Effects of Host Movement Patterns on Helminth Infections in Zebras of Maasai Mara National Reserve and Lake Nakuru National Park

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PhD Proposal Presentation

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Introduction (1)

Plains zebra (*Equus quagga*)

The mountain zebra (*Equus zebra*)

Grevys zebra (*Equus grevyi*)

INTRODUCTION (2)

- Nematodes from five families have been reported in Zebras in Kenya;
  i. *Strongylidae (Cyathostominae, Strongylinae*
  ii. *Atractidae*,
  iii. *Oxyuridae*,
  iv. *Habronematidae* and
  v. *Setariidae*

- One Cestode from the family *Anoplocephalidae* also isolated.

- Studies from other countries have revealed similar spectrum of helminths.

INTRODUCTION (3)

- Parasitism is a growing conservation problem;
  - Many wild animal populations are increasingly threatened by parasitic infections
- It is therefore important to understand factors that contribute to parasitic infections;
  - Important in disease management
  - Contribute to our understanding of the role parasites play in host population dynamics
- Zebras harbor diverse species of helminth parasites

INTRODUCTION (4)

- The factors that influence parasite infections in grazing mammalian hosts are multiple and complex.
- The movement pattern and size of space available for a host is one such factor’
  - Restricted host movement (territoriality or sedentary) reduces parasite diversity but increases intensity.
  - Extensive movement leads to higher parasite diversity though effects of long distance movement by the host on parasite intensity are equivocal.

INTRODUCTION (5)

- Migration also reduces disease levels;
  - When infected animals do not migrate successfully, it may lead to the evolution of less-virulent pathogens.
  - Migratory demands can also reduce immune function, with consequences for host susceptibility and mortality (Altizer *et al*., 2011).

AIM OF THE STUDY

- To determine the effect of host movement patterns on the prevalence, intensity and diversity of helminth infections in the plains zebras.

- Focus will be on two populations of plains zebras:
  - Lake Nakuru National Park (LNNP);
    - ✓Insularized and sedentary population (closed system)
  - Maasai Mara National Reserve (MMNR);
    - ✓Zebra population has unlimited grazing range;
      - Some remain in the reserve year round (open-sedentary system) and
      - Others co-migrate (open-migratory system) annually with the wildebeests

Hypothesis

- There is no difference in the prevalence, intensity and diversity of helminth infections among zebra populations in the open (both sedentary and migratory) and insularized systems

Study objectives

Overall objective:-

- To determine the effects of closed systems, open-sedentary and open-migratory systems on helminth parasite infections in Zebras in the Maasai Mara National Reserve and Lake Nakuru National Park.

Specific objectives:-

i. To determine the prevalence, intensity and diversity of gastrointestinal (GI) helminth parasites in zebras in the closed, open-sedentary and open-migratory systems

ii. To determine how time/annual cycles influence prevalence, diversity and intensity of GI helminth parasites in the three range systems.

iii. To genetically identify large and small strongyles from zebras in the three systems.

Justification…1

- As habitat for wildlife is continually lost and human wildlife conflicts escalates, the optional mitigation is to fence off wildlife habitats or set up ring fenced protected areas.
- The size of a sanctuary influences parasite infection patterns;

  - Smaller systems are likely to mimic effects of sedentary systems and allow parasite build up in the environment, leading to high infection
  - This information is important in the design and size of sanctuaries (closed systems) in relation to parasitism.

Justification…2

- Disease and in particular parasitism is likely to be a challenge in such systems, with some being set up as mixed wildlife-livestock ranches.

- Extensive movement is likely to lead to higher parasite diversity though effects of long distance movement by the host on parasite intensity are equivocal.

- This study provides a model to understand how parasitism is patterned in a single host system that is availed variable space and movement options.

Methodology

Study Areas…1

1. Lake Nakuru National Park in the central Rift Valley, 140km north-west of Nairobi, in Nakuru County.

2. Maasai-Mara National Reserve (MMNR)located in the south-western part of Kenya 290 kilometers from Nairobi.

Data collection (1)

- Data to be collected: Fecal samples, Weather, postmortem worm counts.
- Data will be collected from both migratory and resident live zebras in the MMNR and resident zebras in the LNNP.
- Data collection will be done for three time periods;
  - When zebras migrate north into the Mara from Serengeti (July/August),
  - When they migrate south from the Mara (October/November) and
  - The period in between the migrations (August/September).

Data collection (2)

- Sample size will be calculated using the formula:
  - $s = X^2 NP (1- P) \div d 2 (N -1) + X^2P (1- P)$ (Krejcie and Morgan, 1970; Martin *et al*., 1987)

Where:

- s = required sample size.
- X_2 = the chi-square for 1 degree of freedom at the desired confidence level (3.841).
- N = the population size.
- P = the population proportion (assumed to be .50 since this would provide the maximum sample size).
- d = the degree of accuracy expressed as a proportion (.05).

- The current population of resident zebras is estimated to be 30,000, giving a sample size of 379.
- For the migratory zebras, the total population is estimated at 200,000, giving a sample size of 383.

Sampling and parasitological techniques…1

- Fresh faecal droppings will be collected from the pastures and resting places in the reserve and park and refrigerated at +4^{o}C immediately after collection.

- During the sampling, sampling areas measuring 20m x 100m will be marked out.

- Samples will be analyzed at the nearest KWS lab and VIL Nakuru.

Sampling and parasitological techniques…2

- The areas will then be walked in a Z pattern, collecting faecal samples from the front, the right-hand side and the left-hand side as described for collection of herbage samples in MAFF (1986).
- The samples will be processed and analyzed using fecal flotation techniques (McMaster technique) for nematode and cestode eggs (MAFF, 1986).
- Animals found dead during the visits will be necropsied for worm counts

Faecal cultures for larval identification (2)

- Each sample preparation will be examined twice;
  - Samples that are positive for nematode eggs will be cultured at room temperature for 10 days.
  - Larvae isolated from the cultures will be identified according to the MAAF (1986) manual.
  - Fecal samples will be examined for lung worm larvae using the Beamanns Technique (MAFF, 1986)

Molecular identification of the nematodes

- Larvae isolated from faecal cultures will be used for the molecular identification of nematodes following a procedure described by Archie and Ezenwa (2011):
  - Nucleic Acid will be extract from single larvae.
  - The Internally Transcribed Spacer (ITS) region of ribosomal RNA will be isolated and amplified using PCR.
  - Sequencing will be done to determine the order of nucleotides in the region.
  - A BLAST (Basic Local Alignment Search Tools)will then be performed to compare the query sequences with available databases of known nematodes.

Management and analysis of other data

- Data collected during the study will be entered into Excel and analyzed using standard statistical packages.
- Faecal egg counts will be transformed to normalize their distribution.
- The counts will then be compared between resident and migratory zebras and between seasons using ANOVA.
- Prevalence will be calculated as the percentage of animals infected with a particular parasite out of those examined (Margolis *et al*., 1982)

Work Plan

Activity	Months										
	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											
Data collection											
Sample analyses											
Larval isolations and molecular analysis											
Data analysis and thesis write-up											
Thesis submission and defense											

Budget

No	Item/Activity	Unit cost Ksh.	Quantity	Cost (Ksh)
1	Non Sterile gloves	600	50 boxes	30, 000
2	Faecal pots	50	600 pots	30,000
3	Centrifuge tubes (15ml)	50	100 tubes	5,000
4	Sieves/strainers	50	4 pieces	200
5	Bottle brush	200	10 kg	800
6	Table salt	30/kg	10kgs	300
7	Microscope glass slides (packets)	200/case	100 pkts	20,000
8	Cover slips (22x24mm)	150/case	100 cases	15,000
9	Faecal culture cups	50	100 cups	5,000
10	Gauze swab (non sterile)	250/packet	30 pkts	7,500
11	Cool boxes	3000	2 boxes	6,000
12	<u>Molecular analysis reagents</u> • AmpliTaq Gold PCR Master Mix • AmpliTaq DNA Polymerase,with Buffer 11 • GeneAmp dNTP Blend with dTTP • DNAZap TM Solution • rTth DNA Polymerase and EZ Buffer Pack • Qiagen stool Minikit • Agarose for the separation of PCR Products		 800rxts 250 units 1ml 2x250ml 500units 650rxts 250g	800,000
13	<u>Field work</u> • Fuel Cost = • Distance= • Number of days=	 Ksh. 40/km 300km/day 120days		1,440,000
14	Thesis preparation and production			100,000
	Total			2,429,800

THANK YOU ALL FOR LISTENING