# Effects of habitat overlap on helminth transmission between sympatric primates and ungulates in Amboseli ecosystem

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# Overview

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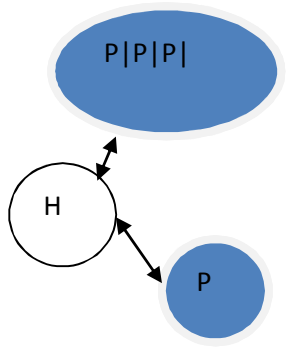
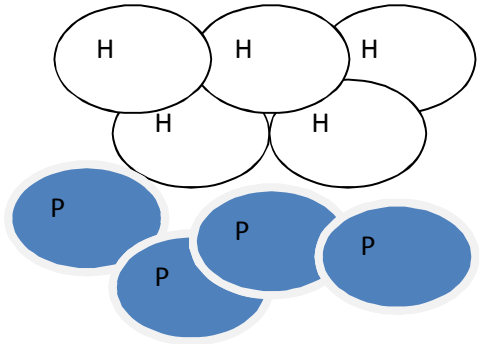
# Introduction

- Important to understand factors that influence parasite transmission in wild populations – better control methods
- Helminths lead to ill-health and sometimes death – risk to conservation of endangered species
- This study focuses on GIT helminths, fecally dispersed in environment, passively transmitted to hosts.




Single (host ï  helminth) system

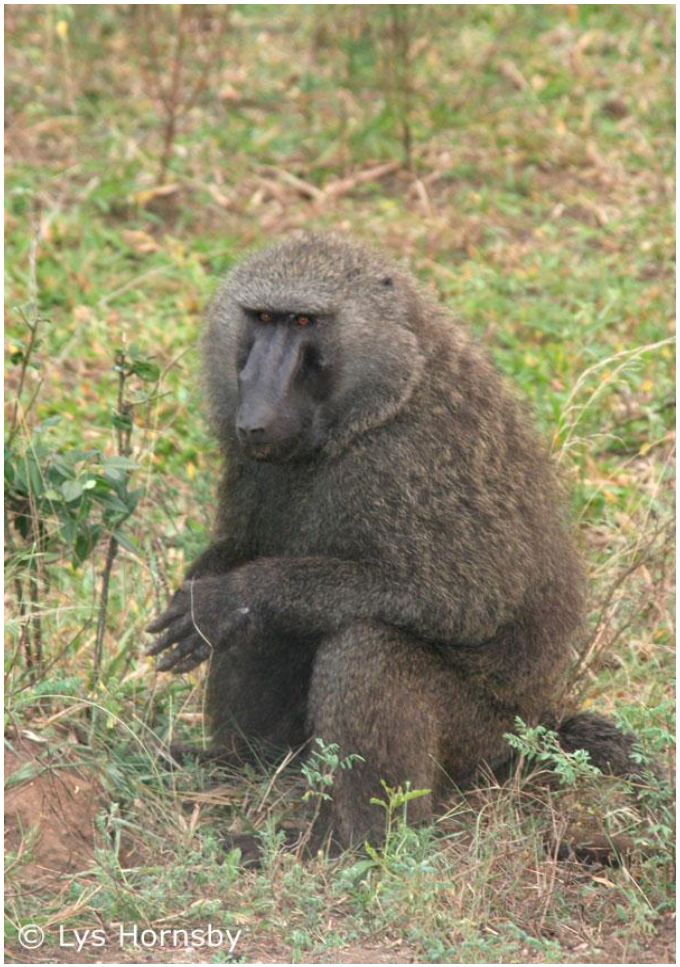



Multiple (host ï  helminth) system

# Introduction

- Focus host is baboons, non-human primate; Yellow (*Papio hamadryas cynocephalus)* and Olive *(Papio h. anubis)*
  - *– how other sympatric hosts influence infection patterns of helminths in baboons.*
- Range of the two species overlap in Amboseli, interbreed – subspecies, *P. c. ibeanus*
- Baboons live in social groups with definable hierachy and home range
- Social groups differ in structure e.g. group size, home range size, age and sex ratio
- Baboons –diverse diet (omnivorous), wide habitat diversity, graze and browse – risk to fecal-oral helminth transmission

# Baboon species




Olive baboon, *P. cynocephalus*

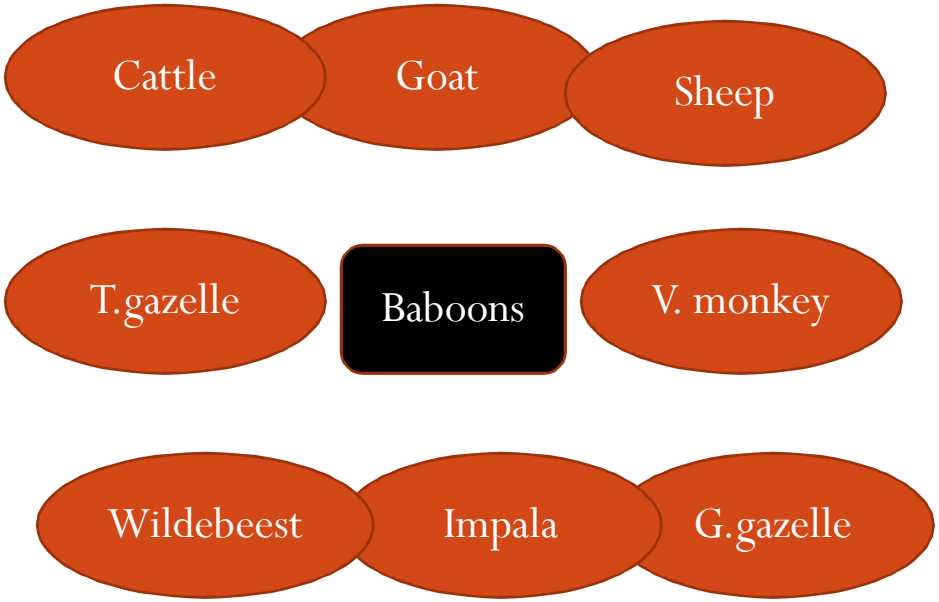
Yellow baboon, *P. anubis*

# GIT helminths of baboons

- Helminths in baboons include cestodes, trematodes and nematodes
- Helminths (e.g. *Trichostrongylus sp*, *Trichuris sp. Oesophagostomum sp, Strongyloides sp. Schistosoma sp*) infect baboons, vervet monkeys and diverse ungulates
- Majority of helminths are 'generalists' – infect multiple hosts across taxa
- Helminth species in different host taxa may be similar or different

# Introduction

- Baboon groups in Amboseli share home ranges with other animal species –




Vervet monkey
*(Cercopithecus aethiops)*

- Share space, pasture and water

# Habitat overlap &Transmission dynamics

- Transmission is the process by which susceptible hosts acquire parasites. The rate of transmission depends on the contact rate between hosts or infectious stage and host
- Patterns and dynamics of transmissions depend on how susceptible hosts and infective stages interact, spatially or socially.
- Habitat overlap leads to overall increase in population size and density, thus contact rate, which influences helminth infection pattern (e.g in African bovids, Ezenwa, 2003)
- Habitat overlap enhances cross-species transmission between related and unrelated hosts (Howells et al, 2011)

# Hypotheses

1. The extent of habitat overlap does not differ among the baboon social groups in Amboseli ecosystem

2. The extent of habitat overlap in baboon groups does not correlate with helminth infection intensity, prevalence and diversity

# Objectives

***Overall objective***: to investigate how habitat overlap among baboons, vervet monkeys and ungulate grazers might influence patterns of infections of gastrointestinal helminth in the baboon population in Amboseli ecosystem.

1. Determine extent to which baboon groups overlap with alternative host species (vervet monkeys, Impalas, Thomson's gazelle, grant gazelle, wildebeests, cattle, sheep and goats)
2. Determine how extent of overlap influences helminth prevalence, infection burden and diversity in baboon groups.
3. Determine genetics of nematodes shared among various hosts

Expected results to benefit helminth control in catlle-wildlife ranching systems and small wildlife sanctuaries

Grant gazelle *(Gazella granti*)

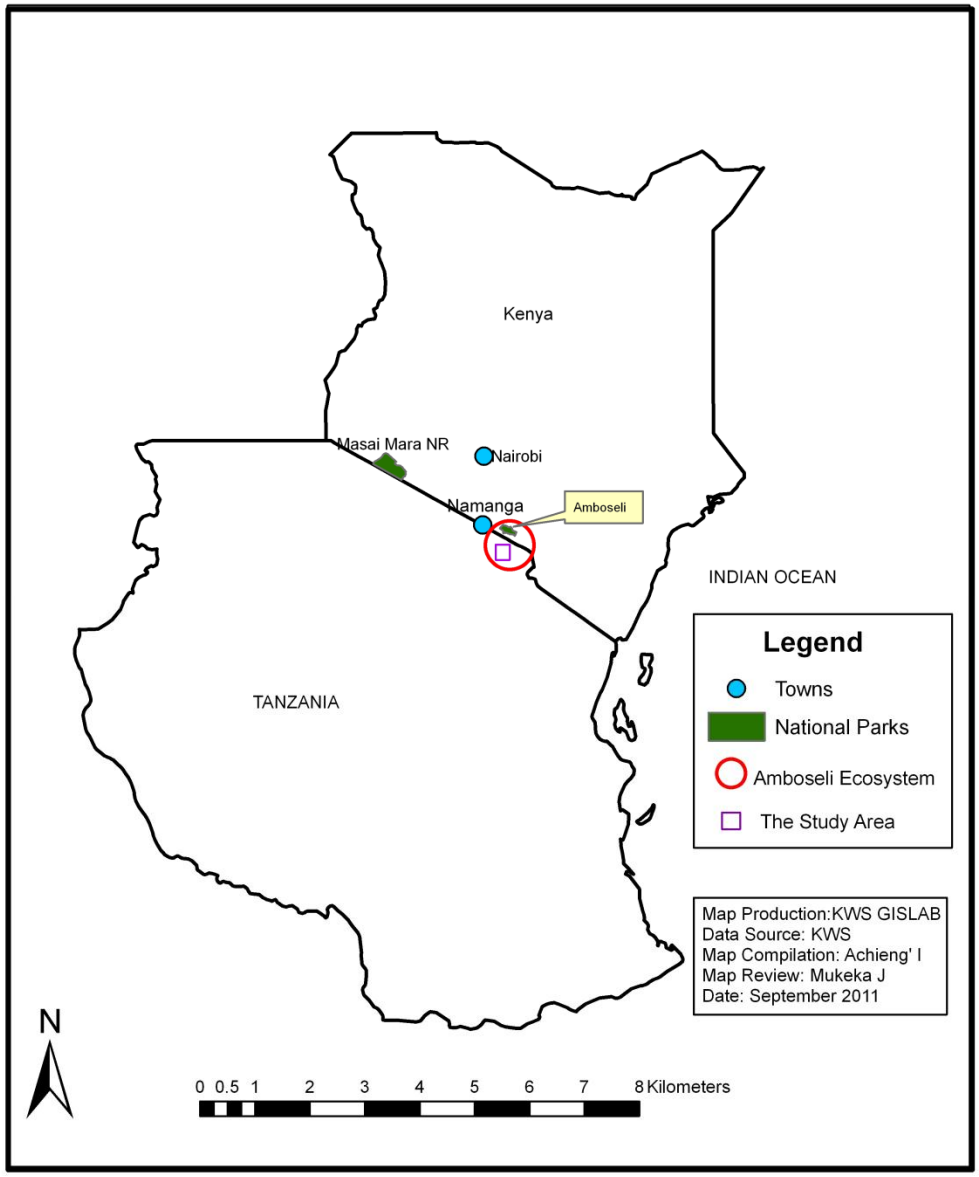
Impala (*Aepyceros melampus*)

# Materials and Methods

**Study area** – Amboseli ecosystem, southern Kajiado District

- 8000 Sq Km, straddles Kenya- TZ boundary, Olgulului
- Wildlife – livestock grazing zone ; Maasai pastoralists
- Flat dry arid area, pools of spring water and water holes
- Vegetation – Open land with short grass, scrubs, pockets of acacia woodlands
- Wet seasons (March - May & November - December)
- Rainfall is unreliable, average annual rainfall : 132 - 532mm
- Dry season (January – February & August – October)
- Mean max Temp – 31.3$^0$C: Mean min Temp - 13.9$^0$C

# Map of the study area

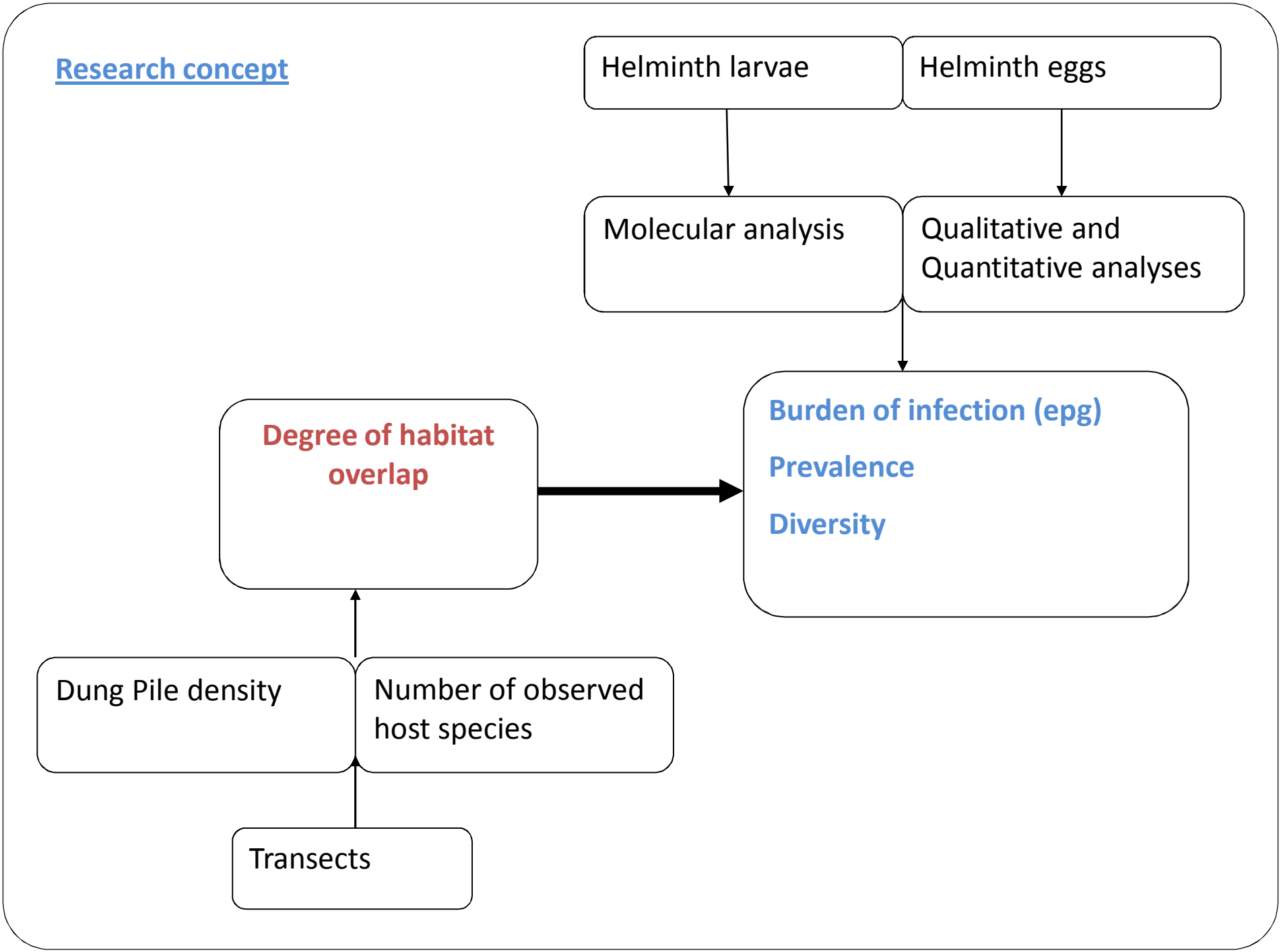


Wildebeest, *Connochaetes gnou*.

Thomsonûs gazelle, *Gazella thomsonii*

# Determine home ranges

- Baboons in the study area are continuously monitored by Amboseli Baboon Research Group (ABRG) – identified six social groups.
- The home ranges of the six groups are known based on continuous tracking and georeferencing movement pattern s of each social group.
- The tracking method described by ABRG will be used to confirm the current baboon groups' home range.


Research concept
Helminth larvae
Helminth eggs
Molecular analysis
Qualitative and Quantitative analyses
Burden of infection (epg)
Prevalence
Diversity
Degree of habitat overlap
Dung Pile density
Number of observed host species
Transects

# Measure of extent of overlap

- Transects – randomly made on each home range
- Transect size – Minimum 200m long by 2 meters wide

1). Transect walked, counting number of ***alternative hosts.***

- Since alternative hosts ($n$ = 8), habitat overlap score will range from 0 – 8. Each baboon group/home range score will be correlated with infection variables e.g., epg.

2). Transect walked, counting ***dung piles*** within the band to get dung density per home range

- Mean dung density values will be used as a measure of extent of habitat overlap
- e.g., 400 dung piles = $\frac{400}{(200 \text{ x} 2)\text{m}^2}$ D= 1 dung pile/$\text{m}^2$

# Faecal sampling and analysis

- Hosts will be tracked, observed for defecation and fresh faecal material collected.
- Part preserved in 10% formalin; part kept fresh for culture

1. **Qualitative analysis** – sedimentation technique to identify helminth ova – *morphologic diversity and prevalence*
2. **Quantitative analysis** – Mcmaster method to count helminth eggs per gram (epg) – *Intensity of infection*
3. **Coproculture** – faeces incubated moist for 10 days at rt for larval development

- Recovered larvae from the culture will be molecular analysed – *genetic diversity*

# Sample size for infinite population

*Based on formula by Naing et al, 2006; Dohoo et al, 2003*

$$n = \frac{Z^2 (P) (1-P)}{d^2}$$

- Where: $n$ = Sample size

  Z = Z value (Confidence level, e.g. 95%)

  $P$ = estimate of the proportion or anticipated prevalence (e.g. 50%, $p = 0.5$)

  $d$ = confidence interval or the required precision (e.g. 5%, d = 0.05)

- For alternative hosts, $n = 384$
- Sample size for each host (384/8) = 48

# Sample size for finite population

- In small populations, the required sample size (*n'*) is calculated by adjusting downward the sample size (*n*) obtained from infinite population.
- Baboon population (N) = 358 and n = 384

$$n' = \frac{1}{1/n + 1/N}$$

- Sample size for baboons = 185
- Six baboon social groups will be sampled according to proportion of the population.

# Sample size for each group

| Baboon group | Group size | Proportion % | Sample size |
|---|---|---|---|
| Weaver | 117 | 33 | 60 |
| Hokey | 72 | 20 | 37 |
| Viola | 64 | 18 | 33 |
| Narasha | 43 | 12 | 22 |
| Mica | 34 | 9 | 18 |
| Snap | 28 | 8 | 17 |
| Total | 358 | 100 | 185 |

# Sampling design

| Host species | Dry season | Wet season |
|---|---|---|
| Wildebeest | 48 | 48 |
| Impala | 48 | 48 |
| Grant gazelle | 48 | 48 |
| T. gazelle | 48 | 48 |
| Sheep | 48 | 48 |
| Goat | 48 | 48 |
| Cattle | 48 | 48 |
| V. Monkey | 48 | 48 |
| Baboon-weaver | 60 | 60 |
| Baboon-hockey | 37 | 37 |
| Baboon-viola | 33 | 33 |
| Baboon-narasha | 22 | 22 |
| Baboon-mica | 18 | 18 |
| Baboon-snap | 15 | 15 |
| **Total** | **569** | **569** |

# DNA extraction

DNA will be extracted from larvae

1. Pooled larvae will be unsheathed to remove outer cuticle by adding sodium hypochlorite for 5 minutes
2. Centrifuge and wash off sodium chloride twice to get unsheathed larval pellet
3. Individual larva will be picked from the unsheathed pool of larvae and mixed with lysis buffer
4. Larva in buffer will be incubated $60^0$C for 98 minutes, then at $94^0$C for 20 minutes to get DNA extract.
5. DNA extract will then be amplified and sequenced

# Genetic identification

- Internal transcribed spacers (ITS) are sequences in all the eukaryotic rRNA genes.
- The spacer regions (ITS1 and ITS2) have high evolution rate, thus, variable.
- Used in phylogenetic analysis among related species and/or among populations within a species.
- ITS2 rRNA of the larvae will be amplified using species specific primers and sequenced according to procedures described by Archie and Ezenwa, 2011. e.g., 72 bp of the ITS2 is specific marker for *Trichostrongylus axei*

# Statistics

- **Data will be entered in excel spreadsheets and exported to Graphpad and SPSS for analysis.**

  - Chi-square and ANOVA will be used to test difference in extents of overlap among the baboon groups
  - ANOVA and further post-hoc tests including Student *t* test will be used to compare differences in infection intensity and prevalence among baboon groups.
  - Correlation and regression analyses to test relationship between extent of overlap and intensity of infection, prevalence and diversity of helminths in baboon population

# WORKPLAN

| Activity | Year | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 2011 | 2012 | | | | 2013 | | | | 2014 | |
| | Oct - Dec | Jan - Mar | Apr - Jun | Jul - Sep | Oct - Dec | Jan - Mar | Apr - Jun | Jul - Sep | Oct - Dec | Jan - Mar | Apr - Jun |
| | 1,2,3 | 4,5,6 | 7,8,9 | 10,11,12 | 13,14,15 | 16,17,18 | 19,20,21 | 22,23,24 | 25,26,27 | 28,29,30 | 31,32,33 |
| Proposal writing and submission | | | | | | | | | | | |
| Determine home ranges | | | | | | | | | | | |
| Transect host counts and dung counts | | | | | | | | | | | |
| Sampling points | | | | | | | | | | | |
| Parasitological analyses | | | | | | | | | | | |
| Larval isolations & Molecular analyses | | | | | | | | | | | |
| Data analysis and thesis write-up | | | | | | | | | | | |
| Thesis submission and defense | | | | | | | | | | | |

# Supervisors

- Prof. Ndichu Maingi

  *University of Nairobi, Kenya*

- Dr. Gerald Muchemi

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  *University of Notre Dame, USA*


Thanks
for
listening