

Integrated non-invasive investigations of captive Abyssinian colobus monkeys (*Colobus guereza*): behaviour, stress and parasitism

Submitted by Johanna Rabineau, to the University of Exeter as a thesis for the degree of Doctor of Philosophy in Biological Sciences, September 2009.

This thesis is available for Library use on the understanding that it is copyright material and that no quotation from the thesis may be published without proper acknowledgement

I certify that all material in this thesis which is not my own work has been identified and that no material has previously been submitted and approved for the award of a degree by this or any other University.

..... Johanna Rabineau

Abstract

The prime aim of the present study was to investigate the welfare of Abyssinian colobus monkeys (*Colobus guereza kikuyuensis*) held in captivity, by behavioural, endocrinological and parasitological approaches. Five groups of colobus monkeys were studied, in the UK at Port Lympne Zoo, Banham Zoo and Paignton Zoo (separate male and female groups) and in France at La Boissière du Doré Zoo.

Overall, the patterns of behavioural activity for captive colobus monkeys agree with observations of wild colobus monkeys, suggesting that there was generally good welfare at all zoos. However, local differences in group composition (i.e. age and gender) or management practices were shown to affect activity budgets. At Banham Zoo, where colobus monkeys had access to a large paddock and food was offered only twice a day, animals spent significantly more time foraging/feeding than other groups which received three meals a day. At Paignton Zoo (adult male only group) animals spent more time resting alone and less time resting socially or being social than other zoos. Generally, across zoos, older, higher ranking animals spent less time moving and more time feeding than younger, more subordinate ones.

Social dominance rank and linearity of the hierarchy in the five groups of colobus was determined. Large mixed groups of colobus monkeys including both males and females displayed strong linear dominance hierarchies, but in single sex groups, the strength of the hierarchy was found to depend on local events and group composition. Generally, male colobus monkeys displayed most of the dominance behaviour, however, females were an integral part of the dominance hierarchy with unidirectional dominant behaviour between both genders in large mixed groups.

Factors such as life history, which may influence social dominance ranking, were investigated. Index of success (another measurement of dominance rank) was mainly explained by age and gender. Social behaviours such as play behaviour and grooming were also associated with ranking. Older, more dominant animals were more often the recipient of grooming behaviour whereas younger more subordinate animals spent more time playing..

Faecal egg counts of the intestinal nematode parasite, *Trichuris trichiura*, were investigated in relation to individual index of success, age, gender, and husbandry practices at each zoo. No significant differences in egg count were observed between genders. Egg count was explained by the index of success and anti-helminthic practices at zoos. The highest count of eggs were observed at La Boissière du Doré Zoo, probably due to the fact animals were locked in for several months in

the winter, therefore favouring re-infection of infective larvae. The lowest count of *Trichuris* egg was at Paignton Zoo female group, who had the largest enclosure, with very low animal density. Counts of eggs were at their lowest in the winter compared to other season of the year. Differences between zoos seem to result from anti-helminthic practices coupled with the influence of enclosure size and husbandry regimes.

Faecal glucocorticoids and their metabolites, in colobus monkeys held in the five groups, were measured by radioimmunoassay and investigated in relation to index of success, levels of aggression, gender, age and reproductive status of females. The overall dominant animals in various groups had higher levels of faecal glucocorticoids. Males had significantly lower faecal glucocorticoid than females, and pregnant females had higher levels than others. The older, higher ranking colobus monkeys initiated significantly more acts of aggression than younger, lower ranking animals. Seasonal variation in faecal cortisol equivalents were observed in the autumn where higher levels were recorded compared to other seasons. Comparison of faecal content of cortisol equivalents of the different groups showed the lowest levels at Port Lympne Zoo and Banham Zoo while the highest levels were at Paignton Zoo (female groups). This suggests that the large multi-male, multi-female groups had the lowest levels of stress and associated high levels of welfare.

These studies lead to the recommendation that single sex groups of captive Abyssinian colobus monkeys should be avoided, particularly when this involves breaking down the social structure of established groups.

Chapter 1: Introduction

1.1 The concept of animal welfare	17
1.2 Assessing welfare in captive animals	18
1.3 Stress and its relevance to animal welfare	20
1.4 Factors affecting stress and welfare in captivity	23
1.5 <i>Colobus guereza</i>: The Eastern black-and-white colobus	24
1.5.1 General physical description	25
1.5.2 Distribution	27
1.5.3 Habitat	27
1.5.4 Diet & digestion	27
1.5.5 Life history	28
1.5.6 Behaviour	29
1.5.7 Conservation of colobus monkeys	30
1.6 Aims	31

Chapter 2: Activity budget of *Colobus guereza*

2.1 Introduction	34
2.2 Methodology	40
2.2.1 Age category definition	40
2.2.2 Study sites and subjects	41
2.2.3 Housing and Husbandry	43
2.2.3 a Port Lympne Zoo	43
2.2.3 b Paignton Zoo	43
2.2.3 c Banham Zoo	46
2.2.3 d La Boissière du Doré	46
2.2.4 Behavioural data collection	47
2.2.5 Data analyses	48
2.3 Results	50
2.3.1 Overall activity budget	50
2.3.1 a Port Lympne Zoo	50
2.3.1 b Paignton Zoo	50

2.3.1 c Banham Zoo	52
2.3.1 d La Boissière du Doré Zoo	52
2.3.2 Feeding behaviour	54
2.3.3 Social behaviour	58
2.3.4 Moving behaviour	61
2.3.5 Resting socially behaviour	64
2.3.6 Resting alone behaviour	68
2.4 Discussion	72
2.4.1 Seasonality	72
2.4.2 Influence of age/sex and index of success on activity budgets	74
2.4.3 Activity budget comparison between zoos	75
2.4.4 Activity budget in captivity compared to in the wild	77
2.5 Summary & Conclusions	79

Chapter 3: Social dominance

3.1 Introduction	81
3.1.1 Social dominance	81
3.1.2 Social dominance hierarchy	82
3.1.3 Linear dominance hierarchy	84
3.1.4 Social dominance hierarchy, life history and social behaviours	85
3.1.4 a Social dominance hierarchy in relation to gender	85
3.1.4 b Social dominance hierarchy and life history	87
3.1.4 c Social dominance hierarchy and grooming behaviour	87
3.2 Methodology	89
3.2.1 Dominance related activities	89
3.2.2 Focal sampling of behaviours	89
3.2.3 Data Analysis	91
3.2.3 a Social dominance hierarchy	91
3.2.3 b Index of success in relation to age, sex, linearity of the hierarchy and social behaviours	93

3.3 Results	94
3.3.1 Dominance matrices and hierarchy indices	94
3.3.2 Life history factors and social behaviours in relation to index of success	101
3.4 Discussion	105
3.4.1 Dominance hierarchy linearity	105
3.4.1 a <i>Social dominance in large mixed sexes groups</i>	106
3.4.1 b <i>Dominance hierarchies at Paignton Zoo male and female groups</i>	108
3.4.1 c <i>Differences in social dominance hierarchy of females in single sex and mixed sexes groups</i>	109
3.4.2. Index of success in relation to age, sex and social behaviours	110
3.5 Summary & Conclusions	113

Chapter 4: *Trichuris trichiura* burden in captive *Colobus guereza*

4.1 Introduction	115
4.1.1 The importance of Trichuriasis in relation to health	115
4.1.2 <i>Trichuris spp</i> life cycle	116
4.1.3 Trichuriasis in primates and factors affecting it	117
4.2 Methods	119
4.2.1. Sample collection	119
4.2.2 Identification, extraction and measurement of <i>Trichuris</i> eggs in faeces	120
4.2.3 Validation of separation methods	123
4.2.3 a <i>Extraction of <i>Trichuris</i> eggs from faecal samples</i>	123
4.2.3 b <i>Assessment of eggs lost in supernatant</i>	123
4.2.3 c <i>Floatation time</i>	124
4.2.4 Anti-helminthic practices	125
4.2.5 Data analyses	128
4.3 Results	129
4.3.1 Mean <i>Trichuris</i> egg count per data collection period in relation to anti-helminthic practice	129
4.3.2 Generalised estimated equation of factors affecting the counts of <i>Trichuris</i> eggs	132

4.4 Discussion	136
4.4.1 <i>Trichuris</i> egg count, age and index of success	136
4.4.2 <i>Trichuris</i> egg count and gender	137
4.4.3 Seasonality of <i>Trichuris</i> epg	138
4.4.4 The impact of anti-helminthic on <i>Trichuris</i> epg	139
4.4.5 Enclosure size and husbandry	140
4.5 Conclusions	142

Chapter 5: Non invasive monitoring of faecal cortisol equivalents in relation to rank, age, gender and time

5.1 Introduction	143
5.1.1 Measurement of stress from plasma and faecal samples	143
5.1.2 Steroid pathway	145
5.1.3 Presence of glucocorticoid and their metabolites in faeces and urine	147
5.1.4 Faecal glucocorticoid content in relation to social dominance hierarchy	149
5.2 Materials and methods	152
5.2.1 Faecal sample collection	152
5.2.2 Lyophilisation	153
5.2.3 Grinding	154
5.2.4 Extraction of faecal samples	154
5.2.5 Extraction efficiency	155
5.2.6 Radioimmunoassay	155
5.2.6 a Assay methanol concentration	155
5.2.6 b Antiserum and ¹²⁵ I cortisol concentration	156
5.2.6 c ¹²⁵ I cortisol radioimmunoassay	160
5.2.7 Assay checks	162
5.2.7 a RIA coefficients of variation and minimum detectable limit	162
5.2.7 b Parallelism of faecal extracts and standard curve	162
5.2.7 c Biological validation	163
5.2.7 d Assay cross-reactivity	164

5.2.8 Cross-reacting compounds investigated using HPLC	165
<i>5.2.8 a Sep-Pak (C18) purification of faecal samples</i>	167
<i>5.2.8 b HPLC system</i>	167
<i>5.2.8 c HPLC analysis</i>	167
5.2.9 Data analyses	168
5.3 Results	170
5.3.1 Antiserum cross-reactivity tests	170
5.3.2 HPLC results for faecal samples and cross-reacting compounds	173
5.3.3 GEE of predictors affecting faecal contents of cortisol equivalents.	178
5.3.4 Detailed results of cortisol equivalents at La Boissière du Doré Zoo	184
5.4 Discussion	184
5.4.1 Radioimmunoassay (RIA) of faecal cortisol equivalents	184
5.4.2 Faecal cortisol equivalents in relation to index of success, gender and reproductive status	188
5.4.3 Faecal cortisol equivalents in relation to aggression	190
5.4.4 Comparison of faecal content of cortisol equivalents of colobus monkeys in different zoos	191
5.5 Conclusions	194
<u>Chapter 6: General discussion and future directions</u>	196
<u>Reference List</u>	205

List of Tables

Chapter 2:

<i>Table 2.1:</i> Summary of colobus group composition at all zoos and times of visits.	41
<i>Tables 2.2 a-b:</i> Group composition, date of birth and age class of colobus monkeys at Port Lympne Zoo and Paignton Zoo during different visits.	42
<i>Tables 2. 3a-b:</i> Group composition, date of birth and age class of colobus monkeys at Banham Zoo and La Boissière du Doré Zoo during different visits.	44
<i>Table 2.4:</i> Enclosure characteristics and husbandry practices at Paignton Zoo, La Boissière du Doré Zoo, Banham Zoo and Port Lympne Zoo.	45
<i>Table 2.5:</i> Ethogram of state behaviours recorded in colobus monkeys.	47
<i>Table 2.6:</i> Total number of scans per visit per zoo.	48
<i>Table 2.7:</i> Factors affecting the % time spent feeding by colobus monkeys	55
<i>Table 2.8:</i> Factors affecting the % time spent socially by colobus monkeys	58
<i>Table 2.9:</i> Factors affecting the % time spent moving by colobus monkeys	61
<i>Table 2.10:</i> Factors affecting the % time spent resting socially by colobus monkeys	65
<i>Table 2.11:</i> Factors affecting the % time spent resting alone by colobus monkeys	68

Chapter 3:

<i>Table 3.1:</i> Ethogram of dominant and submissive behaviours recorded and used in the establishment of the social dominance hierarchy.	90
<i>Table 3.2:</i> Ethogram of behaviours recorded during focal sampling.	90
<i>Table 3.3:</i> Example of dominance matrix at Port Lympne, October 2006.	92
<i>Table 3.4:</i> Summary of dominance order and hierarchy indices (h') at all zoos during each data collection period.	97
<i>Table 3.5:</i> Factors affecting the index of success of colobus monkeys.	101

Chapter 4:

<i>Table 4.1:</i> Number of samples collected for each colobus monkey at each zoo during each data collection period.	129
<i>Table 4.2:</i> Factors affecting colobus monkeys faecal egg count of <i>Trichuris sp.</i>	133

Chapter 5:

<i>Table 5.1:</i> Standard number and cortisol in pg/tube and ng/ml.	160
<i>Table 5.2:</i> List of compounds used in radioimmunoassay cross-reactivity checks.	165
<i>Table 5.3:</i> Compounds standard dilution in pg/tube and ng/ml.	165
<i>Table 5.4:</i> Number of faecal samples for each colobus monkey at each zoo for each data collection period.	169
<i>Table 5.5:</i> Factors affecting colobus monkeys faecal cortisol equivalent levels.	179

List of Figures

Chapter 1:

- Figure 1.1:** Mode of communication between the central nervous system, the endocrine system and the immune system, hormones (from von Borell, 2001). 22
- Photo 1.1:** *Colobus guereza* 26

Chapter 2:

- Figure 2.1:** Age in months of colobus monkeys according to index of success. 49
- Figure 2.2:** Mean % time spent performing state behaviours between March 2005 and October 2006 for each colobus monkey at Port Lympne Zoo. 51
- Figure 2.3:** Mean % time spent performing state behaviours (from August 2005 to October 2006) for each colobus monkey at Paignton Zoo all male group. 51
- Figure 2.4:** Mean % time spent performing state behaviours (from June 2005 to October 2006) for each colobus monkey at Paignton Zoo female group. 52
- Figure 2.5:** Mean % time spent performing state behaviours (in March 2005 and October 2005) for each colobus monkey at Banham Zoo. 53
- Figure 2.6:** Mean % time spent performing state behaviours (in March 2006 and November 2006) for each colobus monkey at La Boissière du Doré Zoo. 53
- Figure 2.7:** Mean (+SE) % time spent feeding by the groups of colobus monkeys at each zoo. 56
- Figure 2.8:** Mean (+SE) % time spent feeding by the groups of colobus monkeys during each season. 56
- Figure 2.9:** Mean (+SE) % time spent feeding by the groups of colobus monkeys according to index of success. 57
- Figure 2.10:** Percentage time spent feeding by colobus monkeys according to their age. 57

Figure 2.11: Mean (+SE) % time spent socially by the groups of colobus monkeys at each zoo.	59
Figure 2.12: Mean (+SE) % time spent socially by the groups of colobus monkeys during each season.	60
Figure 2.13: Mean (+SE) % time spent socially by the groups of colobus monkeys according to index of success.	60
Figure 2.14: Mean (+SE) % time spent moving by the groups of colobus monkeys at each zoo.	62
Figure 2.15: Mean (+SE) % time spent moving by the groups of colobus monkeys according to index of success.	63
Figure 2.16: Mean (+SE) % time spent moving by the groups of colobus monkeys according to gender.	63
Figure 2.17: Percentage time spent resting moving by colobus monkeys according to their age.	64
Figure 2.18: Mean (+SE) % time spent resting socially by the groups of colobus monkeys at each zoo.	66
Figure 2.19: Mean (+SE) % time spent resting socially by the groups of colobus monkeys during each season.	66
Figure 2.20: Mean (+SE) % time spent resting socially by the groups of colobus monkeys according to index of success.	67
Figure 2.21: Mean (+SE) % time spent resting socially by the groups of colobus monkeys according to gender.	67
Figure 2.22: Mean (+SE) % time spent resting alone by the groups of colobus monkeys at each zoo.	70
Figure 2.23: Mean (+SE) % time spent resting alone by the groups of colobus monkeys during each season.	70
Figure 2.24: Mean (+SE) % time spent resting alone by the groups of colobus monkeys according to index of success.	71
Figure 2.25: Mean (+SE) % time spent resting alone by the groups of colobus monkeys according to gender.	71

Chapter 3:

Figures 3.1 a-d: Dominance matrices in March 2005, July 2005 October 2005 and January 2006 at Port Lympne Zoo.	95
Figures 3.2 a-c: Dominance matrices in April 2006, July 2006 and October 2006 at Port Lympne Zoo.	96
Figures 3.3 a-b: Dominance matrices in March 2005 and October 2005 at Banham Zoo	98
Figures 3.4 a-b: Dominance matrices in March 2006 and November 2006 at La Boissière du Doré Zoo.	98
Figures 3.5 a-c: Dominance matrices in June 2005, March 2006 and October 2006 at Paignton Zoo female group.	100
Figures 3.6 a-c: Dominance matrices in August 2005, March 2006 and October 2006 at Paignton Zoo all male group.	100
Figure 3.7: Age in months and gender of colobus monkeys according to index of success.	102
Figure 3.8: Percentage time spent playing by the colobus monkeys according to their index of success.	102
Figure 3.9: Percentage time spent providing grooming by the colobus monkeys according to their index of success and gender.	103
Figure 3.10: Percentage time spent receiving grooming by the colobus monkeys according to their index of success.	103
Figure 3.11: Percentage time spent providing grooming by the colobus monkeys according to gender and index of success.	104

Chapter 4:

Photo 4.1-4.3: <i>Trichuris</i> eggs	122
Figure 4.1: Mean <i>Trichuris</i> egg counts in samples left to float for up to 180 min in McMaster chambers.	124

Figure 4.2: Faecal-parasitological work, anti-helminthic treatments and data collection periods at Port Lympne Zoo.	126
Figure 4.3: Anti-helminthic treatments and data collection periods at Paignton Zoo female group.	127
Figure 4.4: Anti-helminthic treatments and data collection periods at Paignton Zoo all male group.	127
Figure 4.5: Mean (+ SE) <i>Trichuris</i> count of eggs per gram (epg) of faeces for the group of colobus monkeys at Port Lympne Zoo for each data collection period.	130
Figure 4.6: Mean (+ SE) <i>Trichuris</i> count of eggs per gram (epg) of faeces for colobus monkeys at La Boissière du Doré Zoo for each data collection period.	130
Figure 4.7: Mean (+ SE) <i>Trichuris</i> count of eggs per gram (epg) of faeces for colobus monkeys at Banham Zoo for each data collection period.	131
Figure 4.8: Mean (+ SE) <i>Trichuris</i> count of eggs per gram (epg) of faeces for male group of colobus monkeys at Paignton Zoo for each data collection period.	131
Figure 4.9: Mean (+ SE) <i>Trichuris</i> count of eggs per gram (epg) of faeces for female group of colobus monkeys at Paignton Zoo for each data collection period.	132
Figure 4.10: <i>Trichuris</i> egg count according to age in colobus monkeys.	134
Figure 4.11: Mean (+SE) <i>Trichuris</i> egg count per gram of faeces (epg) for colobus monkeys at various zoos.	134
Figure 4.12: Mean (+SE) <i>Trichuris</i> egg count per gram of faeces (epg) for colobus monkeys during each season.	135
Figure 4.13: Mean (+SE) <i>Trichuris</i> egg count per gram of faeces (epg) for colobus monkeys according to anti-helminthic treatment.	135

Chapter 5:

Figure 5.1: Summary of metabolic pathway from cholesterol to cortisol.	146
Figure 5.2: Mean drying time for samples lyophilised in vacuum freeze drier.	153
Figures 5.3 a-c: Standard curves showing percentage binding of standards made up in different methanol concentrations.	157
Figure 5.4: Antiserum dilution curve showing the percentage binding of ¹²⁵ I cortisol at a	

range of antibody dilutions.	158
Figure 5.5: Effect of the dilution of the antiserum on the standard curve using the ¹²⁵ I cortisol RIA.	159
Figure 5.6: Effect of the amount of ¹²⁵ I cortisol on the standard curve using the ¹²⁵ I cortisol RIA.	159
Figure 5.7: Serially diluted pooled sample compared to cortisol standard curve to show parallelism between the curves.	163
Figure 5.8: Skeleton structure of hormones used in the cross-reactivity assays.	166
Figure 5.9: % binding of cortisol and test compounds at various concentrations.	171
Figure 5.10: % binding of cortisol and test compounds at various concentrations.	171
Figure 5.11: % binding of cortisol and test compounds at various concentrations.	172
Figures 5.12 a-b: Comparison between an original faecal sample aliquot and an aliquot of the same faecal sample, spiked with cortisol.	174
Figures 5.13 a-b: Comparison between the original faecal sample aliquot and an aliquot of the same faecal sample, spiked with cortisol and cortisone.	174
Figures 5.14 a-b: Comparison between the original faecal sample aliquot and an aliquot of the same faecal sample, spiked with cortisol, cortisone and cortexolone.	175
Figures 5.15 a-d: HPLC results for unspiked faecal sample from four colobus monkeys at Port Lympne Zoo with the marked position of cortisol, cortisone and cortexolone.	176
Figure 5.16: Radioimmunoassay results of a faecal sample from Port Lympne Zoo fractionated by HPLC separation.	177
Figure 5.17: Faecal cortisol equivalents according to the proportion of aggression given.	180
Figure 5.18: Faecal cortisol equivalents according to the proportion of aggression received.	180
Figure 5.19: Mean faecal glucocorticoids for colobus monkeys at various zoos.	181
Figure 5.20: Mean faecal glucocorticoids for colobus monkeys during each season.	181
Figure 5.21: Mean faecal glucocorticoids for colobus monkeys according to index of success.	182
Figure 5.22: Mean faecal glucocorticoids for colobus monkeys according to gender.	182

Figure 5.23: Mean faecal glucocorticoids for female colobus monkeys according to reproductive status. *183*

Figure 5.24: Faecal cortisol equivalents for each colobus monkeys at La Boissière du Doré Zoo in November 2006. *183*