equations are solved over a range of values for t , and all equations are written with respect to t .

5. Output points: This is the number of output points for plotting or displaying in a table. It is possible to set different output points in ModelMaker. In the present ModelMaker run, we set the output points to 120, each representing one month for 10 years simulation.

References

- Adair, H.S. (1990) What is your diagnosis? *J. Am. Vet. Med. Assoc.*, **196**, 2023-2024.
- Adams, D.B. (1982) Time of onset and target of immune reactions in sheep with acquired immunity against *Haemonchus contortus*. *Int. J. Parasitol.*, **12**, 439-443.
- Admassie, Y., Abebe, M., Ezra, M. and Gay, J. (1983) Ethiopian high lands reclamation study: report on sociological survey and sociological considerations in preparing a development strategy. Institute of Development Research Working Paper No.4. University of Addis Ababa, Ethiopia.
- Agneessens, J. Engelen, S., Debever, P. and Vercruysse, J. (1998) *Gasterophilus intestinalis* infections in horses in Belgium. *Vet. Parasitol.* **77**, 199-204.
- Alcaino, H.M., Gorman, T.R., Guevara, T.R. and Fernandez, M.V. (1983) Fascioliasis and parasites of the large intestine of horses in the central south region of Chile. *Archivos de Medicina Veterinaria*. **15** (1), 27-35.
- Alkahane, H., Harada, Y. and Oshima, T. (1970) Patterns of the variation of the common liver fluke (*Fasciola* sp.) in Japan. III. Comparative studies on the external forms, size of eggs and number of eggs in the uterus of fluke in cattle goat and rabbit. *Japanese Journal of Parasitology*. **19**, 619-627.
- Alkahane, H., Harada, Y. and Oshima, T. (1974) Patterns of the variation of the common liver fluke (*Fasciola* sp.) in Japan. IV. Comparative studies on the external forms, size of eggs and number of eggs in the uterus of fluke in pig and horse. *Japanese Journal of Parasitology*. **23**, 207-212.
- Alves, R.M.R., Rensburg, L.J.van. and Wyk, J.L. van. (1988) Fasciola in horses in the Republic of South Africa: A single natural case of *Fasciola hepatica* and the failure to infest ten horses either with *F. hepatica* or *F. gigantica*. *Onderstepoort J Vet Res*. **55**(3), 157-163.
- Amborski, G.F., Bello, T.R. and Torbert, B.J. (1974) Host response to experimentally induced infection of *Strongylus vulgaris* in parasite-free and naturally infected ponies. *Am. J. Vet. Res.*, **35**, 1181-1188.
- Anderson, N., Luong, T.T., Vo, N.G., Bui, K.L., Smooker, P.M. and Spithill, T.W. (1999) The sensitivity and specificity of two methods for detecting *Fasciola* infections in cattle. *Vet. Parasitol.*, **83**, 15-24.
- Anderson, R.M. and May, R.M. (1978) Regulation and stability of host-parasites population interactions. I. Regulatory processes. *J. Anim. Ecol.*, **47**, 219-247.

- Anderson, R.M. and May, R.M. (1979) Population biology of infectious diseases: part 1. *Nature*, **280**, 361-367.
- Anderson, R.M. and May, R.M. (1991) Infectious diseases of human; dynamics and control . Oxford Science publications, Oxford. p16.
- Anderson, R.M. and May, R.M. (1992) Infectious diseases of humans: Dynamics and control. Oxford University Press, Oxford, New York.
- Anderson, R.M. and Nokes, D.J. (1991) Mathematical models of transmission and control. In: Holland, W.W, Detels, R., Knox, G., Fitzsimmons, B., and Gardner, L. (eds). Oxford textbook of public health (2nd ed, vol. 2), pp 225-252. Oxford Medical Publications.
- Andonova, S. (1997) An economic analysis of the benefits of donkeys' utilization in Bulgaria. Paper presented for Animal Traction Network in Eastern and Southern Africa (ATNESA) workshop on improving donkey utilization and management. 5-9 May, 1997. Debre Zeit, Ethiopia.
- Andrews, S.J. (1999) The lifecycle of *Fasciola hepatica*. In: J. P. Dalton (ed). Fasciolosis. CAB International, Oxon. pp. 1-29.
- Angus, M. (1978) Veterinary helminthology (2nd ed.). William Heinmann Medical Book Ltd, London.
- Anon, (1972) A review of Animal Health and Livestock Productivity Factors in Ethiopia, 1965-1971. Ethiopian Ministry of Agriculture, Addis Ababa.
- Ansel, M. and Thibaut, M. and Saez, H. (1974) Scanning electron microscopy on *Parascaris equorum* (Goeze, 1782), Yorke and Maplestone, 1926. *Int. J. Parasitol.*, **4**, 17-23.
- Applewhaite, L.M. and Ruiz, A. (1983) *Gastrodiscus aegyptiacus* in horses in Guyana. *Br Vet J.* **139**(3): 265-266.
- Armour, J. (1980) The epidemiology of helminth disease in animals. *Vet. Parasitol.*, **6**, 7-46.
- Armour, J. Urquhart, G.M., Duncan and J.L. (1997) Veterinary Parasitology (2nd ed).
- Aron, J.L. and Schwartz, I.B. (1984) Seasonality and period-doubling bifurcation in an epidemic model. *J.Theor. Biol.*, **100**, 665-679.
- Arslan, M.O. and Umur, S. (1998) The helminth and eimeria (protozoa) species in horses and donkeys in Kars province of Turkey. *Acta Parasitologica Turcica*, **22** (2), 180-184.

- Ashmawy, K.I. and Diab, M.R. (1998) Fasciola infestation among donkeys in Egypt. Eighth Scientific Congress, November, 1998. Assiut University, Faculty of Veterinary Medicine, Egypt. pp. 485-495.
- Audige, L., Doherr, M.G., Hauser, R. and Salman, M.D. (2001) Stochastic modelling as tool for planning animal health surveys and interpreting screening-test results. *Prev. Vet. Med.*, **49**(1-2), 1-17.
- Austin, S.M. (1994) Large strongyles in horses. *Compend. Contin. Educ. Pract. Vet.*, **16**, 650-656.
- Austin, S.M., Dipietro, J.A., Foreman, J.H., Baker, G.J. and Todd, K.S. (1990) *Parascaris equorum* infections in horses. *Compend. Contin. Educ. Pract. Vet.*, **12**(8), 1110-1118.
- Azzie., M.A. (1975) Pathological infection of thoroughbred horses with *Gastrodiscus aegyptiacus*. *J. South Af. Vet. Ass.* 46(1): 77-78.
- Bailey, C.J., Reid, S.W., Hodgson, D.R. Susann, C.J. and Rose, R.J. (1997) Risk factors associated with musculo-skeletal injuries in Australian thoroughbred racehorses. *Prev. Vet. Med.*, **32**(1-2), 47-55.
- Bailey, C.J., Reid, S.W., Hodgson, D.R., Bourke, J.M. and Rose, R.J. (1998) Flat, hurdle and steeple racing: risk factors for musculo-skeletal injury. *Equine Vet. J.*, **30**(6), 498-503).
- Bain, S.K. and Kelly, J.D. (1977) Prevalence and pathogenecity of *A. perfoliata* in a horse population in South Auckland. *NZ. Vet J.*, **25**, 27-28.
- Baker, D.W., Salisbury, G.W. and Britton, J.W. (1939) Control of equine strongylosis. I. The effects of natural factors on development of strongylosis in foals. *Cornell. Vet.*, **29**, 297-308.
- Ball, F. (1983) The threshold behaviour of epidemic models. *J. Appl. Prob.*, **20**, 227-241.
- Banks, D.J.D., Singh, R., Barger, I.A., Pratap, B. and Le Jambre, L.F. (1990) Development and survival of infective larvae of *Haemonchus contortus* and *Trichostrongylus colubriformis* on pasture in tropical environment. *Int. J. parasitol.*, **20**, 155-160.
- Barclay, W.P., Phillips, T.N. and Foerner, J.J (1982) Intussusceptions associated with *Anoplocephala perfoliata* infection in five horses. *J Am Vet Med Assoc.*, **180**, 752-753.
- Barger, I.A. (1989) Genetic resistance of hosts and its influence on epidemiology. *Vet. Parasitol.*, **32**, 21-35.
- Barger, I.A. and Le Jambre, L.F. (1988) Regulation of *Haemonchus contortus* in sheep: mortality of established worms. *Int. J. Parasitol.*, **18**, 269-273.

- Bargues, M.D. et al (1996) Natural water, an additional source of human infection by *Fasciola hepatica* in the Northern Bolivia, Altiplano. *Parasitologia*, 38: 251.
- Barlow, N.D. (1994) Bovine tuberculosis in New Zealand: epidemiology and models. *Trends Microbiol*, 2(4), 119-124.
- Barlow, N.D., Kean, J.M., Caldwell, N.P. and Ryan, T.J. (1998) Modelling the regional dynamics and management of bovine tuberculosis in New Zealand cattle herds. *Prev. Vet. Med*, 36(1), 25-38.
- Barnes, E.H. and Dobson, R.J. (1990) Population dynamics of *Trichostrongylus colubriformis* in sheep: Mathematical model of worm fecundity. *Int. J. Parasitol.*, 20, 375-380.
- Barnes, E.H., Dobson, R.J. and Barger, I.A. (1995) Worm control and Anthelmintic resistance: adventure with model. *Parasitol Today*, 11(2), 56-63.
- Barrett, E.J., Farlam, J. and Proudman, C.J. (2004) Field trial of the efficacy of a combination of ivermectin and praziquantel in horses infected with roundworms and tapeworms. *Vet. Rec.* 154, 323-325.
- Barrowman, P.R. (1991) Equine trypanosomiasis. In: D. Fielding and R.A. Pearson (eds). Donkeys, mules and horses in tropical agricultural development. *Proceedings of a Colloquium*, 3-6 September, 1990. Edinburgh, Scotland. pp. 106-112.
- Bartlett, M.S. (1955) Stochastic processes. Cambridge University press, UK.
- Bartlett, M.S. (1960) Stochastic population models in ecology and epidemiology. Methuen, London.
- Baylis, M., Meiswinkel, R. and Venter, G.J. (1999) A preliminary attempt to use climatic data and satellite imagery to model the abundance and distribution of *Culicoides imicola* (Diptera: Ceratopogonidae) in South Africa. *J. South Af. Vet. Ass*, 70(2), 80-89.
- Beal, V.C. and McCallon, W.R. (1983) The use of mathematical models in animal disease programme evaluation. In: Proceedings of the third international symposium on veterinary epidemiology and economics. 6-10 September 1982, Arlington, Virginia. Pp 400-407.
- Beelitz, P. Gobel, E. and Gothe, R. (1996) Endoparasites of donkeys and horses kept together in upper Bavaria: range of species and intensity of infestation. *Tierärztliche Praxis*, 24(5), 471-475.

- Behm, C. A. and Sangster, N.C. (1999) Pathology, pathophysiology and clinical aspects. In: Dalton, J.P. (ed). Fasciolosis. CAB International. Oxon. pp. 185-224
- Belemlih, A. (1991) SPA/SPANNA's education programme in Morocco. In: D. Fielding and R.A. Pearson (eds). Donkeys, mules and horses in tropical agricultural development. *Proceedings of a Colloquium*, 3-6 September, 1990. Edinburgh, Scotland. pp. 319-321.
- Bello, T.R. (1979) Perspectives on current equine anthelmintic therapy: misunderstanding and classification. *Proceedings Am. Assoc. Equine Practit.*, **25**, 261-265.
- Bello, T.R. (1982) Endoparasitism. In: R. Mansmann, E. McAllister and P. Pratt (eds). Equine Medicine and Surgery. American Veterinary Publications. Santa Barbara. pp. 68-70.
- Bello, T.R. (1985) The insidious invasive verminous antigens of the horse. *J. Eq. Vet. Sci.*, **5**, 163-168.
- Bello, T.R., Amborski, G.F. and Torbert, B.J. (1977) Practical equine parasitology - based upon recent research. *Proc. Am. Assoc. Equine Pract.*, **20**, 97-118.
- Bello, T.R., Amborski, G.F., Torbert, B.J. and Green, G.J. (1973) Anthelmintic efficacy of cambendazole against gastrointestinal parasites of the horse. *Am. J. Vet. Res.*, **34**, 771-777.
- Bemark, W. J. (1978) Tolerance of equine strongylids to desiccation and freezing. *Cryobiology*, **15**, 214-218.
- Benitez-Usher, C., Maciel, S. Rebollo, C. and Armour, J. (1984) A study of bovine parasitic gastro-enteritis in Paraguay. *Prev. Vet. Med.*, **2**, 295-308.
- Benton, R.E. and Lyons, E.T. (1994) Survey in ventral Kentucky for the prevalence of *Anoplocephala perfoliata* in horses at necropsy in 1992. *Vet. Parasitol.* **55**, 81-86.
- Bergeon, P. and Laurent, M. (1970) Differences between testicular morphology of *Fasciola hepatica* and *Fasciola gigantica*. *Revue. Elve. Med. Vet. Pays. Trop.*, **23**, 223-227.
- Bernard, N. collobert, C., Tariel, G. and Lamidey, C. (1994) Epidemiological survey of bot infection in horses at necropsy in Normandy from April 1990 to March 1992. *Rec. Med. Vet.* **170**, 231-235.
- Bernoulli, M.D. (1766) Essai d'une nouvelle, analyse de la mortalite causee par la petite verole et des avantages de l'inoculation pour la prevenir. In: Histoire de l'Academie Royale de Science, 1760, Avec les Memoires de Mathematique et de physique.

- L’Imprimerie Royal, Paris. Translated in: Bradley, L (1971) Smallpox inoculation: an eighteen century mathematical controversy. University of Nottingham Adult Education Department, Nottingham, UK.
- Beroza, G.A., Barclay, W.P., Phillips, T.N., Foerner, J.J. and Donawick, W.J. (1983). Caecal perforation and peritonitis associated with *Anoplocephala perfoliata* infections in three horses. *J. Am. Vet. Med. Ass.*, **183**, 804-806.
- Beroza, G.A., Marcus, L.C., Williams, R. and Bauer, S.M. (1986) Laboratory diagnosis of *A. perfoliata* infection in horses. *Proceedings of the 3rd Convention of the American Association of Equine Practitioners*. Nashville, Tennessee.
- Beroza, G.A., Williams, R., Marcus, L.C. and Mille, P. (1985) Prevalence of tapeworm infection and associated with large bowel disease in horses. *Proceedings of the 2nd equine colic symposium*. Georgia, pp. 21-25.
- Beyersbach, M., Gerlach, G.F and Kreienbrock, L. (2001) Modelling of the prevalence development in paratuberculosis control programme in a dairy herd. *Dtsch Tierarztl Wochenschr*, **108**(9), 363-370.
- Bizuneh, A., Birrie, H. and Bebele K. (1995) Colonization of irrigation canals by *Bullinus abyssinicus* and upsurge of urinary schistosomiasis in the middle Awash Valley of Ethiopia. *Ethiopian Med. J.*, **33**(4):256-263.
- Blanc, F., Martin, G.B. and Bocquier, F. (2001) Modelling reproduction in farm animals: a review. *Reprod. Fertil. Dev.*, **13**(5), 337-353.
- Bliss, D.H., Svendsen, E.D., Georgoulakis, I.E., Grosomanidis, S., Taylor, F. and Jordan, W. (1985) Strategic use of anthelmintics in working donkeys in Mediterranean climatic conditions. *Ve. Rec.*, **117**, 613-614.
- Boag, B. and Thomas, R.J. (1975) The population dynamics of nematode parasites of sheep in northern England. *Res. Vet. Sci.*, **19**, 293-295.
- Boray J.C. (1982) CRC Handbook series in zoonoses. Section C: Parasitic zoonoses, fascioliasis. pp. 71-88.
- Boray, J.C. (1969) Experimental fascioliasis in Australia. *Adv Parasitol.* **7**, 95-210.
- Boray, J.C. (1985) Flukes of domestic animals. In: S.M. Gaafar, W.E. Howard and R.E. Marsh (eds.). *Parasites, pests and predators*. Elsevier, New York, pp. 179-218.
- Boray, J.C., Crowfoot, P.D., Strong, M.B., Allison, J.R., Schellenbaum, M., Vo Orelli, M. and Sarasin, G. (1983) Treatment of immature and matured *F. hepatica* infection in sheep with triclabendazole *Vet. Rec.*, **113**(14), 315-317.

- Bracegirdle, J.R. (1973) A case of *Gastrodiscus aegyptiacus* infection in a horse in Ethiopia. *Vet Rec.* **93**, 561-562.
- Britton, J.W.(1938) Studies on the normal variations in the strongyle egg counts of horse faeces. *Cornnel. Vet.* **28**, 228-239.
- Brown, P.J and Clayton, H.M. (1979) Hepatic pathology of experimental *Parascaris equorum* infection in worm-free foals. *J. Comp. Path.*, **89**, 115-123.
- Brown, C.C. and Dardiri, A.H. (1990) African Horse Sickness: a continuing menace. *J. Am. Vet. Med. Ass.*, **196**, 2019-2021.
- Brown, D.S. (1967) A review of the fresh water mollusca of Natal and their distribution. *Annals of the Natal museum.* **18**, 477-494.
- Bucknell, D.G., Gasser, R.B. and Beveridge, I. (1995) The prevalence and epidemiology of gastrointestinal parasites of horses in Victoria, Australia. *Int. J. Parasitol.*, **25**, 711-724.
- Burgu, A., Doganay, A., Oge, H., Sarimehmetoglu, O. and Ayaz, E. (1995) Helminth species found in donkeys. *Veteriner Fakultesi Dergisi*, **42**(2), 207-215.
- Callinan, A.P.L. (1978a) The ecology of the free-living stages of *Ostertagia circumcincta*. *Int. J. Parasitol.*, **8**, 233-237.
- Callinan, A.P.L. (1978b) The ecology of the free-living stages of *Trichostrongylus axei*. *Int. J. Parasitol.*, **8**, 453-456.
- Callinan, A.P.L. (1979) The ecology of the free-living stages of *Trichostrongylus Vitrinus*. *Int. J. Parasitol.*, **9**, 133-136.
- Camel, D.K. (1988) Tapeworm infection in horses. *J. Equine Vet. Sci.* **8**, 343.
- Cannon, R.M. and Roe, R.T, (1982) Livestock disease surveys: a field manual for veterinarians. Australian Bureau of animal health, Commonwealth of Australia. pp 35.
- Carpenter, T.E., McBride, M.D. and Hird, D.W. (1998) Risk analysis of quarantine station performance: a case study of the importation of equine infectious anaemia virus-infected horses into California. *Journal of Diagnostic Investigation*, **10**(1), 11-16.
- Carvalho, R.O., Silva, A.V.M., Santos, H.A. and Costa, M.M.A. (1998) Nematodes Cyathostominae parasites of *Equus caballus* in the state of Minas Gerais, Brazil. *J. Vet. Parasitol.*, **7**, 165-168.
- Cats, A., Scholten, P., Meuwissen, S.G.M. and Kuipers, E.J. (2000) Acute *F. hepatica* infection attributed to chewing khat. *Gut*, **47**, 584-585.

- Celano, G., Zizzo, N. and Iaffaldano, d. (1991) Incidence of fascioliasis in imported horses slaughtered in Puglia: gross and histopathological changes and meat inspection. *Obiettivi e Documenti Veterinari*. **12**(2), 45-47.
- Central Statistic Authority (1995) Number and percentage distribution of horses, asses, mules and camels used for draught purposes by types and regions for private holdings. Statistical Bulletin No. 132. Addis Ababa, Ethiopia.
- Chapman, M.R., French D.D., Klei, T.R. (2002) Gastrointestinal helminths of ponies in Louisiana: a comparison of species currently prevalent with those present 20 years ago. *J. Parasitol.* **8**, 1130-1134.
- Chen, M.G. and Mott, K.E. (1990) Progress in assessment of morbidity due to *F. hepatica* infection: a review of recent literature. *Trop. Dis. Bull.*, **87**, R1-R38.
- Chiejina, S.N. and Fakae, B.B. (1984) Development and survival of infective larvae of gastro-intestinal nematode parasites of cattle in Eastern Nigeria. *Res. Vet. Sci.*, **37**, 148-153.
- Clayton, H.M. (1986) Ascarids: Recent advances. *Vet. Clns. N. Am. Equine practice*. **2**(2), 313-329.
- Clayton, H.M. and Duncan, J.L. (1977) Experimental *Parascaris equorum* infection of foals. *Res. Vet. Sci.*, **23**, 109-114.
- Clayton, H.M. and Duncan, J.L. (1978) Clinical signs associated with *Parascaris equorum* in worm-free pony foals and yearlings. *Vet. Parasitol.* **4**, 69-78.
- Clayton, H.M. and Duncan, J.L. (1979a) The migration and development of *Parascaris equorum* in the horses. *Int. J. Parasitol.*, **9**, 285-292.
- Clayton, H.M. and Duncan, J.L. (1979b) The development of immunity to *Parascaris equorum* infection in the foal. *Res. Vet. Sci.*, **26**, 383-384.
- Clayton, H.M., Duncan, J.L. and Dargie, J.D. (1980) Pathophysiological changes associated with *Parascaris equorum* infection in the foal. *Equine Vet. J.*, **12**, 23-25.
- Cliff, A.P., Haggett, P. and Ord, J.K. (1983) Forecasting epidemic pathways for measles in Iceland: the use of simultaneous equation and logic models. *Ecol. Disease*, **2**, 377-396.
- Coetzer, J.L.W. and Erasmus, B.J. (1994) African horse sickness. In: J.A.W. Coetzer, G.R. Thomson and R.C. Tustin (eds). *Infectious Diseases of Livestock with Special Reference to South Africa*. Vol I. Oxford University Press. Cape Town, South Africa. pp. 460-475.

- Cogley, T.P and Cogley,M.C. (1999) Inter-relationship between *Gasterophilus* larvae and the horses' gastric and duodenal wall with special reference to penetration. *Vet. Parasitol.* **86**, 127-142.
- Cogley, T.P and Cogley,M.C. (2000) Field observations of the host-parasite relationship associated with the common horse bot fly, *Gasterophilus intestinalis*. *Vet. Parasitol.* **88**, 93-105.
- Cohen, N.D. and Chaffin, M.K. (1994) Intestinal obstruction and other causes of abdominal pain in foals. *Compend. Contin. Educ. Pract. Vet.*, **16**(6), 780-790.
- Coles, G.C., Bauer, C., Borgsteede, F.H.M., Geerts, S., Klei, T.R., Taylor, M.A. and Waller, P.J. (1992) World association for the advancement of Veterinary parasitology (WAAVP). Methods for detection of anthelmintic resistance in nematodes of veterinary importance. *Vet. Parasitol.* **44**, 35-44.
- Coles, G.C., Hillyer, M.H., Taylor, F.G.R. and Villard, I. (2003) Efficacy of an ivermectin-praziquantel combination in equids against bots and tapeworms. *Vet. Rec.* **152**, 178-179.
- Colglazier, M.L. (1979) Critical anthelmintic trials in ponies with oxfendazole and cavitaphos and concomitant studies on the spontaneous elimination of small strongylids. *Am. J. Vet. Res.*, **40**(3), 384-386.
- Collins, D.R. (1961) Fascioliasis in Mexican burro. *J. Am. Vet. Med. Ass.* **139**(12), 1321-1323.
- Conceicao, M.A.P, Durao, R.M., Costa, I.H and Correia da Costa, J.M. (2002) Evaluation of a simple sedimentation method (Modified McMaster) for diagnosis bovine fasciolosis. *Vet. Parasitol.* **105**, 337-343.
- Connor, R.J. (1994) African animal trypanosomiasis. In: J.A.W. Coetzer, G.R. Thomson and R.C. Tustin (eds). *Infectious Diseases of Livestock with Special Reference to South Africa*. Vol I. Oxford University Press. Cape Town, South Africa. pp. 165-207.
- Cosgrove, S.S., Sheeran, J.J. and Sainty, T.V. (1986) *Irish Vet. J.* **40**, 35-36.
- Coyne, M.J. (1991) Fecundity of gastrointestinal trichostrongylid nematodes of sheep in the field. *Am. J. Vet. Res.*, **52**(7), 1182-1188.
- Craig, P.S. and Nelson, G.S. (1984) The detection of circulating antigen in human hydatid disease. *Ann. Trop. Med. Parasitol.* **78**, 219-227.
- Craig, T.M., Bowen, J.M. and Ludwig, K.G. (1983) Transmission of equine cyathostomes (strongylidae) in central Texas. *Am. J. Vet. Res.*, **44**, 1867-1869.

- Crane, M. (1997) Medical. In: E.D. Svendsen (ed). The Professional Handbook of The Donkey (3rd ed). Whittet Book Limited, London. pp. 19-36.
- Crofton, H.D. (1954) The ecology of the immature phases of trichostrongyle nematodes. V. The estimation of pasture infestation. *Parasitology*, **44**, 313-324.
- Crofton, H.D. (1965). Ecology and biological plasticity of sheep nematodes. I. The effect of temperature on the hatching of eggs of some nematodes parasites of sheep. *Cornnel. Vet.* **55**, 242-50.
- Dagnew, M., Hailu, T., Tessema, S., Asmera, J. and Ferejo, M (1995) Intensity of infection and reinfection rate of *A. lumbricoides* in a rural farming village, Dembia district, northwest Ethiopia. *Ethiopian Med. J.* **33**(4): 243-249.
- Daoud, M.S. and Al-Alousi, T.I. (1995) A note on some parasites of donkeys in Mosul – Iraq. *Iraqi Journal of veterinary Science*, **8**(1), 169-170.
- Dart, A. J. Hutchins, D.R. and Begg, A.P. (1987) Suppurative splenitis and peritonitis in a horse after gastric ulceration caused by larvae of *Gasterophilus intestinalis*. *Aust. Vet. J.* **64**, 155-158.
- De Aluja, A.S. and Lopez, F. (1991) Donkeys in Mexico. In: D. Fielding and R.A. Pearson (eds). Donkeys, mules and horses in tropical agricultural development. *Proceedings of a Colloquium*, 3-6 September, 1990. Edinburgh, Scotland. pp. 1-7.
- De Aluja, A.S., Nunez, E., Acevedo, A. Neyra, J. and Ochoa, P. (1990). Evaluation of different worming schedules for internal nematodes in donkeys in Mexico. *Veterinaria Mexico*, **21**(3), 269-273.
- De Jong, M.C.M. (1995) Mathematical modelling in veterinary epidemiology: why model building is important. *Prev. Vet. Med.*, **25**, 183-193.
- De Wall, D.T. and Van Heerden, J. (1994) Equine babesiosis. In: J.A.W. Coetzer, G.R. Thomson and R.C. Tustin (eds). Infectious Diseases of Livestock with Special Reference to South Africa. Vol I. Oxford University Press. Cape Town, South Africa. pp. 293-304.
- Demir, S., Tinar, R., Aydin, L., Ciark, V.Y. and Ergul, R. (1995) Prevalence of helminth species by faecal examination in equines of Bursa. *Turkiye Parazitoloji Dergisi*, **19**(1), 124-131.
- Dietrich, R.A., Amosson, S.H. and Hopkin, J.A. (1980) Epidemiologic and economic analysis of the USA bovine brucellosis programme and selected programme alternatives via an open-ended simulation model. In: W.A. Roe and L.A. Champan

- (eds). *Proceedings of the second international symposium*. 7-11 May, 1979, Canberra, Australia. Pp 623-632.
- Diez, K., Molineaux, L. and Thomas, A. (1974). A malaria model tested in the African Savannah. *Bull. Wld. Org.*, 50, 347-357.
- Dineen, J.K. and Wagland, B.M. (1966) The dynamics of the host-parasite relationship IV. The response of sheep to graded and to repeated infection with *Haemonchus contortus*. *Parasitology*, 56, 639-650.
- Dipietro, J.A. and Todd, K.S. (1988) Chemotherapeutic treatment of larvae and migratory stages of *Parascaris equorum*. *Proc. Am. Assoc. Equine Pract.*, 34, 611-618.
- Dipietro, J.A., Boero, M. and Ely, R.W. (1983) Abdominal abscess associated with *Parascaris equorum* infection in foal. *J. AM. Vet. Med. Ass.*, 182, 991-992.
- Dipietro, J.A., Klei, T.R. and French D.D. (1990) Contemporary topics in equine parasitology. *Compend. Contin. Educ. Pract. Vet.*, 12(5), 713-721.
- Dobson, R.J., Waller, P. J. and Donald, A.D. (1990a) Population dynamics of *Trichostrongylus colubriformis* in sheep: the effect of infection rate on the establishment of infective larvae and parasite fecundity. *Int. J. Parasitol.*, 20, 347-352.
- Dobson, R.J., Waller, P. J. and Donald, A.D. (1990b) Population dynamics of *Trichostrongylus colubriformis* in sheep: The effect of host age on the establishment of infective larvae. *Int. J. Parasitol.*, 20, 353-357.
- Doherty, J.F., Price, N., Moody, A.H., Wright, S.G. and Glynn, M.J (1995) Fascioliasis due to imported khat. *The Lancet*, 34, 462.
- Dorchies, P., Lahitte, J.D.de. and Donat, F. (1990) Treatment of experimental fascioliasis in horses with closantel. *Revue de Medecine Veterinaire*. 141(5), 383-387.
- Dorman, A. (1994) Educational means and measures. In: M. Bakkoury and R.A. Prentis (eds). Working equines. *Proceedings of The Second International Colloquium*, 20-22 April, 1994. Rabat, Morocco. pp. 323-326
- Drudge, J.H. and Lyons, E.T. (1977) Pathology of infections with internal parasites of horses. *The Blue Book for Veterinarian*, 27, 267-275.
- Drudge, J.H. and Lyons, E.T. (1983) Ascariasis. In: N.E. Robinson (ed). Current Therapy in Equine Medicine (1st ed). WB. Saunders co., Philadelphia, USA. pp. 262-267.
- Dumenigo, B.E., Espino, A.M. and Finlay, C.M. (1996) Detection of *Fasciola hepatica* in cattle faeces by a monoclonal antibody-based sandwich immunoassay. *Res. Vet. Sci.*, 60, 278-279.

- Duncan, J.L. (1973) The life cycle, pathogenesis and epidemiology of *S. vulgaris* in the horse. *Equine. Ve. J.*, **5**, 20-25.
- Duncan, J.L. (1974) Field studies on the epidemiology of mixed strongyle infection in the horse. *Vet. Rec.*, **94**, 337-345.
- Duncan, J.L. (1975) Immunity to *Strongylus vulgaris* in the horse. *Equine Vet. J.*, **7**, 192-197.
- Duncan, J.L. and Love, S. (1991) Preliminary observations on an alternative strategy for the control of horse strongyles. *Equine Vet. J.*, **23**, 226-228.
- Duncan, J.L. and Pirie, H.M. (1972) The life cycle of *S. vulgaris* in horses. *Res. Vet. Sci.*, **13**(4), 374-379.
- Duncan, J.L., Abbot, E.M., Arundel, J.H., Eysker, M., Klei, T.R., Krecek, R.C., Lyons, E.T., Reinemeyer, C. and Slocombe, J.O.D. (2002). World Association for the advancement of veterinary parasitology (WAAVP): second edition of guidelines for evaluating the efficacy of equine anthelmintics. *Vet. Parasitol.*, **103**, 1-8.
- Dunn, A.M. (1978) veterinary helminthology (2nd ed). Buttler and Tanner, London. pp. 122-212.
- Dunsmore, J.D., Jue Sue, L.P. (1985) Prevalence and epidemiology of the major gastrointestinal parasites of horses in Perth, Western Australia. *Equine Vet. J.*, **17**, 208-213.
- Dykhuisen, A.A. (1993) Modelling animal health economics. In: Thrusfield, M.V. (ed). Proceedings of Society for Veterinary Epidemiology and Preventive Medicine. 31March-2 April, 1993, Exeter, UK. pp 9-22.
- EARO (2004) Ethiopian Agricultural Research Organization, Soil Department. Addis Ababa, Ethiopia.
- Ebenezer, J.S. (1991) Human and animal power for rural areas. In: D. Fielding and R.A. Pearson (eds). Donkeys, mules and horses in tropical agricultural development. *Proceedings of a colloquium*, 3-6 September 1990. Edinburgh, Scotland. pp. 240-244.
- Edwards, G.B. (1986) Surgical management of intussusceptions in the horse. *Equine Vet. J.*, **18**, 313-321.
- Edwards, G.T. (1982) The prevalence of *Gasterophilus intestinalis* in horses in Northern England and Wales. *Vet. Parasitol.* **11**, 215-222.

- El-Abdin, Y.Z., Selim, M.K., Hamza, M. and Abdel-Gawad, A.M. (1983). Study on the efficacy of ivermectin as an anthelmintic in Egyptian equines. *J. Egyptian Vet. Med. Ass.*, **43**, 227-234
- EMA (1988) National atlas of Ethiopia. Ethiopian Mapping Authority. Berhanena Selam Printing Press, Addis Ababa. pp.80.
- English, A.W. (1979a) The epidemiology of equine strongylosis in southern Queensland. 1. The bionomics of the free-living stages in faeces and on pasture. *Aust. Vet. J.*, **55**, 299-305.
- English, A.W. (1979b) The epidemiology of equine strongylosis in southern Queensland. 2. The survival and migration of infective larvae on pasture. *Aust. Vet. J.*, **55**, 306-309
- English, A.W. (1979c) The epidemiology of equine strongylosis in Southern Queensland. 3. Seasonal variation in arterial population of *Strongylus vulgaris* and the prevalence of some helminths. *Aust. Vet. J.*, **55**, 310-314.
- ENMSA (2004) Ethiopian National Meteorological Service Agency, Addis Ababa, Ethiopia
- Erich, J. (1983) Investigation of fascioliasis in the Ethiopian highlands around Debre Berhan. Msc. Thesis. Leiden University, The Netherlands.
- Erko, B. and Medhin, G. (2003) Human helminthiasis in Wondo Genet, southern Ethiopia, with emphasis on geohelminthiasis. *Ethiopian Med. J.* **41**(4): 333-344.
- Espino, A.m. and Finlay, C.M. (1994) Sandwich enzyme-linked immunosorbent assay for detection of excretory-secretory antigens in human with fascioliasis. *J. Clin. Microbiol.*, **32**, 190-193.
- Esteban, J.G., Flores, A., Angels, R. and Mas-Coma, S. (1999). High endemicity of human fasciolosis between lake Titicaca and La Pa Valley, Bolivia. *Trans. R. Soc. Trop. Med. Hyg.*, **93**, 151-156.
- Esteban, J.G., Flores, A., Angels, R., Strauss, W., Aguirre, C. and Mas-Coma, S. (1997) A population based coprological study of human fascioliasis in a hyperendemic area of the Bolivian Altiplano. *Trop. Med. Int. Health.*, **2**(7), 695-699.
- Evans, J.W. (2001) Horses: A guide to selection, care and enjoyment. W.H. Freeman and Company. New York, USA, pp. 30-38.
- Eysker, M. (1987) Over-wintering of non-migrating strongyles in donkeys in the highveld of Zimbabwe. *Res. Vet. Sci.*, **42**, 262-263.

- Eysker, M. and Pandey, V.S. (1989) Small strongyle infections in donkeys from the Highveld in Zimbabwe. *Vet. Parasitol.*, **30**, 345-349.
- Eysker, M., Boersema, J.H. and Kooyman, F.N.J. (1990) Seasonally inhibited development of cyathostomine Nematodes in Shetland Ponies in The Netherlands. *Vet. Parasitol.*, **36**, 259-264.
- Eysker, M., Jansen, J. and Mirck, M.H. (1984) Inhibited development of cyathostominae in the horse in the early third stage. *Res. Vet. Sci.*, **37**, 355-356.
- Eysker, M., Jansen, J. and Mirck, M.H. (1986) Control of strongylosis in horses by alternative grazing of horses and sheep and some other aspects of the epidemiology of strongylidae infections. *Vet. Parasitol.*, **19**, 103-115.
- Fabiyi, J.P. (1987) Production losses and control of helminths in ruminants of tropical regions. *Int. J. Parasitol.*, **17**, 435-442.
- Fabiyi, J.P., Copeman, D.B. and Hutchinson, G.W. (1988) Abundance and survival of infective larvae of the cattle nematodes *Cooperia punctata*, *Haemoncus contortus* and *Oesophagostomum radiatum* from faecal pats in a wet tropical climate. *Aust. Vet. J.*, **65**(8), 229-231.
- Fagbemi, B.O. and Guobadia, E.E. (1995) Immunodiagnosis of fasciolosis in ruminants using a 28-kDa cysteine protease of *F. gigantica* adult worm. *Vet. Parasitol.*, **57**, 309-318.
- Fahmy, M.F.M. and El-Attar, S.R. (1990) Pathological study on fascioliasis in camel and solipeds. *Egyptian Journal of Comparative Pathology and Clinical Pathology*. **3**(2), 285-291.
- Fairbairn, D. (1957) The biochemistry of *Ascaris*. *Exp. Parasitol.*, **6**, 491-554.
- Fairweather, I. and Boray, J.C. (1999) Fasciolicides: efficacy, actions, resistance and its management (review). *The Vet. J.* **158**, 81-112.
- FAO (1989) Food and Agriculture Organization Production Yearbook, Vol.42. Rome, Italy.
- FAO (2002). Food and Agricultural Statistical Database. Home page at <http://apps.fao.org>.
- FAO (2004) Food and Agricultural Statistical Database. Home page at <http://apps.fao.org>.
- Fernando, P. (1997) Donkeys and development: socio-economic issues in the use and management of donkeys. Paper presented for Animal Traction Network in Eastern and Southern Africa (ATNESA) workshop on improving donkey utilization and management. 5-9, May 1997. Debre Zeit, Ethiopia (In press).

- Feseha, G. (1991) Use of equines in Ethiopia. In: *Proceedings of Institute of Agricultural research (IAR) No. 4*. Fourth livestock improvement conference, 13-15 November, 1991. Addis Ababa, Ethiopia. pp. 51-58.
- Feseha, G. (1997) Disease and health problems of donkeys abroad. In: D.E. Svendsen (ed). *The Professional Handbook of The Donkey*, (3rd ed). Whittet Books Lmt, UK. pp. 207-226.
- Feseha, G. (1998) Helminth parasites of working equids: the African perspective. In: Wenery, U., Wade, J.F., Mumford, J.A. and Kaaden, O.R (eds). *Proceedings of the Eighth International Conference*. Dubai, 23-26 March 1998. pp. 318-323
- Feseha, G., Alemu, G., Friew, K., Abule, I. And Ketema, Y. (1997) An overview of donkey utilization and management in Ethiopia. Paper presented for Animal Traction Network in Eastern and Southern Africa (ATNESA) workshop on improving donkey utilization and management. 5-9 May 1997. Debre Zeit, Ethiopia (In press).
- Feseha, G., Mohammed, A. and Yilma, J.M. (1991) Vermicular endoparasitism in donkeys of Debre Zeit and Menagesha, Ethiopia: strategic treatment with ivermectin and Fenbendazole. In: D. Fielding and R.A. Pearson (eds). *Donkeys, mules and horses in tropical agricultural development. Proceedings of a colloquium*, 3-6 September 1990. Edinburgh, Scotland. pp. 156-166.
- Fielding, D. and Krause, P. (1998) *Donkeys*. Tropical Agricultural Series, CTA Publications. Nezerland. 119p.
- Fischer, K.L.A. (1982) *Veterinary Medical Dissertation*. Hanover, Germany.
- Foerner, J.J., Phillips, T.N. and Barclay, W.P. (1980) Surgical diseases of the large intestine. *Proceedings of the 26th Convention of the American Association of Equine Practitioners*. Anaheim, CA. pp. 213-234.
- Fogarty, U., del Piero, F., Purnell, R.E. and Mosurski, K.R. (1994) Incidence of *Anoplocephala perfoliata* in horses examined at the Irish abattoir. *Vet. Rec.*, **134**, 515-518.
- Foreyt, W. J. (2001) *Veterinary parasitology. Reference manual* (5th ed.). Iowa State University press. Pp. 125-136.
- Francy, D.B. and Wagner, B.A. (1992) Equine encephalomyelitis in the united states, 1980-1990. Use of weather variables to develop a protective model for equine cases. *Animal Health Insight*. Spring, 1-8.
- French, D.D. and Chapman, M.R. (1994) Tapeworms of the equine gastrointestinal tract. *Compend. Contin. Educ. Pract. Vet.*, **14**(5), 655-661.

- Fuentes, M.V., Valero, M.A., Bargues, M.D. and Esteban, J.G. (1999) Analysis of climatic data and forecast indices for human fascioliasis at a very high altitude. *Ann Trop Med Parasitol.* **93**(8), 835-850.
- Fukui, M. (1960) Studies on equine tapeworms and their intermediate hosts. 1. *Anoplocephala perfoliata* Goeze 1782 and *Anoplocephala magna* Abildgaard 1789 and the experimental studies on the removal of these cestodes with bithionol. *Jpn, J. Parasitol.*, **9**, 190-194.
- Garlanda, A. (1958) Contributo allo studio della distomatosis epiatica negli equini. *Annali della facolta di medicina veterinaria di torino.* **8**, 291-300.
- Garret-Jones, C. (1964) The human blood index of malaria vectors in relation to epidemiological assessment. *Bull. Wld. Org.*, **40**, 531-545.
- Gasqui, P., Pons, O. and Coulon, J.B. (2000) An individual modelling tool for consecutive clinical mastitis during the same lactation in dairy cows: a method based on survival model. *Vet. Res.*, **31**(6), 583-602.
- Geering, R.R. and Johnson, P.J. (1990) equine tapeworms more prevalent. *Vet. Rec.*, **127**, 96
- Gemechu, B. and Mamo, E. (1979) A preliminary survey of bovine fascioliasis in Ethiopia. *Ethiopian journal of Agricultural science.* **1**, 5-12
- Georgi, J.R. (1974) Parasitology for veterinarians (2nd ed) WB Saunders Co. Philadelphia.
- Getachew, M A. (1999) Epidemiological study on the health and welfare of Ethiopian donkeys, with particular reference to parasitic diseases. MVM thesis. University of Glasgow Veterinary School. Glasgow, Scotland, UK.
- Gettinby, G. (1989) Computational veterinary parasitology with an application to chemical resistance. *Vet. Parasitol.*, **32**, 57-72.
- Gettinby, G. and Paton, G. (1981) The role of temperature in predicting the pattern of bovine *Ostertagia* infection on pasture. *J. Theor. Biol.*, **6**, 395-402.
- Gettinby, G., Bairden, K., Armour, J. and Benitez-Usher, C. (1979) A prediction model for bovine ostertagiasis. *Vet Rec.*, **105**, 57-59.
- Gettinby, G., Hope-Cawdery, M.J. and Grainger, J.N.R. (1974) Forecasting the incidence of fascioliasis from climatic data. *Int. J. Biometeor.*, **18**, 319-323.
- Gibson, T.E. and Parfitt, J.W. (1972) the effect of age on the development by sheep of resistance to *Trichostrongylus colubriformis*. *Res. Vet. Sci.*, **13**, 529-535.
- Gibson, T.E. and Whitehead, J.D. (1981) Changes in the worm burden of lambs under continuous infection with *Ostertagia circumcincta*. *Brit. Vet. J.*, **137**, 192-195.

- Gizaw, N. (1987) Policy and strategy options towards rapid development of Ethiopian livestock industry. *Proceedings of The first Livestock Improvement Conference*, 21-22 June, 1986. Addis Ababa, Ethiopia.
- Glass, K., Wood, J.L.N., Mumford, J.A., Jesset, D. and Grenfell, B.T. (2002) Modelling equine influenza 1: a stochastic model of within-yard epidemics. *Epidemiol Infect*, **128**(3), 491-502.
- Gloster, J., Blackall, R.M., Sellers, R.F. and Donaldson, A.I. (1981) Forecasting the airborne spread of foot-and-mouth disease. *Vet. Rec.*, **108**, 370-374.
- Goll, H.P. and Scott, J.M. (1978) The interrelationship of *Lymnaea truncatula* and ovine fascioliasis in the Ethiopian central highlands. *Br. Vet. J.*, **134**, 551-555.
- Gonenc, B. (1999) Digestive tract helminths of donkeys (*Equus asinus*, L). *Veteriner, Fakulitesi Dergisi*, **44** (2/3), 325-335.
- Gordon, G., O'Callaghan, M. and Tallis, G.M. (1970) A deterministic model for the life cycle of a class of internal parasites of sheep. *Math. Biosci.*, **8**, 209-226.
- Gordon, H.M. (1967) The diagnosis of helminthosis in sheep. *Vet. Med. Rev.*, 140-168.
- Gorman, T., Sanchez, R., Fredes, F. (1998) Immunodiagnostico de fasciolosis bovina mediante ELISA y Western blot. *Parasitol al dia*. **22**(1-2), 16-22.
- Gopal, R. M., Pomroy, W.E. and West, D.M. (199). Resitance of field isolates of *T. colubriformis* and *O. circumcincta* to ivermectin. *Int. J. Parasitol.*, **29**, 781-786.
- Gothe, R. and Heil, H.G. (1984) Internal parasites and lungworms of donkeys in Germany: Age related evaluation of prevalence of infections and specific composition. *Deutsche Tierarztliche Wochenschrift*, **91**(4), 144-145.
- Graat, E.A., de Jong, M.C., Frankena, K. and Franken, P. (2001) Modelling the effect of surveillance programmes on spread of bovine herpes virus 1 between certified cattle herds. *Vet. Microbiol.*, **79**(3), 193-208.
- Graber and Perrotin (1983) Helminths et Helminthoses des ruminants domestiques d'Afrique tropicale. Institut d'Elevage et de Medecine Veterinaire des Pays Tropicaux. Editions du point veterinaire, France.
- Graber, M. (1970) Helminthes et helminthiasis des equids (anes et chevaux) de la republique du Tchad. *Revue de Elevage et de Medicine Veterinaire des Pays Tropicaux*, **23**, 207-222.
- Graber, M. (1975) Helminths and helminthiasis of domestic and wild animals of Ethiopia. *Bulletin of Animal disease and Production in Africa*. **23**, 57-86.

- Graber, M. and Gruvel, J. (1964) Etude des agents des myiasis des animaux domestiques et sauvages d'Afrique equatoriale. *Revue d'Elevage et de Medicine Veterinaire des Pays Tropicaux*, **17**, 535-554.
- Grant, J.L. (1982) The epizootiology of nematode parasites of sheep in a high-rainfall area of Zimbabwe. *J. S. Afr. Vet. Assoc.*, **52**(1):33-37.
- Green, D.A., Leiper, J.W.G. and Green, C.M. (1968) Use of hilomid against *Fasciola hepatica* in donkeys. *Equine Vet. J.*, **1**, 89-90.
- Green, L. (1994) The practice of farriery in a few less developed countries. In: M. Bakkoury and R.A. Prentis (eds). Working equines. *Proceedings of The Second International Colloquium*, 20-22 April 1994. Rabat, Morocco. pp.169-170.
- Grelck, H., Horchner, F. and Wohrl, H. (1977) Zur experimentellen infection des Pferdes mit *Fasciola hepatica*. *Berliner und Munchener Tierarztliche Wochenschrift*. **90**, 371-373.
- Grenfell, B.T. and Smith, G. (1983) Population biology and control of ostertagiosis in first-year grazing calves. *Proceedings of the Society for Veterinary Epidemiology and Preventive Medicine*. 12-13 April 1983, Southampton. pp 70-77.
- Grenfell, B.T., Smith, G. and Anderson, R.M. (1987) A mathematical model of the population biology of *Ostertagia ostertagi* in calves and yearlings. *Parasitology*, **95**, 389-406.
- Grenfell, B.T., Smith, G. and Anderson, R.M. (1987) The regulation of *Ostertagia ostertagi* in calves: the effect of past and current experience of infection on proportional establishment and parasite survival. *Parasitology*, **95**, 363-372.
- Grenfell, B.T., Wilson, K., Isham, V.S., Boyd, H.E.G. and Dietz, K. (1995) Modelling patterns of parasite aggregation in natural populations: Trichostrongylid nematode-ruminant interactions as a case study. *Parasitology*, **111**, S135-S151.
- Gronvold, J. and Høgh-Schmidt, K. (1989) Factors influencing rain splash dispersal of infective larvae of *Ostertagia ostertagi* (Trichostrongylidae) from cow pats to the surroundings. *Vet. Parasitol.*, **31**, 57-70.
- Grubbs, S.T., Amodie, D., Rulli, D., Wulster-Radcliffe, M., Reinemeyer, C., Yazwinski, T. Tucker, C., Hutchens, D., Smith, D. and Patterson, D. (2003) Field evaluation of moxidectin/praziquantel oral gel in horses. *Vet. Ther.* **4**(3): 249-256.

- Guyatt, H.L., Bundy, D.A., Medley, G.F. and Grenfell, B.T. (1990) The relationship between the frequency distribution of *Ascaris lumbricoides* and the prevalence and intensity of infection in human communities. *Parasitology.*, **101**, 139-143.
- Haas, D.K. (1979) Equine parasitism. *Vet. Med. SAC.*, **74**, 980-988.
- Hall, N.C. and Koskin, H.P. (1918) The occurrence of tapeworms, *Anoplocephala* spp., of the horse in the United States. *Cornell Vet.*, **8**, 287-292.
- Halls, M.J. and Wall, R. (1995) Myiasis of human and domestic animals. *Adv Parasitol.*, **35**, 257-334.
- Hamer, W.H.(1906) Epidemic disease in England. *The lancet*, **1**, 733-739.
- Hammond, J.A. and Sewell, M.M.H. (1990) Diseases caused by helminths. In: M.M.H. Sewell and D.W. Brocklesby (eds). *Handbook of Animal Diseases in the tropics* (4th ed.). Bailliere Tindal, London. pp. 105-159.
- Haridy, F.M., Morsy, T.A., Gawish, N.I., Antonios, T.N. and Gawad, A.G.E.A. (2002) The potential role of donkeys and horses in zoonotic fascioliasis in Gharbia Governorate, *J Egypt Soc Parasitol.* **32**(2), 561-570.
- Harnett, W. (1988) The anthelmintic action of praziquantel. *Parasitol. Today.* **4**, 144-146.
- Haroun, E.M. and Hillyer G.V. (1986) Resistance to fascioliasis: a review. *Vet Parasitol.*, **20**, 63-93.
- Harrison, G.B.L., Pulford, H.D., Gatehouse, T.K., Shaw, R.J., Pfeiffer, A. and Shoemaker, C.B. (1999). Studies on the role of mucus and mucosal hypersensitivity reactions during rejection of *Trichostrongylus colubriformis* from the intestine of immune sheep using an experimental challenge model. *Int. J. Parasitol.*, **29**, 459-468.
- Haseeb, A.N., El-shazly, A.M., Arafa, M.A.S. and Morsy, A.T.A. (2002) A review of fascioliasis in Egypt. *J Egypt Soc Parasitol.* **32**(1), 317-354.
- Hass, D.K. (1979) Equine parasitism. *VM. SAC*, **74**, 980-988.
- Hasslinger, M.A. and El-Seify, M.A. (1996) Paramphistomatid infestation in equids in Egypt. *Berl Munch Tierarztl Wochenschr.* **109**(6-7), 224-226.
- Hatch, c. (1966) *Fasciola hepatica* in donkeys. *Irish Vet. J.*, **20**, 130.
- Herd, R. P. (1986) Pasture hygiene: a non-chemical approach to equine endoparasite control. *Mod. Vet.Prac.*, **67**, 36-38.
- Herd, R.P. (1990a) The changing world of worms: the rise of the cyathostomes and the decline of *S. vulgaris*. *Compen. Con. Educ. Prac. Vet.*, **12**, 732-736.

- Herd, R.P. (1990b) Equine parasite control: problems associated with intensive anthelmintic therapy. *Equine Vet. Educ.*, **2**, 41-47.
- Herd, R.P. and Willardson, K.L. (1985) Seasonal distribution of infective strongyle larvae on horse pasture. *Equine Vet. J.*, **17**(3), 235-237.
- Hethcote, H.W., Stech, H.W. and van de Driessche, P. (1981) No-linear oscillations in epidemic models. *SIAM J. Appl. Math*, **40**, 1-9.
- Hilali, M., Derhalli, F.S. and Baraka, A. (1987) Incidence and monthly prevalence of *Gasterophilus* species larvae (Diptera: Gasterophilidae) in donkeys (*Equus asinus*) in Egypt. *Vet. Parasito.*, **30**, 111-113.
- Hillyer, G.V. (1993) Serological diagnosis of *Fasciola hepatica*. *Parasitol. Al dia*. **17**, 130-136.
- Hillyer, G.V. and Apt, W. (1997) Food borne trematodes infections in the Americas. *Parasitol. Today*. **13**, 87-88.
- Hoglund J. Nilsson, O., Ljungstrom, BL., Hellander, J., Lind, EO. And Uggla, A. (1998) Epidemiology of *Anoplocephala perfoliata* infection in foals on a stud farm in South-western Sweden. *Vet. Parasitol.* **75**, 71-79.
- Hoglund, J., Ljungstrom, B., Nilsson, O. and Uggla, A. (1995) Enzyme-linked immunosorbent assay (ELISA) for the detection of antibodies to *A. perfoliata* in horse sera. *Vet. Parasitol.*, **59**, 97-106.
- Hong, H.A., Michel, J.F. and Lancaster, M.B. (1987). Observations on the dynamics of worm burdens in lambs infected daily with *O. circumcincta*. *Int. J. Parasitol.*, **17**, 951-957.
- Hopkins, D.R. (1992) Homing in on helminths. *Am. J. Trop. Med. Hyg.*, **46**, 626-634.
- Horak, I.G. (1971) Paramphistomiasis of domestic ruminants. *Adv. Parasitol.*, **9**, 33-72
- Hosmer, D.W. and Lemeshow, S. (1989) Applied logistic regression. John Wiley and Sons, New York, USA.
- Howell, P.G. (1963) African Horse Sickness: emerging diseases of Animals. FAO Agricultural Studies No. 61. Rome, Italy.
- Hugh-Jones, M.E. (1986) A mathematical modelling of antigenic drift. *Mathematical modelling*, **7**, 765-775.
- Hugh-Jones, M.E., Ellis, P.R. and Felton, M.R. (1976) The use of a computer model of brucellosis in the dairy herd. In: P.R. Ellis, A.P.M. Shaw and A.J. Stephens Eds. New

- techniques in veterinary epidemiology and economics. Proceedings of a symposium, 12-15 July 1976. University of Reading. Pp 96-112.
- Hurd, H.S. and Kaneene, J.B. (1993) The application of *simulation* models and systems analysis in epidemiology: a review. *Prev. Vet. Med.*, **15**, 81-99.
- Hutchinson, G.W., Abba, S.A. and Mfitilodze, M.W. (1989) Seasonal translation of equine strongyle infective larvae to herbage in tropical Australia. *Vet. Parasitol.*, **33**, 251-263.
- Ihler, C.F. (1995) The distribution of *Parascaris equorum* eggs in the soil profile of bare paddocks in some Norwegian studs. *Vet. Res. Coms.*, **19**, 495-501.
- Ihler, C.F., Rootwelt, V., Heyeraas, A. and Iolvik, N.I. (1995) The prevalence and epidemiology of *Anoplocephala perfoliata* infection in Norway. *Vet. Res. Coms.*, **16**(6), 487-494.
- Imrie, H. and Jacobs, D.E. (1987) Prevalence of horse tapeworm in north London and Hertfordshire. *Vet. Rec.*, **120**, 304.
- Innocent, G.T. (1998) An investigation using mathematical modelling techniques, into the effects of bovine viral diarrhoea virus infection on a closed dairy herd, with particular reference to the effects of various control strategies. PhD Thesis. University of Strathclyde, UK.
- Isham, V.S. and Medley, G.F. (1996) Models for infectious human diseases: their structure and related to data. Cambridge University Press.
- Islam, A.W.M.S. (1986) The prevalence of helminth parasites in horses. *Livestock Adviser*. **11**(6): 44-46.
- Itagaki, T., Ohta, N., Hosaka, Y., Iso, H., Konishi, M. Chinone, S. and Itagaki, H. (1989) Diagnosis of *Fasciola* sp. infections in cattle by Enzyme-linked immunosorbent assay. *Jpn. J. Vet. Sci.*, **51**(4), 757-764.
- Itepu, F. (1997) Donkeys: An alternative source of draft animal power for rural women in Kavango, Namibia. Paper presented for Animal Traction Network in Eastern and Southern Africa (ATNESA) workshop on improving donkey utilization and management. 5-9 May 1997. Debre Zeit, Ethiopia.
- Iversen, E. (1991) Equine based transport. In: D. Fielding and R.A. Pearson (eds). Donkeys, mules and horses in tropical agricultural development. *Proceedings of a colloquium*, 3-6 September 1990. Edinburgh, Scotland. pp. 245-250.
- Jacobs, D.E. (1986) Tapeworm infection – *Anoplocephala* spp. A colour atlas of equine parasites. Gower Publishing Ltd, London. pp. 5-16.

- Jemaneh, L. (1998) Comparative prevalences of some common intestinal helminth infections in different altitude regions in Ethiopia. *Ethiopian medical journal*. 36(1): 1-8.
- Jones, P. (1991) Overcoming ignorance about donkeys in Zambia: a case study. In: D. Fielding and R.A. Pearson (eds). *Donkeys, mules and horses in tropical agricultural development. Proceedings of a colloquium*, 3-6 September 1990. Edinburgh, Scotland. pp. 311-318
- Joseph, S.A.(1986) The large round worm of horses, *Parascaris Equorum*. *Centaur*, **II**(4), 149-150.
- Kaboet, Y., Pangui, L.J. and Vercruysse, J. (1986) Note sur la gasterophilose des anes au Burkina Faso. *Revue d'Elevage et de Medicine Veterinaire des Pays Tropicaux*, **39**, 211-212.
- Kallen, A., Arcuri, P. and Murray, J.D. (1985) A simple model for the spatial spread and control of rabies. *J. Theor. Biol.*, **116**, 377-393.
- Kania, S.A. and Reinemeyer, C.R. (2005) *Anoplocephala perfoliata* coproantigen detection: a preliminary study. *Vet. Parasitol.* **127**, 115-119.
- Kao, R.R. (2002) The role of mathematical modelling in the control of the 2001 FMD epidemic in the UK. *Trends in Microbiology*, **10**(6), 279-286.
- Kao, R.R., Leathwick, D.M., Roberts, M.G. and Sutherland, I.A. (2000) Nematode parasites of sheep: a survey of epidemiological parameters and their application in a simple model. *Parasitology*, **121**, 85-103.
- Karanja, D.N.R., Ngatia, T.A. and Wandera, J.G. (1994) Some common gastrointestinal parasites observed in Kenyan donkeys. *Bull. Anim. Prod. Afr.*, **42**, 75-76.
- Kearney, A. (1974) *Fasciola hepatica*: equines as reservoir hosts on hill and marginal land. *Proceedings of third International Congress of Parasitology*, August 25-31, 1974, Munich. Vol 1. Facta Publication, Vienna, Australia. pp. 511-512.
- Keelen, M.J., Woolhouse, M.E., May, R.M., Davies, G. and Grenfell, B.T. (2003) Modelling vaccination strategies against foot-and-mouth disease. *Nature*, **421**(6919), 136-142.
- Kelly, J.C. and Fogarty, U.G.M. (1993) Out break of larval cyathostomiasis on a thoroughbred stud farm. *Ir. Vet. J.*, **46**, 133-136.
- Kendall, S.B. (1967) Host specificity as evidenced by species of *Fasciola*. *Helminthologia*, **8**, 223-233.
- Kermack, W.O. and McKendrick, A.G. (1927) A contribution to the mathematical theory of epidemics. *Proc. R. Soc.*, A115, 700-721.

- Khallaayoune, K. (1991) Benefit of strategic deworming programme in working donkeys in Morocco. In: D. Fielding and R.A. Pearson (eds). Donkeys, mules and horses in tropical agricultural development. *Proceedings of a colloquium*, 3-6 September, 1990. Edinburgh, Scotland. pp.174-180.
- Kilani, M., Soussi, K., Dorchies, P. and Franc, M. (1986) Observations epidemiologiques sur les gastrophiles des equide de la region de Tunis (Tunisie). *Revue de Medicine Veterinaire*, **137**, 537-540.
- Kimura, S., Shimizu, A. and Kawano, J. (1984) Morphological observation on liver fluke detected from naturally infected carabaos in the Philippines. Science reports of faculty of agriculture Kobe University, Vol. 16.
- Kiper, M.L. Traub-Dargatz, J.L., Salman, M.D. and Rikihisa, Y. (1992) Risk factors for equine monocytic ehrlichiosis seropositivity in horses in Colorado. *Prev. Vet. Med.*, **13**(4), 251-259.
- Klein, T.R. and Torbert, B.J. (1980) Efficacy of ivermectin (22, 23, Dihydroavermectin B₁) against gastrointestinal parasites in ponies. *Am J Vet Res*, **41**, 1747-1750.
- Kotwal, S.K., Sharma, R.K. and Kalita, G. (2000) Epidemiological studies on internal parasites of equines of cold arid regions of Ladakh. *Centaur*. **17**(2), 39-41.
- Krawiecki, J.M. (1986) A clinical case of hepatic fascioliasis in a one-day eventer. *Pratique Veterinaire Equine*. **18**(4), 173-176.
- Krecek, R.C., Guthrie, A. J. (1999) Alternative approaches to control cyathostomes: an African perspective. *Vet. Parasitol.* **85**, 151-162.
- Krecek, R.C., Guthrie, A.J., Kriek, N.J.P., Hrak, I.J. and Malan, F.S. (1994) Helminth parasites of Cape mountain zebra from Cape Province, South Africa. *J Wildl Dis*. **30**, 277-280.
- Krecek, R.C., Malan, E.S., Reinecke, R.K. and de Vos V. (1978) Nematode parasites from Burchell's zebras in South Africa. *J. Wildl. Dis*, **23**, 404-411.
- Lee, C.G. and Zimmerman., G.L (1993) Bandding patterns of *Fasciola hepatica* and *Fasciola gigantica* (trematoda) by isoelectric focusing. *J. Parasitol.* **79**, 120-123.
- Lee, C.G., Zimmerman., G.L. and Mulrooney, D.M. (1992) Isoelectric focusing of soluble proteins from *Fasciola hepatica* and *Fascioloides magna*. *Am. J. Vet Res*. **53**, 246-250.

- Lemma, B. (1985) Studies on fascioliasis in four selected sites in Ethiopia. *Vet. Parasitol.*, **18**, 29-37.
- Lewa, A.K., Munyua, W.K., Ngatia, T.A. and Maingi, N.E. (1998) The common internal parasites encountered in donkeys in Kiambu district (Kenya), their seasonal prevalence and the pathological lesions associated with them. *Proceedings of 3rd colloquios International sobre equidos de Trabajo*, 5-9 Octob., Mexico. pp. 169-177.
- Lichtenfels, J.R. (1975) Helminths of domestic equids. Illustrated keys to genera and species with emphasis on North American forms. *Proceedings of The Helminthological Society of Washington* (special issue), **42**, 1-92.
- Lichtenfels, J.R., Kharchenko, V.A., Krecek, R.C. and Gibbons, L.M. (1998) An annotated checklist by genus and species of 93 species level names for 51 recognized species of small strongyles (Nematoda: Strongyloidea: Cyathostominae) of horses, asses and zebras of the world. *Vet. Parasitol.*, **79**, 65-79.
- Lichtenfels, J.R., McDonnell, A., Love, S. and Matthews, J.B. (2001) Nematode of the tribe cyathostominae(Strongylidae) collected from horses in Scotland. *Compar. Parasitol.*, **68**, 265-269
- Little, P.B., Lwin, U.S. and Fretz, P. (1974) Verminous encephalitis of horses: experimental induction with *Strongylus vulgaris* larvae. *Am. J. Vet. Res.*, **35**, 1501-1510.
- Lofty, W.M., El-Morshedy, H.N., Abou El-Hoda, M., El-Tawila, M.M., Omar, E.A. and Farag, H.F. (2002) Identification of the Egyptian species of Fasciola. *Vet. Parasitol.* **103**, 323-332.
- Lord, C.C., Woolhouse, M.E. and Mellor, P.S. (1997) Simulation studies of vaccination strategies in African horse sickness. *Vaccine*, **15**(5), 519-524).
- Lord, C.C., Woolhouse, M.E. and Mellor, P.S. (1998) Simulation studies of African horse sickness in Spain. African horse sickness. Springer-verlag, Vienna, Austria. pp 103-111.
- Love, S. (1992) Parasite-associated equine diarrhoea. *Compend. Contin. Educ. Pract. Vet.*, **14**(5), 642-649.
- Love, S. (1995) Recognizing disease associated with strongyles in horses. *Compend. Contin. Educ. Pract. Vet.*, **17**(14), 564-567.
- Love, S. and Duncan, J.L. (1992) The development of naturally acquired cyathostome infection in ponies. *Vet. Parasitol.*, **44**, 127-142.
- Love, S., Duncan, J.L., Parry, J.M. and Grimshaw, W.T.R (1995) Efficacy of oral ivermectin paste against mucosal stages of cyathostomes. *Vet. Rec.*, **136**, 18-19.

- Love, S., Mair, T.S. and Hillyer, M.H. (1992) Chronic diarrhoea in adult horses: a review of 51 referred cases. *Vet. Rec.*, **130**, 217-219.
- Love, S., Murphy, D. and Mellor, D. (1999) Pathogenecity of cyathostome infection. *Vet. Parasitol.*, **85**, 113-122.
- Lynn, R.C. (1995) Antiparasitic drugs: In: D.D. Bowman (ed). Georgi's parasitology for veterinarian. W.B. Saunders Co., Philadelphia, pp. 247-292.
- Lyon, S., Stebbings, H.C. and Coles, G.C. (2000) Prevalence of tapeworms, bots and nematodes in abattoir horses in southwest England. *Vet. Rec.*, **147**, 456-457.
- Lyons , E.T., Drudge, J.H. and Tolliver, S.C. (1976) Studies on the development and chemotherapy of larvae of *Parascaris equorum* (Nematoda: Ascaridoidea) in experimentally and naturally infected foals. *J. Parasitol.*, **62**, 453-459.
- Lyons, E.T., Drudge, J.H., Swerczek, T.W. (1981) Prevalence of *Strongylus vulgaris* and *Parascaris equorum* in Kentucky Thoroughbreds at necropsy. *J. Am. Vet. Med. Ass.*, **179**(8), 818-819.
- Lyons, E.T., Drudge, J.H., Tolliver, S.C., Swerczek, T.W. and Collins, S.S. (1989) Determination of the efficacy of pyrantel pamoate at the therapeutic dose rate against the tapeworm *Anoplocephala perfoliata* in equids using a modification of the critical test method. *Vet. Parasitol.* **31**, 13-18.
- Lyons, E.T., Swerczek, T.W., Tolliver, S.C., Bair, H.D., Drudge, J.H. and Ennis, L.E. (2000) Prevalence of selected species of internal parasites of equids at necropsy in central Kentucky (1995-1999). *Vet. Parasitol.*, **92**(1), 51-62.
- Lyons, E.T., Tolliver, B.S. and Drudge, J.H. (1985) Prevalence of some internal parasites recovered at necropsy of thoroughbreds born in 1982 in Kentucky. *Am. J. Vet. Res.* **46**(3), 679-683.
- Lyons, E.T., Tolliver, S.C. and Drudge, J.H. (1999). Historical perspective of cyathostomes: prevalence, treatment and control programme. *Vet. Parasitol.*, **85**, 97-112.
- Lyons, E.T., Tolliver, S.C., Drudge, J.H., Granstrom, D. E. and Stamper, S. (1992) Activity of praziquantel against *Anoplocephala perfoliata* (cestoda) in horses. *J. helminthol. Soc. Wash.* **59**, 1-4.
- Lyons, E.T., Tolliver, S.C., Drudge, J.H., Swerczek, T.W. and Crowe, M.W. (1984) Prevalence of *A. perfoliata* and lesions of *D. megastoma* in thoroughbreds in Kentucky at necropsy. *Am. J. Vet. Res.*, **45**, 996-999.

- Macdonald, D.W. and Bacon, P.J. (1980) To control rabies: vaccinate foxes. *New Sci*, **87**, 640-645.
- Macdonald, G. (1957) The epidemiology and control of malaria. Oxford University press, London.
- MacGregor, M.C. (1994) ILPH working together for equines programme. In: M. Bakkoury and R.A. Prentis(eds). Working equines. *Proceedings of The Second International Colloquium*, 20-22 April, 1994. Rabat, Morocco. pp. 175-178.
- Mackenzie, K. and Bishop, S.C. (2001) utilizing stochastic genetic epidemiological models to quantify the impact of selection for resistance to infectious diseases in domestic animals. *J Anim Sci*, **79**(8), 2057-2065.
- MAFF (1984) Manual of Veterinary Investigation Laboratory Techniques. Reference book 390, Vol. 2. London UK.
- Mair, T.S. (1993) recurrent diarrhoea in aged ponies associated with larval cyathostomiasis. *Equine Vet. J.*, **25**, 161-163.
- Mair, T.S. (1994) Outbreak of larval cyathostomiasis among a group of yearling and two-year-old horses. *Vet. Rec.*, **135**, 598-601.
- Maizels, R.M., Bundy, D.A.P., Selkirk, M.E., Smith D.F. and Anderson, R.M. (1993). Immunological modulation and evasion by helminth parasites in human population. *Nature*. **365**, 797-805
- Malan, F.S., Horak, I.G., De Vos, V. and Van Wyk, J.A. (1997) Wildlife parasites: lessons for parasites control in livestock. *Vet. Parasitol.*, **71**, 137-153.
- Malan, F.S., Reinecke, R.K. and Scialdo-Krecek, R.C. (1981a) Recovery of helminths post-mortem from equine. I. Parasites in arteries, sub-peritoneum, liver and lungs. *Onderstepoort J. Vet. Res.*, **48**, 141-143.
- Malan, F.S., Reinecke, R.K. and Scialdo-Krecek, R.C. (1981b) Recovery of helminths post-mortem from equines. II. Helminths and larvae of gasterophilus in the gastrointestinal tract and oestrids from the sinuses. *Onderstepoort J. Vet. Res.* **48**, 145-147.
- Malan, F.S., Reinecke, R.K. and Scialdo-Krecek, R.C. (1982) Anthelmintic efficacy of fenbendazole in donkeys assessed by the modified non-parametric method. *J S Afr Vet Assoc*, **53**(3), 185-188.

- Mamo, B., Assefa, B. and Lo CT. (1989) Intestinal helminths in Akaki town, with special emphasis on the epidemiology of *Schistosoma mansoni*. *Ethiop Med J.*, **27**(4): 183-191.
- Marley, S.E., Hutchens, D.E., Reinemeyer, C.R., Holste, J.E, Paul, A.J. and Rebbein, S. (2004) Antiparasitic activity of ivermectin and praziquantel combination paste in horses. *Vet. Ther.* **5**(2): 105-109.
- Martin, G.S., Strand, E. and Kearney, M.T. (1996) Use of statistical models to evaluate racing performance in Thoroughbreds. *J. AM. Vet. Med. Ass.*, **209**(11), 1900-1906.
- Mas-Coma, M.S. (1997) Secondary reservoir role of domestic animals other than sheep and cattle in fascioliasis transmission in the Northern Bolivian Altipano. *Research and Reviews in Parasitology.* **57**, 39-46.
- Mas-Coma, M.S., Bargues, M.D and Esteban, J.G. (1999) Human fasciolosis. In: Dalton, J.P. (ed). Fasciolosis. CAB International, Oxon. pp. 411-432.
- Mas-Coma, M.S., Esteban, J.G. and Bargues, M.D. (1999) Epidemiology of human fascioliasis: a review and proposed new classification. *Bull. World Health Organisation*, **77**(4), 340-346.
- Matthee, S. Krecek, R.C. and Guthrie, A.J. (2002) Effect of management interventions on the helminth parasites recovered from donkeys in South Africa. *J. parasitol.*, **88**(1), 171-179.
- Matthee, S. Krecek, R.C. and Milne, S.A. (2000) Prevalence and biodiversity of helminth parasites in donkeys from South Africa. *J. Parasitol.*, **86**(4), 756-762.
- Mattioli, R.C., Zinsstag, J. and Pfister, K. (1994) Frequency of trypanosomosis and gastrointestinal parasites in draught donkeys in the Gambia in relation to animal husbandry. *Trop. Anim. Health Prod.* **26**, 102-108
- May, R.M. and Anderson, R.M. (1979) Population biology of infectious diseases. Part II. *Nature*, **280**, 455-461.
- Mayhew, I.G., Brewer, B.D., Reinhard, M.K. and Greiner, E.C. (1984) Verminous (*Strongylus vulgaris*) myelitis in a donkey. *Cornell Vet.*, **74**, 30-37
- Meana, A., Luzon, M. and Corchero, J. (1998) Reliability of coprological diagnosis of *A. perfoliata* infection. *Vet. Parasitol.*, **74**, 79-83.

- Mellor, P.S., Baylis, M., Hamblin, C., Calisher, C.H. and Mertens, P.P.C. (1998) Modelling the distribution and abundance of *Culicoides imicola* in Morocco and Iberia using climatic data and satellite imagery. African horse sickness. Springer-verlag, Vienna, Austria. pp 137-153.
- Menzies, F.D. and Reid, S.W.J. (2002) Modelling equine influenza: epidemiology, vaccination, spatial spread and strain variation. Proceedings of the society for Veterinary Epidemiology and preventive Medicine. 3rd-5th April, 2002, University of Cambridge, UK. pp 48-60.
- Mfitilodze, M.W. and Hutchinson, G.W. (1987). Development and survival of free-living stage of equine strongyles under laboratory conditions. *Vet. Parasitol*, **28**, 121-133.
- Mfitilodze, M.W. and Hutchinson, G.W. (1988) development of free-living stages of equine strongyle in faeces on pasture in a tropical environment. *Vet. Parasitol.*, **26**, 285-296.
- Michel, J.F. (1969) The epidemiology and control of some nematode infections of grazing animals (review). *Adv. Parasitol.*, **7**, 211-282.
- Michel, J.F. (1982) Some thoughts on the control of parasitic gastro-enteritis. In: L.E.A. Symsons, A.D. Donald and J.K., Dineen (ed). Biology and control of endoparasites. McMaster Lab. 50th anniversary symposium in parasitology, Nov 5-6, 1981. Sydney. Academic Press Australia. pp 113-131
- Miller, Wm. C. (1953). The general problem of parasitic infestation in horses. *Vet. Rec.*, **65**(14), 213-217.
- MOA (2000) Ministry of Agriculture, Agro-ecological Zonations of Ethiopia. Addis Ababa, Ethiopia
- MOA (2004) Ministry of Agriculture, Ada-liben, Akaki, Bereh and Boset Bureaus of Agriculture, Ethiopia.
- Moisant, C., Jolivet, G. and Pitre, J (1972) Equine fascioliasis. Observation in Normandy. Treatment trial of rafoxanide. *Receuil de Medicine Veterinaire*. **148**(4), 443-450.
- Mollison, D. (1977) Spatial contact model for ecological and epidemic spread. *J.R. Statist. Soc.*, **B39**, 743-747.
- Montes, O.G., Zurita, A.L. and Uhart, R.E. (1984) Measurement of aspartate aminotransferase, gamma-glutamyl transpeptidase, total protein and serum albumin in horses with fascioliasis. *Zentralblatt fur Veterinarmedizin, A.*, **31**(5), 338-342.
- Morales, M.A., Luengo, J. and Vasquez, J. (2000) Distribution and prevalence of fascioliasis in meat animals in Chile, 1989-1995. *Parasitologia Al Dia.*, **24**(3/4), 115-118.

- Morel, P.C. (1959) Les helminths des animaux domestiques de l'Afrique occidentale. *Revue Elev.Med. Vet. Pays Trop.*, **12**, 153-174.
- Moss, R.A. and Vlassoff, A. (1993) Effect of herbage species on gastro-intestinal roundworm populations and their distribution. *New Zealand J. Agr. Res.*, **36**, 371-375.
- Mueller, P.O.E. and Baxter, G.M. (1992) Small intestinal diseases of horses. Diagnosis and surgical intervention. *Vet. Med.*, **87**, 1030-1036.
- Mukbel, R., Torgreson, P.R. and Abo-Shehadeh (2001) Seasonal variation in the abundance of *Gasterophilus* spp. Larvae in donkeys in Northern Jordan. *Trop. Anim. Health Prod.*, **33**, 501-509.
- Mulcahy, G., Joyce, P., Dalton, J.P. (1999) Immunology of *Fasciola hepatica* infection. In: Dalton, J.P. (ed). Fasciolosis. CAB International, Oxon. pp. 341-375.
- Mulugeta, H.S., Getachew, T., Tafesse, M., Getachew, W.M., Kinfu, G. and Teshome, Y. (1989) The significance of helminth parasites in livestock production. Paper presented at the third livestock improvement conference held in Addis Ababa, Ethiopia. 24-26 May.
- Murphy H.F. (1959) A report on the fertility status of some soils of Ethiopia. Imperial Ethiopian College of Agriculture and Mechanical arts, Dire Dawa, Ethiopia.
- Murphy, D., Keane, M.P., Chandler, K.J. and Goulding, R. (1997) Cyathostome-associated disease in the horse: investigation and management of four cases. *Equine Vet. Educ.*, **9**, 247-252.
- Nansen, P., Andersen, S. and Hesselholt, M. (1975) Experimental infection of horses with *Fasciola hepatica*. *Exp Parasitol.*, **37**(1), 15-19.
- Ndamukong, K.J.L. and Ngone, M.M. (1996) Development and survival of *Haemoncus contortus* and *Trichostrongylus spp.* on pasture in Cameroon. *Trop. Anim. Health Prod.*, **28**, 193-197.
- Ngategize, P.K., Bekele, T. and Tilahun, G. (1993) Financial losses caused by ovine fasciolosis in the Ethiopian highlands. *Trop. Anim. Health Prod.*, **25**, 155-161.
- Nicholls, J. and Obendorf, D.L (1994). Application of a composite faecal egg count procedure in diagnostic parasitology. *Vet. Parasitol.* **52**, 337-342.
- Nicholls, J.M., Clayton, H.M., Pirie, H.M. and Duncan, J.L. (1978) A pathological study of the lungs of foals infected experimentally with *Parascaris equorum*. *J. Comp. Path.*, **88**, 261-274.

- Niezen, J.H., Waghorn, G.C. and Charleton, W.A.G. (1998) Establishment and fecundity of *Ostertagia circumcincta* and *Trichostrongylus colubriformis* in lambs fed lotus (*Lotus pedunculatus*) or perennial ryegrass (*Lolium perenne*). *Vet. Parasitol.*, **78**, 13-21.
- Nilsson, O., Ljungstrom, B.L., Hoglund, J., Lundquist, H. and Uggla, A. (1995) *Anoplocephala perfoliata* in horses in Sweden: prevalence, infection levels and intestinal lesions. *Act. Vet. Scand.*, **36**, 319-328.
- Njau, B.C. and Scholtens, R.G. (1991) The role of traditionally harvested hay in the transmission of ovine fasciolosis in the Ethiopian highlands. *Vet. Res. Coms.*, **15**, 369-372.
- Njau, B.C. Kasali, O.B., Scholtens, R.G. and Mesfin, D. (1988) Review of sheep mortality in the Ethiopian highlands, 1982-1986. *ILCA Bulletin*. **31**, 19-22.
- Njau, B.C. Kasali, O.B., Scholtens, R.G. and Akalework, N. (1989) The influence of watering practices on the transmission of *Fasciola* among sheep in the Ethiopia highlands. *Vet. Res. Coms.*, **13**, 67-74.
- Ogborne, C.P. (1973) Survival on herbage plots of infective larvae of strongylids nematodes of the horse. *J. Helminth*, **47**, 9-16.
- Ogbourne, C.P. (1971) Variation in fecundity of strongylid worms of the horse. *Parasitology*, **63**, 289-298.
- Ogbourne, C.P. (1972) Observations on the free-living stages of strongylids nematodes of the horses. *Parasitology*, **64**, 461-477.
- Ogbourne, C.P. (1976) The prevalence, relative abundance and site distribution of nematodes of the subfamily cyathostominae in horses killed in Britain. *J. Helminthol.*, **50**, 203-214
- Ogbourne, C.P. (1978a) Studies on the epidemiology of *Strongylus vulgaris* infection of the horse. *Int. J. Parasitol.*, **5**, 423-426.
- Ogbourne, C.P. (1978b) Pathogenesis of cyathostome (*Trichonema*) infection of the horse: a review. Commonwealth Institute Helminthology, commonwealth Agricultural Bureaux of the United Kingdom. *Miscellaneous Publication*, No. 5, 25 pp.
- Ogbourne, C.P. and Duncan, J.L. (1985) *Strongylus vulgaris* in the horse: its biology and veterinary importance (2nd ed). Commonwealth Institute of Parasitology, Miscellaneous Publication No.9, 68pp.
- Oliver, D.F., Jenkins, C.T. and Walding, D. (1970) Duodenum rapture in a nine month-old colt due to *Anoplocephala magna*. *Vet. Rec.*, **101**, 80.

- Ollerenshaw, C.B. (1971) Forecasting liver fluke disease in England and Wales, 1958-1968 with a comment on the influence of climate on the incidence of disease in some other countries. *Vet. Med. Rev.* 2/3, 289-312.
- Ollerenshaw, C.B. (1974) Forecasting liver fluke disease. In A.E.R Taylor and R. Muller (Eds). The effect of meteorological factors upon parasites. Blackwell Scientific publications, Oxford, London. pp. 33-52.
- Ollerenshaw, C.B. and Rowlands, W.T. (1959) A method of forecasting the incidence of fascioliasis in Anglesey. *Vet. Rec.*, **71**, 591-598.
- Ollerenshaw, C.B. and Smith, L.P. (1966) An empirical approach to forecasting the incidence of *nematodirus* over England and Wales. *Vet. Rec.*, **79**, 536-540.
- Ollerenshaw, C.B., Graham, E.G. and Smith, L.P. (1978) Forecasting the incidence of parasitic gastroenteritis in lambs in England and Wales. *Vet. Rec.*, **103**, 461-465.
- Olsen, O.W. (1938) Anoplocephalosis in Minnesota horses. *J. Am. Vet. Med. Ass.*, **92**, 557-559.
- Onyali, I.O., Onwuliri, C.O.E. and Ajayi, J.A. (1990) Development and survival of *Haemoncus contortus* larvae on pasture at Vom, Plateau state, Nigeria. *Vet. Res. Com.*, **14**, 211-216.
- Orr, J.P. (1972) Perforated duodenal ulcer in a foal. *Vet Rec.*, **90**, 571.
- Owen, J.M. (1977) Liver fluke infection in horses and ponies. *Equine Vet. J.*, **9**(1), 29-31.
- Owen, R., Jagger, D.W. and Quan-Taylor, R. (1988) Prevalence of *A. perfoliata* in horses and ponies in Clwyd, Powys and adjacent English marshes. *Vet. Rec.*, **123**, 562-563.
- Owen, R., Jagger, D.W. and Quan-Taylor, R. (1989) Caecal intussusceptions in horses and the significance of *Anoplocephala perfoliata*. *Vet. Rec.*, **124**, 34-37.
- Pandey, V.S. (1980a) Epidemiological observation on lungworm, *Dictyocaulus arnfieldi*, in donkeys from Morocco. *J Helminthol*, **54**, 275-279.
- Pandey, V.S. (1980b) Hydatidosis in donkeys in Morocco. *Annals of Tropical Medicine and Parasitology*, **74**, 519-521.
- Pandey, V.S. (1980c) Seasonal prevalence of *Strongylus vulgaris* in anterior mesenteric artery of the donkey in Morocco. *Ve. Parasitol*, **7**, 357-362.
- Pandey, V.S. (1981) Epidemiology of *Strongylus vulgaris* infection of horse in Morocco. *Trop. Anim. Health Prod.*, **13**, 119-121.

- Pandey, V.S. (1983) Observation on *Fasciola hepatica* in donkey from Morocco. *Ann. Trop. Med. Parasitol.*, **77**, 159-162.
- Pandey, V.S. and Cabaret, J. (1993) Stomach parasites of donkeys in Morocco: habitat and interspecific interaction. *Ve. Parasitol*, **49**, 331-337.
- Pandey, V.S. and Eysker, M. (1988) Parasites in donkeys of the highveld of Zimbabwe. *Vet Q.*, **10**, 246-248.
- Pandey, V.S. and Eysker, M. (1989) *Strongylus vulgaris* in donkeys (*Equus asinus*) from the highveld of Zimbabwe. *Ve. Parasitol*, **32**, 173-179.
- Pandey, V.S. and Eysker, M. (1990) Internal parasites of donkeys from the highveld of Zimbabwe. *Zimbabwe Veterinary Journal*, **21**, 27-32.
- Pandey, V.S. and Eysker, M. (1991) Internal parasites of equines in Zimbabwe. In: D. fielding and R.A. Pearson (eds). Donkeys, mules and horses in tropical agricultural development. *Proceedings of a colloquium*, 3-6 September, 1990. Edinburgh, Scotland. pp. 167-173.
- Pandey, V.S., Khallaayoune, K., Ouhelli, H. and Kakkak, A. (1994) Parasites of donkeys in Africa. In: M. Bakkoury and R.A. Prentis (eds). Working equines. *Proceedings of The Second International Colloquium*, 22-24 April, 1994. Rabat, Morocco. pp. 35-44.
- Pandey, V.S., Ouhelli, H. and Verhulst, A. (1992a) Epidemiological observations on stomach worms of donkeys in Morocco. *Vet. Res. Coms*, **16**, 273-279.
- Pandey, V.S., Ouhelli, H. and Verhulst, A. (1992b) Epidemiological observation on *Gasterophilus intestinalis* and *Gasterophilus nasalis* in donkeys from Morocco. *Vet. Parasitol.*, **41**, 285-292.
- Pankhurst, J. W. (1963) Liver flukes in donkeys. *Vet. Rec.*, **75**, 434.
- Paton, G. (1983) Mathematical models for the prediction and control of ovine parasitic gastroenteritis. PhD Thesis. University of Strathclyde, |Scotland, UK.
- Paton, G. (1987) A model for predicting parasitic gastroenteritis in lambs in mixed nematode infections. *Res. Vet. Sci.*, **43**, 67-71.
- Paton, G., Thomas, R.J. and Waller, P.J. (1984) A prediction model for parasitic gastroenteritis in lambs. *Int. J. Parasitol.*, **14**, 439-445.
- Paul, J.W. (1998) Equine larval cyathostomosis. *Compend. Contin. Educ. Pract. Vet.*, **20**(4), 509-514.
- Pearson, G.R., Davies, L.W., White, A.L. and O'Brien, J.K. (1993) Pathological lesions associated with *Anoplocephala perfoliata* at the ileo-caecal junction of horses. *Vet. Rec.*, **132**, 179-182.

- Pearson, R.A., Nengomasha, E. and Krecek, R. (1997) The challenges in using donkeys for work in Africa. Paper presented for Animal Traction Network in Eastern and Southern Africa (ATNESA) workshop on improving donkey utilization and management. 5-9 May 1997. Debre Zeit, Ethiopia (In press).
- Perler, L., Gottstein, B. and Borioli, A. (1997) Fascioliasis in horses: practical approach. *Pratique Veterinaire Equine*. **29**(3), 159-161.
- Petrie, A. and Watson, P. (1999) Statistics for veterinary and animal science. Black Science Ltd. London, Oxford.
- Pfeiffer, D.U. (2002). Veterinary epidemiology. An Introduction. Royal veterinary college, London, UK. Pp. 28-36. www.vetschools.co.uk/EpiVetNet/epidivision/Pfeiffer/
- Pfister, K. and Brocard, P. (1996) Gasterophilosis. *Parasitologia*. **38**, 405.
- Pilliner, S. and Davie, Z. (1996) Equine science, health and performance. Black-well Science Ltd. Oxford, London, UK. pp. 262-263.
- Poynter, D. (1954) Seasonal fluctuation in the number of strongyle eggs passed by horses. *Vet. Rec.*, **66** (5), 74-78.
- Poynter, D. (1970) Some observations on the nematode parasites of horses. *Proceedings of the Second International Conference on Equine Infectious Diseases*. New York, 1970. pp. 269-289.
- Pradhan, S.M.S., Mahato, S.N. and Joshi, B.R. (1991) Equines in Nepal. In: D. Fielding and R.A. Pearson (eds). Donkeys, mules and horses in tropical agricultural development. *Proceedings of a colloquium*, 3-6 September 1990. Edinburgh, Scotland. pp. 22-25.
- Prentis, R.A. (1994) Issues for working equines: key note address. In: M. Bakkoury and R.A. Prentis (eds). Working equines. *Proceedings of The Second International Colloquium*, 22-24 April, 1994. Rabat, Morocco. pp. 9-16.
- Proudman, C.J and Matthews, J. (2002) Control of internal parasites in horses. *In Practice*, **22**(2), 90-97.
- Proudman, C.J. and Edwards, G.B. (1992) Validation of a centrifugation/flotation technique for the diagnosis of equine cestodias. *Vet. Rec.*, **131**, 71-72.
- Proudman, C.J. and Edwards, G.B. (1993) Are tapeworms associated with equine colic? A case control study. *Equine Vet. J.*, **25**, 224-226.
- Proudman, C.J. and Ellis, R.N.W. (1992) Tapeworm infection in donkeys. *Vet. Rec.*, **131**, 71.
- Proudman, C.J. and Holdstock, N.B. (2000) Investigation of an outbreak of tapeworm-associated colic in a training yard. *Equine Vet J Suppl.*, **32**:37-41.

- Proudman, C.J. and Trees, A.J. (1996a) Use of excretory/secretory antigens for the serodiagnosis of *A. perfoliata* cestodosis. *Vet. Parasitol.*, **61**, 239-247.
- Proudman, C.J. and Trees, A.J. (1996b) Correlation of antigen specific IgG and IgG(T) responses with *A. perfoliata* infection intensity in the horse. *Parasite Immunol.*, **18**, 499-506.
- Proudman, C.J. and Trees, A.J. (1999) Tapeworms as a cause of intestinal disease in horses. *Parasitol Today*, **15**(4), 156-159.
- Proudman, C.J., French, N.P. and Trees, A.J. (1998) Tapeworm infection is a significant risk factor for spasmodic colic and ileal impaction colic in the horse. *Equine Vet. J.*, **30**(3), 194-199.
- Proudman, C.J., Holmes, M.A., Sheoran, A.S., Edwards, S.E.R. and Trees, A.J. (1997) Immunoepidemiology of the equine tapeworm *Anoplocephala perfoliata*: age-intensity profile and age-dependency of antibody subtype responses. *Parasitology*. **114**, 89-94.
- Proudman, C.J., Smith, J.E., Edwards, G.B. and French, N.P. (2002) Long-term survival of equine surgical colic cases. Part 2: Modelling postoperative survival. *Equine, Vet. J.*, **34**(5), 438-443.
- Proudman, C.J., Swan, J.D. and Trees, A.L. (1995) Efficacy of pyrantel embonate and praziquantel against the equine tapeworm *Anoplocephaloides mamillana*. *Vet. Rec.*, **137**, 45-45.
- Pukov, V.I. (1941). Investigation on nematodes of radical prophylaxis against strongylosis of horses kept in stud, Nauki Moskva, pp. 173-180 (in Russian).
- Quinnell, R.J., Wollhouse, M.E.J., Walsh, E.A. and Pritchard, D.I. (1995) Immunoepidemiology of human necatoriasis: correlations between antibody responses and parasite burdens. *Parasite immunol.* **17**, 313-318.
- R Development Core Team (2004) R: A language and environment for statistical computing. R foundation for Statistical computing, Vienna, Australia. ISBN 3-900051-07-0, URL <http://www.R-project.org>.
- Ramachandran, S. and Srinivas, R.P. (1991) The contribution of donkeys and pony to the economy of rural and urban low-income group in Karnataka state, South India. In: D. Fielding and R.A. Pearson (eds). Donkeys, mules and horses in tropical agricultural

- development. *Proceedings of a colloquium*, 3-6 September 1990. Edinburgh, Scotland. pp. 20-21.
- Rangel-Ruiz, L. J., Marquez-Izquierdo, R. and Bravo-Nogueira, G. (1999) Bovine fasciolosis, Mexico. *Vet. Parasitol.* **81**, 119-127.
- Reeves, M.J. and Smith G. (1995) The application and use of epidemiological modelling in the study of equine health problems. *Equine Vet. J.*, **27**(2), 84-85.
- Reeves, M.J., Curtis, C.R., Salman, M.D. and Hilbert, B.J. (1989) Prognosis in equine colic patients using multivariable analysis. *Can. J. Vet. Res.*, **53**, 87-94.
- Reeves, M.J., Curtis, C.R., Salman, M.D., Reif, J.S. and Stashak, T.S. (1990) A multivariable prognostic model for equine colic patients. *Prev. Vet. Med.*, **9**, 241-257.
- Rehbein, S., Holste, J.E., Doucet, M.Y., Fenger, C., Paul, A.J., Reinemeyer, C.R., Smith, L.L., Yoon, S. and Marley, S.F. (2003) Field efficacy of ivermectin plus praziquantel oral paste against naturally acquired gastrointestinal nematodes and cestodes of horses in North America and Europe. *Vet. Ther.* **4**(3): 220-227.
- Reid, S.W.J., Mair, T.S., Hillyer, M.H. and Love, S. (1995) Epidemiological risk factors associated with a diagnosis of clinical cyathostomiasis in the horse. *Equine Vet. J.*, **27**(2), 127-130.
- Reid, S.W.J., Mellor, D.J., Irwin, T., Love, S. and Gettinby, G. (1996) A hypertext knowledge base medium for the delivery of epidemiological models and expert system. *Prev. Vet. Med.*, **26**(2), 97-105.
- Reilly, G.A.S., Cassidy, J.P. and Taylor, S.M. (1993) Two cases of diarrhoea in horses associated with larvae of the small strongyle. *Vet. Rec.*, **132**, 267-268.
- Reinecke, R.K. (1960) A field study of some nematode parasites of bovines in a semi-arid area, with special reference to their biology and possible methods of prophylaxis. *Onderstepoort J. Vet. Res.*, **28**, 365-464
- Reinemeyer, C.R. (1986) Small strongyles: recent advances. *Vet. Clin. North Am. Equine Pract.*, **2**, 281-312.
- Reinemeyer, C.R., Herd, R.P. and Gabel, A.A. (1988) Distribution of adult and larval cyathostomes in helminth-naïve foals after primary infection. *Equine Vet. J.*, **20**, 296-297.
- Reinemeyer, C.R., Smith, S.A., Gabel, A.A. and Herd, R.P. (1984) The prevalence and intensity of internal parasites in horses in the USA. *Vet. Parasitol.*, **15**, 75-83.

- Reinemeyer, C.R., Smith, S.A., Gabel, A.A. and Herd, R.P. (1986) Observations on the population dynamics of five cyathostome nematode species of horses in northern USA. *Equine vet. J.*, **18**(2), 121-124.
- Ricci, N. and Sabatini, A. (1992) Reports on the helminth parasites of caecum and colon of equids in Italy. *Parasitologia (Roma)*, **34**(1/2/3), 53-60.
- Rim, H.J., Farag, H.F., Sornmani, S. and Cross, J.H. (1994) Foodborne trematodes: ignored or emerging? *Parasitol. Today*. **10**, 207-209.
- Roberts, J.A., Estuningsih, E., Widjayanti, S. and Wiedosari, E. (1997) Resistance of Indonesian Thin Tailed sheep against *F. hepatica* and *F. gigantica*. *Vet. Parasitol.*, **68**(1-2), 69-78.
- Roberts, J.A., Widjayanti, S. and Estuningsih, E. (1996) Acquired resistance of merino sheep against *F. gigantica*. *Parasitol. Res.*, **82**, 743-746.
- Roberts, M.G. (1995) A pocket guide to host-parasite models. *Parasitolo Today*, **11**(5), 172-177.
- Roberts, M.G. (1999) The immunoepidemiology of nematode parasites of farmed animals: a mathematical approach. *Parasitolo Today*, **15**(6), 246-251.
- Rodriguez-Bertos, A., Corchero, J., Castano, M., Pena, L. and Luzon, M. (1999) Pathological alterations caused by *Anoplocephala perfoliata* infection in the ileocaecal junction of equids. *J. Vet. Med.*, **46**, 261-269.
- Rodriguez-Perez, J. and Hillyer, G.V. (1995) Detection of excretory-secretory circulating antigens in sheep infected with *F. hepatica* and with *schistosoma mansoni* and *F. hepatica*. *Vet. Parasitol.*, **56**, 57-66.
- Rogers, R.W. (1966) Tapeworm infection causing intussusception in a horse. *Mod. Vet. Pract.*, **47**(4), 72-73.
- Rooney, J.R. (1964) Gastric ulceration in foals. *Path. Vet.* **1**, 497-503.
- Ross, J.C. (1967) A comparison of the resistance status of hosts to infection with *Fasciola hepatica*. Proceedings of the 3rd international conference of the world association for the advancement of veterinary parasitology. Lyons, 96-105.
- Ross, R. (1908) Report on the prevention of malaria in Mauritius. London.
- Ross, R. (1911) The prevention of malaria (2nd eds). Murray, London.
- Ross, R. and Hudson, H.P. (1917) An application of the theory of probabilities to the study of a priori pathometry, III. *Pro. R. Soc.*, **A93**, 225-240.

- Rossanigo, C.E. and Gruner, L. (1994) Relative effect of temperature and moisture on the development of strongyle eggs to infective larvae in bovine parts in Argentina. *Vet. Parasitol.*, **55**, 317-325.
- Round, M.C. (1969) The prepatent period of some horse nematodes determined by experimental infection. *J. Helminthol*, **43**, 185-192.
- Rua-Domenech, R. de la., Reid, S.W.J., Gonzalez-Zariquey, A.E., Wood, J.L.N. and Gettinby, G. (2000) Modelling the spread of a viral infection in equine populations managed in Thoroughbred racehorse training yards. *Prev. Vet. Med.*, **47**(1-2), 61-77.
- Rubilar, L., Cabreira, A. and Giacaman, L. (1988) Treatment of *Fasciola hepatica* infection in horses with triclabendazole. *Vet. Rec.*, **123**, 320-321.
- Rupasinghe, D. and Ogbourne, C.P. (1978) Laboratory studies on the effect of temperature on the development of the free-living stages of strongylid nematodes of the horse. *Zeit. Parasitenk*, **55**, 249-253.
- Russell, A.F. (1948) The development of helminthiasis in thoroughbred foals. *J. Com. Path. Ther.*, **58**, 107-127.
- Sahba, G.H., Arfaa, F., Farahmandian, I. And Jalali, H. (1972). Animal fascioliasis in Khuzestan, southern Iran. *J. Parasitol.*, **58**, 712-716.
- Sanda, Y. and Tsukada, T (1985) Efficacy trial with pyrantel pamoate against *Anoplocephala perfoliata* in horses. *Japanese J. Vet. Med.* **772**, 722-725.
- Sanson, R.L. and Thornton, R.N. (1997) A modelling approach to the quantification of the benefits of a national surveillance programme. *Prev. Vet. Med.*, **30**(1), 37-47.
- Santiago, N. and Hillyer, G.V. (1988) Antibody profiles by EITB and ELISA of cattle and sheep infected with *F. hepatica*. *J. Parasitol.*, **74**, 810-818.
- Scott, J.M. and Goll, P.H. (1977) The epidemiology and anthelmintic control of ovine fascioliasis in the Ethiopian central highlands. *Br. Vet. J.*, **133**, 273-280.
- Seaton, D.S., Jackson, F., Smith, W.D. and Angus, K.W. (1989). Development of immunity to incoming radiolabbed larvae in lambs continuously infected with *Ostertagia circumcincta*. *Res. Vet. Sc.* **46**, 241-246.
- Scott, M.E. and Smith, G. (1994) Parasitic and infectious diseases: epidemiology and ecology. Academic Press, Ltd. London, UK.
- Selim, M.K., Abdel-gawad, A.F., Ragheb, M.M. and Abd el-Malek, M.H. (1994) Control of different strongyle worms in donkeys under the Egyptian environmental condition. In: M. Bakkoury and R.A. Prentis (eds). Working equines. *Proceedings of The Second International Colloquium*, 22-24 April, 1994. Rabat, Morocco. pp. 149-154.

- Sengupta, P.P. and Yadav, M.P. (1998) Incidence of gastrointestinal in organized and unorganised equid farms of Haryana. *Indian Journal of Animal Science*, **68**(12), 1218-1220.
- Sequeira, J.L. Yostes, R.A. and Oliver-Sequeira, T.C.G. (2001) Prevalence and macro and microscopic lesions produced by *Gasterophilus nasalis* (Diptera: Oestridae) in the Botucatu region, SP, Brazil. *Vet. Parasitol.* **102**, 261-266.
- Sewell, M.M.H. (1991) Uniformity and control of the helminth diseases of equids. In: D. Fielding and R.A. Pearson (eds). Donkeys, mules and horses in tropical agricultural development. *Proceedings of a colloquium*, 3-6 September, 1990. Edinburgh, Scotland. pp. 141-150.
- Sisay, Z. and Tilahun, F. (1997) The role of pack donkeys in the major grain market (Yehil Berenda) of Addis Ababa, Ethiopia. Paper presented for Animal Traction Network in Eastern and Southern Africa (ATNESA) workshop on improving donkey utilization and management. 5-9, May 1997. Debre Zeit, Ethiopia (In press).
- Sherriff, A. (1996) A mathematical study of Environmental effects and genetic drug resistance on the lifecycle of the nematode *Teldorsagia circumcincta*. Thesis. University of Strathclyde. Glasgow, UK.
- Slocombe, J.O.D. (1979) Prevalence and treatment of tapeworms in horses. *Can. Vet. J.*, **20**, 136-140.
- Slocombe, J.O.D. (1985) Pathogenesis of helminths in equines. *Vet. Parasitol.*, **18**, 139-153.
- Slocombe, J.O.D. McCraw, B.M. (1973) Gastrointestinal nematodes in horses in Ontario. *Can. Vet. J.*, **14**, 101-105.
- Slocombe, J.O.D., Valenzuela, J. and Lake, M.C. (1987) Epidemiology of strongyles in ponies in Ontario. *Can. J. Vet. Res.*, **51**, 470-474.
- Slocome, J.O.D. (1997) The critical test for *Anoplocephala perfoliata* in equids and efficacy of B1995 oral paste for the parasites. In : Penzhorn, B.L. and Krecek, R.C. (eds), World association for the advancement of veterinary parasitology (WAAVP). 16th international conference, Sun City, South Africa. August 10-15, 1997. 78p.
- Smith G. (1990) A mathematical model for the evolution of anthelmintic resistance in a direct life cycle nematode parasites. *Int. J Parasitol*, **20**(7), 913-921.
- Smith, G. (1989) The population biology of the parasitic phase of *Ostertagia circumcincta*. *Int. J. Parasitol.*, **19**, 385-393.
- Smith, G. (1994) Population biology of the parasitic phase of trichostrongylid nematode parasites of cattle and sheep. *Int. J. Parasitol.*, **24**, 167-178.

- Smith, G. and Galligan, D.T. (1988) Mathematical models of the population biology of *Ostertagia ostertagi* and *Teladorsagia circumcincta*, and the economic evaluation of disease control strategies. *Vet. Parasitol.*, **27**, 73-83.
- Smith, G. and Grefell, B.T. (1994) Modelling of parasite populations: gastrointestinal nematode models. *Vet. Parasitol.*, **54**, 127-143.
- Smith, G. and Guerrero, J. (1993) Mathematical models for the population biology of *Ostertagia ostertagi* and the significance of aggregated parasite distributions. *Vet. Parasitol.*, **46**, 243-257.
- Smith, G.C. (2001) Models of mycobacterium bovis in wildlife and cattle. *Tuberculosis*, **81**(1-2), 51-64.
- Smith, H.J. (1976a) Strongyle infections in ponies. I. Response to intermittent thiabendazole treatments. *Can. J. Comp. Med.*, **40**, 327-333.
- Smith, H.J. (1978) Experimental trichonema infections in mature ponies. *Vet. Parasitol.*, **4**, 265-273.
- Sorensen, J.T. and Enevoldsen, C. (1992) Modelling the dynamics of the health-production complex in livestock herd: a review. *Prev. Vet. Med.*, **13**, 287-297.
- Sotiraki, S.T., Badouvas, A.G. and Himonas, C.A. (1997). A survey on the prevalence of internal parasites of equines in Macedonia and Thessalia- Greece. *J. Equine Vet. Sci.*, **17**(10), 550-552.
- Soule, C., Boulard, C., Levieux, D., Barnouin, J. and Plateau, E. (1989) Experimental fascioliasis in horses: evolution of serum antibodies, plasma enzymes and faecal egg count. *Annales de Recherches Veterinaire*. **20**(3), 295-307.
- Soulsby, E.J.L. (1965) Cestodes of horses. Textbook of Veterinary Clinical Parasitology, Vol. 1. FA Davis Co., Philadelphia
- Soulsby, E.J.L. (1968) Helminths, arthropods and protozoa of domesticated animals. Bailliere, Tindall and Cassell. London, UK. pp.96.
- Soulsby, E.J.L. (1982) Helminths, arthropods and protozoa of domesticated animals (7th ed). Baillier Tindall. London, UK.
- Southcott, W.H. and Barger, I.A. (1975) Control of nematode parasites by grazing management 2. Decontamination of sheep and cattle pasture by varying periods of grazing with the alternate host. *Int. J. parasitol.*, **5**, 45-48.
- Southwood, L.L., Baxter, G.M., Bennet, D.G. and Ragle, C.A. (1998) Ascarid impaction in young horses. *Compend. Contin. Educ. Pract. Vet.*, **20**(1), 100-106).

- Spasskii, A.A. (1951) Anoplocephalate tapeworms of domestic and wild animals. *Osnovy Tsestodologii*, Vol. I. Akad. Nauk. SSSR, Moscow. pp. 730.
- Spithill, T.W., Smooker, P.M. and Copeman, D.B. (1999) *Fasciola gigantica*: epidemiology, control, immunology and molecular biology. In: J.P. Dalton (ed). *Fasciolosis*. CAB International, Oxon. pp. 465-525.
- Srihakim, S. and Swerczek, T.W. (1978) Pathologic changes and pathogenesis of *Parascaris equorum* infection in parasite-free pony foals. *Am. J. Vet. Res.*, **39**, 1155-1159.
- Starkey, P. (1994a) Donkey utilisation in Sub-Saharan Africa: recent changes and apparent needs. In: M. Bakkoury and R.A. Prentis (eds). *Working equines. Proceedings of The Second International Colloquium*, 22-24 April 1994. Rabat, Morocco. pp. 289-302.
- Starkey, P. (1994b) Improving animal traction technology. In: P. Starkey, E. Mwenya and J. Stares (eds). *Proceedings of The First Workshop of the Animal Traction Net Work for Eastern and Southern Africa (ATNESA)*, 18-23 January 1992. Lusaka, Zambia.
- Starkey, P. (1995) The donkey in South Africa: myths and misconceptions. In: P. Starkey (ed). *Animal power in South Africa- empowering rural communities*. Development Bank of South Africa, Gauteng, South Africa. 160p.
- Starkey, P. (1997) Donkey work. In: E.D. Svendsen (ed). *The Professional Handbook of The Donkey*. Whittet Books Limited. London, UK. pp. 183-206.
- Starkey, P. and Starkey, M. (1997) Regional and world trends in donkey population. Paper presented for Animal Traction Network in Eastern and Southern Africa (ATNESA) workshop on improving donkey utilization and management. 5-9 May, 1997. Debre Zeit, Ethiopia (In press).
- Stear, M.J. and Bishop, S.C. (1999) The curvilinear relationship between worm length and fecundity of *Teladorsagia circumcincta*. *Int. J. Vet. Parasitol.*, **29**, 777-780
- Stear, M.J., Bairden, K. and Bishop, S.C. (1998) The processes influencing the distribution of parasitic nematodes among naturally infected lambs. *Parasitology*, **117**, 165-171.
- Stear, M.J., Bishop, S.C., Doligalska, M., Duncan, J.L., Holmes, P.H., Irvine, J., McCririe, L., McKellar, Q.A., Sinski, E. and Murray, M. (1995) Regulation of egg production, worm burden, worm length, and worm fecundity by host responses in sheep infected with *Ostertagia circumcincta*. *Parasite Immunol*, **17**, 643-652.
- Storay, G.W. and Phillips, R.A (1985) The survival of parasite eggs throughout the soil profile. *Parasitology*. **91**, 585-590.

- Stromberg, B.E. (1997) Environmental factors influencing transmission. *Vet. Parasitol.*, **72**, 247-264.
- Sutherland, I.A., Leathwick, D.M., brown, A.E. and Miller C.M. (1997) Prophylactic efficacy of persistent anthelmintics against challenged with drug resistant and susceptible *Ostertagia circumcincta*. *Vet. Rec.*, **141**, 120-123.
- Svendsen, E.D. (1991) Work to improve the condition of working donkeys and mules worldwide. In: D. Fielding and R.A. Pearson (eds). Donkeys, mules and horses in tropical agricultural development. *Proceedings of a colloquium*, 3-6 September 1990. Edinburgh, Scotland. pp. 181-184.
- Svendsen, E.D. (1994) The main types of parasites affecting donkeys' health in various parts of the world. In: M. Bakkoury and R.A. Prentis (eds). Working equines. *Proceedings of The Second International Colloquium*, 20-22 April, 1994. Rabat, Morocco. pp. 23-34.
- Svendsen, E.D. (1997a) Parasites abroad. In: E.D. Svendsen (ed). The Professional Handbook of The Donkey (3rd ed). Whittet Books Limited. London, UK. pp. 227-238.
- Svendsen, E.D. (1997b) Donkeys abroad. In: E.D. Svendsen (ed). The Professional Handbook of The Donkey (3rd ed). Whittet Books Limited. London, UK. pp. 166-182.
- Tan, Y.T., Tillett, D.J. and Mckay, I.A. (2000) Molecular strategies for overcoming antibiotic resistance in bacteria. *Mol. Med. Today*, **6**(8), 309-314.
- Taylor, E. L.(1954) Grazing behaviour and helminthic disease. *Brit. J. Anim. Behaviour*, **2**, 61-62.
- Thamsborg, S.M., Leifsson, P.S., Grondahl, C, Larsen, M. and Nansen, P. (1998) Impact of mixed strongyle infections in foals after 1 month on pasture. *Equine Vet. J.*, **30**, 240-245.
- Thomas, R.J. and Starr, J.R. (1978) Forecasting the peak of gastrointestinal nematode infection in lambs. *Vet. Rec.*, **103**, 465-468.
- Thrushfield, M. (1995) Veterinary epidemiology (2nd ed). Modelling. Black well science Ltd. Oxford, UK. pp 296- 311.
- Timbely, S. (1998) Development and survival of infective larvae of nematode parasites of sheep on pasture in a cool tropical environment. *Vet. Parasitol.*, **79**, 81-87.

- Timbely, S., Lahlou-Kassi, A., Rege, J.E.O., Sovani, S., Diedhiou, M.L. and Baker, R.L. (1997) The epidemiology of nematode infections in sheep in a col tropical environment. *Vet. Parasitol.*, **70**, 129-141.
- Todd, A.C. and Doherty, L.P. (1951) Treatment of ascariasis in horses in central Kentucky. *J. Am. Vet. Med. Ass.*, **119**, 363.
- Todd, A.C. Hanson, M.F., Kelley, G.W. (1949) Worm parasites in thoroughbred sucklings and weanlings. A survey of incidence, development and control. *Kentucky Experimental Station Bulletin* 541.
- Todd, J.C. (1979) Clinical diagnosis by laboratory methods. Philadelphia, PA. W.B. Saunders Company, USA.
- Toll Vera, J.R. Gamboa, D.E. and Cruz, M.L. (1995) The liver fluke (*F. hepatica*), endoparasite present in the Tambera Valley of Tucuman, Argentina. *Avance Agroindustrial*. **16**(62), 18-21.
- Tolliver S.C., Lyons, E.T. and Drudge, J.H. (1987) Prevalence of internal parasites in horses in critical tests of activity of parasiticides over 28 years period (1956-1983) in Kentucky. *Vet. Parasitol.*, **23**, 273-284.
- Tolliver, S.C., Lyons, E.T. and Drudge, J.H. (1985) Species of small strongyles and other internal parasites recovered from donkeys at necropsy in Kentucky. Proceedings of The Helminthological Society of Washington, **52**, 260-265.
- Torbert, B.J., Klei, T.R. and Lichtenfels, J.R.(1986) A survey in Louisiana of intestinal helminths of ponies with little exposure to anthelmintic. *J. Parasitol.*, **72**, 926-930.
- Torgerson, P. and Claxton, J. (1999) Epidemiology and control. In: J.P. Dalton (ed). Fasciolosis. CAB International, Oxon. pp. 113-149.
- Trawford, A.F. and Tremlett, J.G. (1996) Efficacy of triclabendazole against *Fasciola hepatica* in the donkey (*Equus asinus*). *Vet. Rec.*, **139**, 142-143.
- Trewhella, W.R. and Anderson, R.M. (1983) Modelling bovine tuberculosis in badgers. In: Thrusfield, M.V (ed). Proceedings of the Society for veterinary epidemiology and preventive medicine. 12-13 April, 1983, Southampton, UK.
- Twerda, M., Fielding, D. and Fielding, C. (1997) Role and management of donkeys in Samburu and Turkana pastoralist societies in Northern Kenya. *Trop. Anim. Health Prod*, **29**, 48-54
- Uhlinger, C.A. (1991) Equine small strongyles; epidemiology, pathology and control. *Compend. Contin. Educ. Pract. Vet*, **13**, 863-869.

- Uhlinger, C.A. (1993) Uses of faecal egg count data in equine practice. *Compend. Contin. Educ. Pract. Vet.*, **15**(5), 742-749.
- Upadhyay, R.C. (1991) Equine power: the role and feature potential in India. In: D. Fielding and R.A. Pearson (eds). Donkeys, mules and horses in tropical agricultural development. *Proceedings of a colloquium*, 3-6 September 1990. Edinburgh, Scotland. pp. 13-19.
- Uppal, P.K. (1991) Control of the 1987 equine influenza epidemic in India and the present status of the disease. In: D. Fielding and R.A. Pearson (eds). Donkeys, mules and horses in tropical agricultural development. *Proceedings of a colloquium*, 3-6 September, 1990. Edinburgh, Scotland. pp. 126-132.
- Urch, D.L. and Allen, W.R. (1980) Studies on fenbendazole for treating lung and intestinal parasites in horses and donkeys. *Equine Vet. Educ.*, **12**, 74-77.
- Urquhart, G.M., Armour, J., Duncan, J.L., Dunn, A.M. and Jennings, F.W. (1996) Veterinary Parasitology (2nd ed). Black Well Science Ltd. Oxford and London, UK.
- Vatistas, N.J., Snyder, J.R. and Wilson, W.D. (1996) Surgical treatment for colic in the foal (67 cases): 1980-1992. *Equine Vet. J.*, **28**, 139-145.
- Vercruysse, J., Harriids, E.A., Kaboret, Y., Pangui, L.J. and Gibson, D.I. (1986) Gastrointestinal helminths of donkeys in Burkina Faso. *Zeitschrift fur Parasitenkunde* **72**, 821-825.
- Waddell, A.H. (1972) The pathogenecity of *Gasterophilus intestinalis* larvae in the stomach of the horse. *Aust. Vet. J.* **48**, 332-335.
- Wall, R. and Shearer, D (1991) Veterinary ectoparasites. Biology, pathology and control. (2nd ed) Oxford, Blackwell Science. pp. 114-142.
- Waruiru, R.M., Munyua, S.M., Thamsborg and Nansen, P. (1998) Development and survival of infective larvae of gastrointestinal nematodes of cattle on pasture in central Kenya. *Vet. Res. Coms.*, **22**, 315-323.
- Waruiru, R.M., Weda, E.H., Otieno, R.O. and Ngotho, J.W. (2002) Seasonal availability of gastrointestinal nematode larvae to cattle on pasture in the central highlands of Kenya. *Onderstepoort J. Vet. Res.*, **69**(2), 141-146.
- Wells, D. and Krecek, R.C. (1997) Socio-economic roles of donkeys in Hammanskraal, South Africa. Paper presented for Animal Traction Network in Eastern and Southern Africa (ATNESA) workshop on improving donkey utilization and management. 5-9 May 1997. Debre Zeit, Ethiopia (In press).

- Wells, D., Krecek, R.C., Wells, M. and Guthrie, A.J. (1998) Helminth levels of working donkeys kept under different management systems in the Moretele 1 district of the north west province, South Africa. *Vet. Parasitol.*, **77**, 163-177.
- Wescot, R.B., Farrell, C.J. and Shen, D.T. (1983) Diagnosis of naturally occurring *F. hepatica* infections in cattle with Enzyme-linked immunosorbent assay. *Am. J. Vet. Res.*, **45**, 178-179.
- White, L.J., Evans, N.D., Lam, T.J., Schukken, Y.H., Medley, G.F., Godfrey, Chappell, [M.J. (2002) The structural identifiability and parameter estimation of a multispecies model for the transmission of mastitis in dairy cows with post milking teat disinfections. *Math. Biosci.*, **180**, 275-291.
- Whitlock, H.V. (1948) Some modification of the McMaster helminth egg-counting technique and apparatus. *J. Counc. Sci. Ind Res.* **21**, 177-180.
- WHO (1995) Control of foodborne trematodes infections. Technical report series 849.
- Wickwire, K. (1977) Mathematical models for the control of pests and infectious diseases: a survey. *Theor. Pop. Biol.*, **11**, 182-238.
- Williamson, R.M.C., Beveridge, I. And Gasser, R.B. (1998) Coprological methods for the diagnosis of *A. perfoliata* infection of the horse. *Aust. Vet. J.*, **76**(9), 618-621.
- Williamson, R.M.C., Gasser, R.B., Middleton, D. and Beveridge, I. (1997) The distribution of *Anoplocephala perfoliata* in the intestine of the horse and associated pathological changes. *Vet. Parasitol.*, **73**, 225-241.
- Wilson, K. and Grenfell, B.T. (1997) Generalized linear modelling for parasitologists. *Parasitol Today*, **13**, 33-38.
- Wilson, R.T. (1990) The Donkey. In: W.J.A. Payne (ed). An introduction to animal husbandry in the tropics (4th ed). Longman Scientific Technical. New York USA. pp. 581-603.
- Wilson, R.T. (1991) Equines in Ethiopia. In: D. Fielding and R.A. Pearson (eds). Donkeys, mules and horses in tropical agricultural development. *Proceedings of a colloquium*, 3-6 September, 1990. Edinburgh, Scotland. pp. 33-47.
- Wiltshire, F.H. (1954) Diseases of foals. *Aust. Vet. J.*, **30**, 22-24.
- Wood, I.B., Amaral, N. K., Bairden, K. Duncan, J. L., Kassai, T., Malone, J. B., Pankavich, J.A., Reinecke, R.K., Slocombe, O., Taylor, S.M. and Vercruysse, J. (1995) World association for the advancement of Veterinary parasitology (WAAVP): Second

- edition of guidelines for evaluating the efficacy of anthelmintics in ruminants (bovine, ovine, caprine). *Vet. Parasitol.* **58**, 181-123.
- Yilma, J.M. and Malone, J.B. (1998) A geographical information system forecast model for strategic control of fasciolosis in Ethiopia. *Vet. Parasitol.*, **78**, 103-127.
- Yilma, J.M. Feseha, G. Svendsen, E.D. (1989) Epidemiology and control of helminthiasis of working donkeys in Ethiopia (abstract). World Association for the Advancement of Veterinary Parasitology. August 1989. Berlin. pp. 38.
- Yilma, J.M. Feseha, G. Svendsen, E.D. and Mohammed, A. (1991) Health problems of working donkeys in Debre Zeit and Menagesha regions of Ethiopia. In: D. Fielding and R.A. Pearson (eds). Donkeys, mules and horses in tropical agricultural development. *Proceedings of a colloquium*, 3-6 September, 1990. Edinburgh, Scotland. pp. 151-155.
- Yoshihara, T. Oikawa, M., Katayama, Y. and Kaneko, M. (1993) The prevalence of intestinal parasites in racehorses in Japan; a necropsy survey. *Proceedings WAAVP, Congress*, 1993, Cambridge. pp.151.
- Zealelem. B., Dilnesaw, A. and Mengistu, G. (1997) Local manufacturing and distribution of donkey carts in South Shewa, Ethiopia: lessons and problems. Paper presented for Animal Traction Network in Eastern and Southern Africa (ATNESA) workshop on improving donkey utilization and management. 5-9 May 1997. Debre Zeit, Ethiopia (In press).
- Zumpt, F. (1965) Myiasis in man and animals in the Old World. A Textbook of Physicians, Veterinarians and Zoologists. Butterworths and Co (publishers) LTD. London, UK.