

Robert Dominikus Fyumagwa

**Anthropogenic and natural  
influence on disease prevalence  
at the humanlivestock-  
wildlife interface in the  
Serengeti ecosystem, Tanzania**

Thesis for the degree of Philosophiae Doctor

Trondheim, September 2012

Norwegian University of Science and Technology  
Faculty of Natural Science and Technology  
Department of Biology



**NTNU – Trondheim**  
Norwegian University of  
Science and Technology

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## **Preface**

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**Trondheim, 2012**

**Robert Dominikus Fyumagwa**

## TABLE OF CONTENTS

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PREFACE	1
TABLE OF CONTENTS	3
LIST OF PAPERS	5
SUMMARY	7
1. INTRODUCTION	10
2. AIMS OF THE THESIS	13
3.0 METHODS	15
3.1 STUDY AREA AND STUDY SPECIES .....	15
<i>3.1.2 Death of black rhinos in the Ngorongoro Crater (paper I)</i>	20
<i>3.2 Methodological consideration</i>	20
<i>3.2.1 Sample collection</i>	20
<i>3.3.1 Data Analyses</i>	29
4.0 RESULTS	31
<b>4.1 DIAGNOSIS OF DEAD BLACK RHINOS AND TREATMENT (PAPER I) .....</b>	<b>31</b>
<b>4.2 SEASONAL TICK DENSITY IN THE CRATER GRASSLAND (PAPER II).....</b>	<b>32</b>
<b>4.3 IDENTIFIED TICK SPECIES (PAPERS I, II, III, IV, V).....</b>	<b>32</b>
<b>4.4 PLANT SPECIES (PAPER II).....</b>	<b>33</b>
<b>4.5 EFFECTS OF PRESCRIBED BURNING (PAPER II) .....</b>	<b>34</b>
<b>4.6 PCR AMPLIFICATION AND INFECTED TICK SPECIES (PAPERS III, IV, V).....</b>	<b>34</b>
<b>4.6.1 HAEMOTROPIC MYCOPLASMA SPECIES (PAPER III) .....</b>	<b>34</b>
<b>4.6.2 ANAPLASMA SPECIES (PAPERS IV &amp; V).....</b>	<b>35</b>
<b>4.6.3 BABESIA SPECIES (PAPER V).....</b>	<b>35</b>
<b>4.6.4 THEILERIA SPECIES (PAPER V).....</b>	<b>36</b>
<b>4.7 DISEASE OUTBREAK IN SPOTTED HYENAS (PAPER VI).....</b>	<b>38</b>
<b>4.7.1 CLINICAL SIGNS OF INFECTED HYENAS .....</b>	<b>38</b>
<b>4.7.2 HISTOPATHOLOGICAL AND BACTERIOLOGICAL EXAMINATION OF HYENA SAMPLES (PAPER VI) .....</b>	<b>39</b>
<b>4.7.3 MOLECULAR ANALYSIS OF BACTERIAL ISOLATES FROM HYENAS (PAPER VI).....</b>	<b>40</b>
<b>4.8 SERO-SURVEY IN BUFFALO AND WILDEBEEST AGAINST BRUCELLOSIS (PAPERS VII &amp; IX) .....</b>	<b>40</b>
<b>4.8.1 PREVALENCE OF BRUCELLOSIS IN BUFFALO AND WILDEBEEST .....</b>	<b>40</b>
<b>4.8.2 RISK OF EXPOSURE TO BRUCELLA MICROORGANISM.....</b>	<b>41</b>

<b>4.9 LABORATORY RESULT OF WILD DOG SAMPLES (PAPER VIII)</b> .....	42
<b>4.9.1 HISTOPATHOLOGICAL EXAMINATION OF WILD DOG SAMPLES</b> .....	42
<b>4.9.2 RT- PCR AND CDV P AND F GENE FRAGMENT SEQUENCES</b> .....	42
5.0 DISCUSSION	44
5.1 TICK INFESTATION AND TICK-BORNE DISEASES.....	44
5.2. INFECTIOUS DISEASES .....	46
5.3 CONCLUSION .....	53
5.4. RECOMMENDATIONS.....	54
6.0 REFERENCES	55

## LIST OF PAPERS

The thesis is a compilation of nine published papers:

- i. **Fyumagwa, R.D.**, Mkumbo, S.S., & Morkel, P.B. 2004. Remote treatment of black rhinos against babesiosis in the Ngorongoro Crater, Tanzania. *Pachyderm*. 34: 80-83.
- ii. **Fyumagwa, R.D.**, Runyoro, V., Horak, I.G., & Hoare, R. 2007. Ecology and control of ticks as disease vectors in wildlife of the Ngorongoro Crater, Tanzania. *South African Journal of Wildlife Research*. 37(1): 79-90.
- iii. **Fyumagwa, R.D.**, Simmler, P., Willi, B., Meli, M.L., Sutter, A., Hoare, R., Dasen, G., Hofmann-Lehmann, R., & Lutz, H. 2008. Molecular detection of haemotropic *Mycoplasma* species in *Rhipicephalus sanguineus* tick species collected on lions (*Panthera leo*) from Ngorongoro Crater, Tanzania. *South African Journal of Wildlife Research*. 38(2): 117-122
- iv. **Fyumagwa, R.D.**, Simmler, P., Meli, M.L., Hoare, R., Hofmann-Lehmann, R. & Lutz, H. 2009a. Prevalence of *Anaplasma marginale* in different tick species from Ngorongoro Crater, Tanzania. *Veterinary Parasitology*. 161: 154-157.
- v. **Fyumagwa, R.D.**, Simmler, P., Meli, M.L., Hoare, R., Hofmann-Lehmann, R. & Lutz, H. 2011. Molecular detection of *Anaplasma*, *Babesia* and *Theileria* species in a diversity of tick species from Ngorongoro Crater, Tanzania. *South African Journal of Wildlife Research*. 41 (1): 79-86.
- vi. Höner, O.P., Wachter, B., Speck, S., Wibbelt, G., Ludwid, A., **Fyumagwa, R.D.**, Wohlsein, P., Lieckfeldt, D., Hofer, H. & East, M.L. 2006. Severe *Streptococcus* infection in spotted hyenas in the Ngorongoro Crater, Tanzania. *Veterinary Microbiology*. 115: 223-228.
- vii. **Fyumagwa, R.D.**, Wambura, P.N., Mellau, L.S.B. & Hoare, R. 2009b. Sero-prevalence of *Brucella abortus* in buffalo and wildebeest in the Serengeti ecosystem: a threat to human and animal health. *Tanzania Veterinary Journal*. 26(2): 62-67.
- viii. Goller, K.V., **Fyumagwa, R.D.**, Nikolin, V., East, M.L., Kilewo, M., Speck, S., Muller, T., Matzeke, M., & Wibbelt, G. 2010. Fatal canine distemper infection in a pack of African wild dogs in the Serengeti ecosystem, Tanzania. *Veterinary Microbiology*. 146: 245-252.

- ix. **Fyumagwa, R.D. 2010.** Diseases of economic and conservation significance in the livestock-wildlife interface in Tanzania. In: E.J.Gereta and E. Røskaft (Eds). Conservation of Natural Resources. Some African and Asian examples. Tapir Academic Press, Trondheim, Norway. Chapter 22, pp 419-444.

#### **Declaration of contribution**

Most of the ideas on which the research is described in this thesis were mine. I performed much of the work on which this thesis is based and was involved in the final stages of writing for all the papers in the thesis. MSS & MPB participated in manuscript writing (paper **I**). RV, HIG & RH helped in field work and provided useful comments (paper **II**). SP, WB, MML, SA, DG, HLR& HL helped in laboratory work and provided useful comments (papers **III, IV & V**). HOP & WB are researchers on demographic and behavioral studies of spotted hyenas in the Crater and participated in sample collection and analysis and preparation of manuscript (paper **VI**). SS, WG, LA, WP, LD & GKV helped in samples analyses and HH & EML contributed in analysis and manuscript writing (paper **VI**). WPN, MLSB & HR helped in laboratory work and manuscript writing (paper **VII**). GKV, KM, NV, EML, MT, MM, & WG participated in sample collection, laboratory analysis and manuscript writing (paper **VIII**).



## Summary

Anthropogenic activities in ecosystems interfere with natural processes and cause habitat fragmentation and loss. Habitat fragmentation and loss restrict wildlife movement between populations consequently reducing the gene flow and genetic diversity. Increased human encroachment on wildlife habitat compromises immunity and disturbs host-pathogen relationships resulting in disease outbreaks in naïve populations. Tick-borne and infectious diseases are considered a major threat to the health of people and their domesticated animals and as described in this thesis, can cause increased mortality in endangered wildlife species. There is evidence that ecological changes exacerbated by anthropogenic activities are increasing both the threat and economic cost of emerging diseases. The emergence of these diseases is often associated with ecological factors exacerbated by anthropogenic activities. The Serengeti ecosystem is a useful area to study interactions between disease, anthropogenic factors and wildlife because it has some of the largest wildlife populations in Africa and areas surrounding the ecosystem hold some of the highest livestock populations in Tanzania which can interact freely with wildlife in some areas. The area surrounding the ecosystem is occupied by about 2 million people; agro-pastoralists in the west and pastoralists in the east who are competing with wildlife for limited resources. This is an ideal condition for disease spillover to susceptible animals or humans.

I start this thesis with a description of the first major veterinary intervention to treat free ranging black rhinos (*Diceros bicornis*) in the Ngorongoro Crater (hereafter referred to as Crater) against a perceived threat by tick-borne diseases in 2001. From this initial disease problem in rhino I developed a research interest in tick ecology which was followed by a detailed investigation of tick ecology, vegetation structure and effect of prescribed fire management that were conducted in the Crater grassland. My interest in tick ecology led me to apply molecular methods to investigate the blood parasites in ticks collected from Crater grassland and on animals. My interest in wildlife diseases prompted me to participate in efforts to identify the cause of the appearance of disease in the spotted hyena (*Crocuta crocuta*) population in the Crater in 2002. Bacteriological,

histopathological and molecular analyses were performed on tissue samples collected from dead spotted hyenas in the Crater. News of sick and dying African wild dogs (*Lycaon pictus*) in one pack in 2007 in the Loliondo Game Control Area (LGCA) prompted me to investigate the problem in this pack, and observation of dying animals suggested to me that the pack was infected with canine distemper virus and I decided that this should be verified by histopathology and genetic analyses. A cross-sectional retrospective study was organized for sero-prevalence of *Brucella* micro-organism in buffalo (*Syncerus caffer*) and wildebeest (*Connochaetus taurinus*) in the Serengeti ecosystem using Rose Bengal Plate Agglutination test followed by competitive ELISA.

My results show that a cascade of ecological changes in the Crater grassland caused by anthropogenic and natural influences were the underlying factors associated with the wildlife mortality. The study on tick ecology revealed an unusually high tick density of 42 and 819 per square meter for adults and immature ticks respectively, in unburned Crater grassland, consisting of about 15 tick species. Tick-borne diseases contributed to the mortality of a number of herbivores and carnivores in the Ngorongoro Crater in year 2000/ 2001. It was observed that nine out of 15 tick species were found to contain at least one or more of infectious haemoparasites. Gradual change in the Crater ecology partly caused by good intention of the NCAA Management to relocate the Maasai and prohibiting burning of the Crater grassland for almost three decades (27 years) were probably the contributing factors for the change in herbivore composition in favour of buffalo and decrease in small herbivores particularly wildebeest. This change occurred concurrently with change in vegetation structure and composition which favoured more tick survival leading to high tick infestation in the Crater grassland and on animals. The study described for the first time, detection of the blood parasite *Anaplasma bovis* in the tick species *Amblyomma gemma* and *Rhipicephalus praetextatus* in Tanzania. *Mycoplasma haemofelis* and *Candidatus Mycoplasma haemominutum* were also detected in *Rhipicephalus sanguineus* ticks collected on lions (*Panthera leo*) from Ngorongoro Crater. The study reported the highest diversity of tick species (six) detected with *Anaplasma marginale* including

*Rhipicephalus praetextatus* and *Rhipicephalus pulchellus* which were previously not described to be of importance as disease vectors in Tanzania.

Similarly, *Streptococcus equi* subsp.*ruminatorum*, a bacterium previously described in domestic stock in Europe was identified as the causative agent of severe infection and increased mortality in the spotted hyena population in the Crater in 2002/ 2003. Canine distemper virus (CDV) was identified as the causative agent of high mortality in one wild dog pack in the LGCA in 2007. Genetic sequence data showed this virus to resemble the CDV strain that killed 30% of lions in the Serengeti in 1993/94. A number of other disease pathogens have been discussed that are associated with various anthropogenic activities onto naïve wildlife populations during encroachment onto natural ecosystems.

## 1. Introduction

Wildlife populations are increasingly threatened with extinction because of habitat degradation and loss, diseases, pollution, inappropriate land use practices, climate change, over-exploitation, alien invasive species and a host of other factors (Kennedy-Stoskopf 1998; Daszak et al. 2000; Aguirre & Gomez 2009). Problems affecting wild animals and their habitats should be evaluated and dealt with to ensure sustainable conservation of wildlife populations (Peterson 1991; Aguirre & Gomez 2009). To achieve a successful management of wildlife health, information about the biotic and abiotic factors affecting wildlife and their environment is important (Gulland 1995; Nielsen 1995; Kennedy-Stoskopf 1998; Deem et al. 2001). Adverse weather conditions have diverse effects on many ecosystem components including diseases which have devastating consequences to endangered species (Daszak et al. 2000).

The eastern Serengeti is inhabited by Maasai pastoralists who graze livestock in protected areas. The western Serengeti is occupied by multi-ethnic communities (>20 tribes) who practice extensive peasantry farming and livestock keeping. Poor livestock and crop production cause encroachment to protected areas in search of natural resources including illegal hunting, wood, charcoal burning and grazing (Mfunda & Røskaft 2011). These anthropogenic activities around protected areas have resulted in loss of habitat and increase of both human and livestock interactions with wildlife due to resource competition. This association creates an ideal condition for diseases transmission from reservoir hosts to immunocompromised or naïve susceptible individuals (Thomson et al. 2002; Skinner 2010). There is increasing evidence that global climate change has influenced the distribution range of disease vectors and pathogens (Alekseev 1998; Ubukata 2000; Wilson et al. 2000; Hofer & East 2010; East et al. 2011).

Conversely, global warming may cause a decline in food availability to both human and animals in some regions thereby subjecting them to nutritional stress and increased susceptibility to infections (Daszak et al. 2000; Fyumagwa et al. 2004; 2007). In nature, pathogens (i.e. parasites, protozoa, bacteria, fungi and viruses) are component parts of the ecosystems where wildlife live (East et al.

2011). Millions of years of co-evolution have engendered a *modus vivendi* that assures the survival of both host and pathogen populations, hence, pathogens do not necessarily cause devastation to wild animal population (East et al. 2011).

The Ngorongoro Conservation Area (NCA) is a multiple land use area that was set aside for Maasai pastoralists and is managed by the NCAA. At the time of its inception in 1959, the Maasai were known to co-exist friendly with wildlife particularly herbivores because their taboos and beliefs did not allow them to consume game meat. However, Maasai are traditionally hunters of carnivores. For this reason the Maasai were compatible with wildlife and therefore NCA was given to Maasai in exchange for agreement that they would move out of Serengeti National Park when it was set up. From the time of resettlement in NCA, a few Maasai families settled on the floor of the Crater and burnt the grassland following a traditional rotation practice to reduce tick infestation on livestock and enhance good pasture sprouts. Such a unique conservation system provided a number of different competitive processes amongst wildlife, livestock and humans which had some negative impacts on conservation (Nielsen 1995; Runyoro et al. 1995; Daszak et al. 2000). The appearance of buffalo on the Crater floor in 1974 and the subsequent increase in size of this buffalo population significantly accelerated after the removal of the Maasai in 1974, which deprived the area of traditional burning which caused vegetation composition changes in favour of tall grass in the Crater grassland (Fyumagwa et al. 2007).

High mortality of different wildlife species including black rhinos (*Diceros bicornis*), lions (*Panthera leo*), African buffalo (*Syncerus caffer*) and other herbivore species in the Ngorongoro Crater in 2000 and 2001 was a unique tragedy that has been described by many authors (Nijhof et al. 2003; Fyumagwa et al. 2004; Estes et al. 2006; Fyumagwa et al. 2007; 2008; Munson et al. 2008). The decline in the rhino population in Tanzania in 1970s and 1980s was caused by poaching (Sinclair 1995), therefore, death of rhinos in Ngorongoro Crater from babesiosis was a new phenomenon (Fyumagwa et al. 2004). High mortality of buffalo and wildebeest before 1960 was caused by rinderpest, which was a highly fatal *Morbillivirus* infection to both domestic and wild ungulates (Sinclair 1973) before its eradication worldwide by effective vaccination control. Evidence suggests that

latent infections with a stress trigger (starvation and high tick infestation) were more likely the cause of tick-borne diseases outbreak than sudden exposure to infections due to poor immune response (Dobson & Hudson 1995; Fyumagwa et al. 2004). Presence of high tick numbers and species diversity in the Ngorongoro Crater harbouring a diversity of haemoparasites are likely to have caused mortality of the local population of lions and endangered black rhinos. Buffalo and other wild herbivores are described to be resistant to haemoparasites (Grootenhuis 2000), however, when under high tick challenge and subjected to concurrent stress some species do succumb to infections with clinical symptoms (Pfitzer et al. 2004; Nijhof et al. 2003; 2005).

Animals like spotted hyenas (*Crocuta crocuta*) are hardy and are the most numerous large carnivore in the Serengeti ecosystem. In 2002 and 2003 a number of spotted hyenas in the Ngorongoro Crater displayed clinical signs of an infection, including severe swelling of the head followed by abscess formation at the submandibular region, respiratory distress, mild ataxia, and lethargy suggesting an outbreak of infection (Höner et al. 2006). A total of 16 out of about 400 individual hyenas showed clinical symptoms but many more were infected (Höner et al. 2006; 2011). Two of these 16 (12.5%) individuals died within few days after developing clinical symptoms. Histopathological examination of internal organs from dead hyenas revealed morphological changes consistent with severe bacterial infection (Höner et al. 2006).

Brucellosis was first reported in Tanzania in the 1920's and *Brucella abortus* is endemic in indigenous cattle population while isolated cases are reported in small holder dairy cattle (Kiputa et al. 2008). However, the reported prevalence of brucellosis derived from surveys based on a small sample of tested animals is subject to uncertainty (Fyumagwa 2010). A sero-survey of livestock to the west of Serengeti National Park indicated sero-prevalence of 10.5% and 14% in Bunda district and Serengeti district respectively (Bugwesa et al. 2009). Kiputa et al. (2008) reported a similar average endemic prevalence (11%) of brucellosis in cattle in both dairy herds and pastoral communities. Sero-survey in wildlife against *Brucella abortus* was conducted for the first time in 2007 with the aim to establish

prevalence of exposure to the bacterium in buffalo and wildebeest in the Serengeti ecosystem (Fyumagwa et al. 2009b).

Canine Distemper Virus (CDV) infection is a highly contagious and sometimes fatal disease of carnivores caused by a *Morbillivirus* closely related to the measles virus in humans and rinderpest virus of ungulates (Pomeroy et al 2008). The virus is airborne and aerosol droplet infection from secretions of infected animals is the main route of infection. In Tanzania this disease is not a priority to the livestock sector and individual dog owners are responsible for the control of this disease. CDV is not zoonotic although it causes considerable mortality in dogs, particularly pups (Appel & Summers 1995). The occurrence of clinical CDV infection in susceptible wild carnivores such as lions and wild dogs (*Lycaon pictus*) is unpredictable and occurs sporadically. In 1980's 29% (n=77) of lions in Serengeti ecosystem were seropositive for CDV (Kennedy-Stoskopf, 1998), but the population was free from clinical symptoms of the disease. The Serengeti National Park sustained an outbreak of clinical disease in lions of all age classes and in young spotted hyenas between 1993 and 1994 (Haas et al. 1996; Roelke-Parker et al. 1996). Since this fatal outbreak in the early 1990s, no further fatal outbreaks of CDV has occurred in Serengeti lions or spotted hyenas. A scientific review established the existing information with regard to prevalence of diseases at the livestock-wildlife interface in Tanzania particularly in the Serengeti ecosystem.

## **2. Aims of the Thesis**

This thesis aimed to investigate and identify the possible impact of intrinsic and extrinsic (anthropogenic) factors on pathogens associated with increased mortality and outbreaks of disease in wildlife species in the Serengeti ecosystem in Tanzania between 2000 and 2007.

The specific objectives for the study were:

- i. To investigate the tick ecology and vegetation structure in the Ngorongoro Crater grassland in relation to anthropogenic influence and suggest some mitigation measures in reducing the tick burden in the grassland and eventually on animals (paper II).

- ii. To determine the haemoparasites present in tick vectors and establish the possible risk to susceptible animals in the Ngorongoro Crater (papers I, III, IV, V).
- iii. To establish the pathogen responsible for the disease outbreak in spotted hyena population in the Ngorongoro Crater in 2002/ 2003 and its impact to the sustainability of the affected species (paper VI).
- iv. To establish the exposure status of buffaloes and wildebeest to brucellosis, a common zoonotic disease in pastoral communities and the possible risk of transmitting the infection to susceptible livestock and human who handle game meat without knowledge of the associated risk (paper VII).
- v. To establish the cause of mortality in a pack of endangered African wild dogs in the Loliondo Game Controlled Area to the east of the Serengeti National Park and its consequences to sustainability of the endangered species (paper VIII).
- vi. To conduct a review of known diseases of economic and conservation significance in livestock-wildlife interfaces in Tanzania and establish gaps in knowledge requiring more research (paper IX)



### 3.0 Methods

#### 3.1 Study area and study species

The study was conducted in Serengeti ecosystem which is roughly defined as the area covered by the migratory movements of wild herbivores (Schaller, 1972), and included Ngorongoro Conservation Area (NCA), Serengeti National Park (SNP) and Loliondo Game Controlled Area (LGCA). The NCA encompasses a multiple land use area of about 8300km<sup>2</sup> lying at the western edge of the Great Rift Valley in the Arusha region, northern Tanzania and is populated by wildlife, livestock and people, the latter mostly Maasai pastoralists. The Ngorongoro Crater (03°010'S, 35°035'E), the world's largest intact, inactive caldera occupies approximately 300 km<sup>2</sup> (4%) of the total NCA. The rim is 2200m above sea level and the Crater floor (250 km<sup>2</sup>) is about 600m below, composed predominantly of grassland with small patches of swamps, Acacia and riverine forest. The Ngorongoro Crater is a World Heritage Site and Biosphere Reserve with high interaction of wildlife and domestic animals. Although the Crater is part of the greater Serengeti ecosystem it is regarded 'to some extent' as an ecologically distinct unit because of its uniqueness in terms of location and high density of wild animals it harbours (Runyoro et al. 1995).

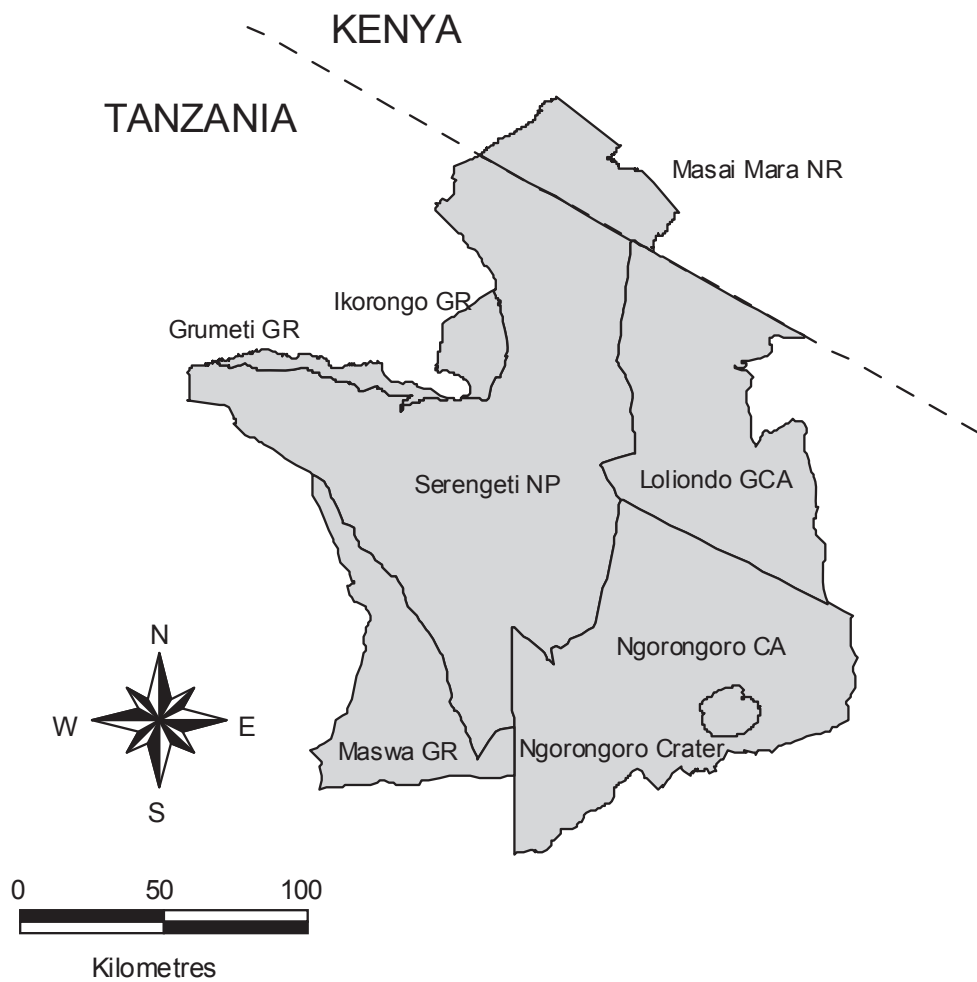
Serengeti National Park, (2°19'.60 S, 34°49'.60 E) is part of the larger Serengeti ecosystem with approximately 25,000 km<sup>2</sup> (Fyumagwa et al. 2007) Serengeti National Park (SNP) is a protected area where human settlement is not allowed except for support staff, researchers and tourists. The park has an area of about 14763 km<sup>2</sup> and is situated on the north-western edge of NCA stretching almost to the shores of Lake Victoria and extends up north to the border with Kenya, it is contiguous to Masai Mara National Reserve (MMNR) (Figure 1). Two third of the park (northern and western) is dominated by bushes and woodland and the southern part is dominated by short grass plains.

The Loliondo Game Controlled Area (LGCA) which is about 4 000 km<sup>2</sup> is part of the great Serengeti ecosystem. LGCA is another conservation category where human settlement especially pastoralists with limited agricultural activities is

allowed. The LGCA is located on the eastern boundary of the Serengeti National Park (Figure 1).

### **3.1.1 Disease outbreak in wildlife in Ngorongoro Crater (papers I & II)**

The disease investigation described in this study was initiated in response to the recommendation made by the team of experts that convened an ad hoc meeting to deliberate on the cause of unusual wildlife mortality including black rhinos (*Diceros bicornis*) in the Ngorongoro Crater in the year 2000/ 2001. Therefore, the study was demand driven focussing on diseases in the Serengeti ecosystem with special attention to the Crater wildlife population. The primary focus was on Crater vegetation, tick infestation and ecology and tick-borne haemoparasites. Secondly the investigation was extended to other diseases of conservation significance in the entire Serengeti ecosystem.



**Figure 1:** Map of Serengeti ecosystem showing different status of protected areas including Serengeti National Park (SNP), Ngorongoro Conservation Area (NCA), Ikorongo-Grumeti Game Reserves (IGGR), Maswa Game Reserve (MGR), Loliondo Game Controlled Area (LGCA) in Tanzania and Maasai Mara National Reserve in Kenya, (Source: Machoke Mwita, TAWIRI).

### **3.1.2 Death of black rhinos in the Ngorongoro Crater (paper I)**

The black rhino is an endangered species and an important tourist attraction in the Crater. In 1997 two female black rhinos were translocated from Addo Elephants National Park, South Africa to Tanzania in an attempt to increase the genetic diversity of the local population in the Ngorongoro Crater. At the beginning of 2000, the population of indigenous rhinos in the Crater was 19 individuals. In May 2000 the Crater experienced unusual mortality of wildlife and cases of mortality in the small rhino population was cause for concerns. In December 2000, two indigenous black rhinos were seen to be lethargic and anorexic. They passed frothy red urine, and their faeces were unusually dark, almost black. The two affected animals spent an unusual amount of time lying down. Fortunately, after showing these signs for almost two weeks these two rhinos recovered spontaneously. In early January 2001 two adult female rhinos who were original crater residents (Maggie and Bahati) died nine days apart, on 5 and 14 January. Both exhibited similar symptoms of lethargy, anorexia and red urine. The two animals died on the same day that the game rangers noticed and reported on the symptoms.

### **3.2 Methodological consideration**

Part of the work presented in this thesis is based on routine disease monitoring in wildlife in Serengeti ecosystem for which disease outbreaks in individual species were investigated on case by case basis, therefore, lack some clear plan and design.

#### **3.2.1 Sample collection**

##### **3.2.1.1 Vegetation structure (paper II)**

The assessment of vegetation structure in the Crater was based on the hypothesis that ticks thrive well at a certain grass height due to favourable humidity and have preference to particular grass species. In March 2001, at the commencement of the study, the canopy height of the grass sward and the corresponding quantity of grass material (grass fuel load) in kg/ha was determined on each transect by means of a tape measure and Disc Pasture Meter (DPM),

respectively (Trollope & Potgieter 1986). The predominant grass and herb species within the sampling locality were also recorded. During subsequent monthly data collections only the canopy height was measured and linear regression was used to establish the corresponding grass fuel load (W.S.W. Trollope & L.A. Trollope 2001, unpublished).

#### **3.2.1.2 Prescribed burning (paper II)**

In the assessment of vegetation structure it was established that ticks thrive well on grass with fuel load above 4,000kg/ha, therefore, NCAA was advised to introduce prescribed burning. The management authority followed a recommendation to burn areas of grassland with a fuel load of >4,000 kg/ha (W.S.W. Trollope & L.A. Trollope 2001; W.S.W. Trollope et al. 2002, unpublished data) starting from September 2001 and repeated annually in September/October, the late dry season. About 10-20% of the Crater floor was burnt on a rotational basis every year in separate blocks, and the tourist roads are acting as firebreaks.

#### **3.2.1.3 Tick sampling (papers II, III, IV, V)**

In the study of tick ecology it was necessary to establish the level of infestation on the grassland in Ngorongoro Crater and Serengeti plains. To quantify for tick density in its different habitats, 24 initial transects were established using GPS coordinates and local names. Each transect had a length of 200 m. Transects were in all types of grassland including areas where the Maasai grazed their cattle, areas with tall grass mainly preferred by buffalo, areas with short grass dominated by smaller herbivores, tall grass near swamps and preferred by buffalo and elephants, and in patches of grass in the Acacia forest which is a refuge for black rhino and elephants. After prescribed burning commenced in late 2001, six additional transects were selected where tick density could be compared in adjacent burned and unburned treatments.

Tick collection was carried out in all study transects over 2-3 days every month by removal sampling for adult ticks and drag sampling for immature ticks (larvae and nymphs) as previously described by Short & Norval (1981). Removal sampling of adult ticks involved throwing a metre square steel quadrant at random

within each of the 30 transects of 200 m long and the ticks within the square were collected. This procedure was repeated nine times on each transect, and the ticks from each collection were stored separately in 70% ethanol in 10 ml labelled silicon tubes and in liquid nitrogen. Adult tick density was calculated by dividing the total number of ticks collected each month by the number of quadrants thrown. Adult *Amblyomma* and *Hyalomma* species usually quest for hosts from the ground, and ticks of both genera are apparently more active at night (Walker et al. 2003). Consequently, these ticks were collected from grass litter or from the road surfaces in the early morning, or from immobilized animals. Data collection was continued for a total of 34 months in the initial 24 transects (from March 2001 to January 2004) and for 27 months in the additional six transects (from October 2001 to January 2004).

#### **3.2.1.4 Non-questing ticks (papers II, III, IV, V)**

Adult *Rhipicephalus (Boophilus)* species and those of *Rhipicephalus evertsi evertsi* and *R. sanguineus* were collected from immobilized animals in 2001 and 2004 including buffalo, lions, zebra, wildebeest and gazelles. These ticks were preserved in liquid nitrogen for subsequent laboratory analysis.

#### **3.2.1.5 Immature tick collection (paper II)**

The immature (larvae and nymphs) ticks of several tick species quest for hosts on vegetation. Immature ticks were collected by 'drag-sampling' -dragging a flannel cloth at a constant walking speed over the pasture. The drag apparatus used in the present study consisted of five white flannel strips, each 10 cm wide and 100 cm long adjacent to one another and attached at one end to a wooden spar. Dragging cord was attached to the ends of the spar. Each drag of 50 m length was repeated nine times in each of the 30 transects of 200 m diameter. Immature ticks were recovered from the flannel strips by means of jeweller's forceps, counted and the tick density per square metre of vegetation calculated by dividing the total number of immature ticks collected by the total surface area covered by the 0.5 x 50 m long drag-samples.

Direct tick collection from hosts was carried out on five wild herbivore species (n=47) using chemical immobilization including buffalo, wildebeest, plain zebra (*Equus burchelli*), Grant's gazelle (*Gazella granti*) and Thomson gazelle (*Gazella thomsoni*). In addition, four domestic species including zebu cattle, sheep, goats and donkeys (n = 125) were similarly sampled in those Maasai 'bomas' from which cattle are still herded into the Crater. Ticks were also collected during post-mortem of two dead black rhinos in 2001 and two dead elephants (*Loxodonta africana*) in 2002 and 2004.

#### **3.2.1.6 Tick identification (papers II, III, IV, V)**

The adult ticks in each silicone tube were poured separately into a petri dish, counted and identified. All ticks were identified to the species level using a dissecting microscope (Wild M38, Heerbrugg, Switzerland) and tick identification manual (Walker et al. 2003) and few unidentified ones were sent to Prof. Ivan G. Horak at the University of Pretoria, South Africa for validation on identification. Ticks of the same species and from the same source were prepared by sorting under the dissecting microscope and separated in groups of five ticks. Sets of five ticks prepared from the same species and collected on the same location or from the same animal species named as pools were then preserved at -80°C for subsequent laboratory procedures.

#### **3.2.1.7 Nucleic acids extraction from tick pools (papers III, IV, V)**

In order to determine for the blood parasites harboured by different tick species collected on grass and on animals the molecular analyses techniques were conducted using nucleic acids extracted from tick pools. The frozen tick pools were thawed and placed on biochemically clean parafilm (American National Can™, USA) previously fitted on a sterile glass frame. One tick pool at a time was mechanically disrupted using sterile scalpel blades followed by decontamination of the glass frame and scalpel with DNA-EX (Inno-Train Diagnostic GmbH, Kronberg, Germany), 70% ethanol and rinsed with distilled water prior to processing the next pool. The mechanically disrupted ticks were transferred into 2 ml microcentrifuge tubes and to each of the macerated pools 250 µl of phosphate

buffered saline (PBS 1x, without MgCl<sub>2</sub> and CaCl<sub>2</sub>, Invitrogen, Basel, Switzerland) and 375 µl of external lysis buffer (Guanidinium thiocyanate Triton X-100, MagNA Pure LC Total Nucleic acid isolation kit, Roche Diagnostics, Rotkreuz, Switzerland) were added together with a 5 mm silver bead (Schieritz & Hauenstein AG, Arlesheim, Switzerland). The samples were homogenized at 30'000 Hz for 2 min in a Mixer Mill device (Retsch GmbH, Haan, Germany), cooled in ice for 15 min, homogenized again for 2 min and cooled for 15 min then centrifuged at 8'000 rpm (Ilettich-EBA12, Switzerland) for 1min. From each sample, 500 µl of the lysate was used for nucleic acid extraction using the MagNA Pure LC automated system according to the manufacturer's instructions (Roche Diagnostics GmbH,). Sterile aerosol-barrier tips were used during all procedures. At the end of the extraction procedure 90 µl of the eluate was transferred and preserved at -80°C for subsequent molecular analysis. Nucleic acids extraction and PCR reactions were performed in separate laboratories. Extraction controls using distilled water were included in each extraction process to monitor absence of cross-contamination.

#### **3.2.1.8 Real- time PCR amplification from tick DNA (papers III, IV)**

Real-time TaqMan PCR was used for the amplification of the haemotropic *Mycoplasma* species and *Anaplasma marginale* with the ABI PRISM™ 7700 Sequence Detector System (Applied Biosystems, Rotkreuz, Switzerland) using fluorogenic probe and laser detection system as previously described (Willi et al. 2005; 2006). The ABI PRISM™ 7700 Sequence detector provides cycle by cycle measurement of the fluorescence emission from each PCR reaction. Positive, negative and extraction controls were included in each PCR reaction. Haemotropic *Mycoplasma* species amplified in the tick DNA samples included *M. haemofelis*, 'Candidatus *M. haemominutum*' and 'Candidatus *M. turicensis*'. The number of DNA pools tested for each haemoplasma species was 415, 507 and 440 respectively. Of note is that the *M. haemofelis* assay also amplifies *M. haemocanis* (Willi et al. 2005; 2006) due to the high degree of sequence similarity between the two microorganisms (>99% sequence identity across the 16S rRNA gene sequence) (Sykes et al. 2005). The forward and reverse primers for *Mycoplasma haemofelis*



were Mhf: 5'-GAA AGT CTG ATG GAG CAA TAC CAT-3' and Mhr: 5'-CTG GCA CAT AGT TWG CTG TCA CTT A-3'; the fluorogenic probes (reporter/ quencher dye) was: 5'-VIC-AGT ACT ATC ATA ATT ATC CCT CG- MGB-3'. The forward and reverse primers for "*Candidatus M. haemominutum*" were Mhmf: 5'-GAA AGT CTG ATG GAG CAA TAC CAT-3' and Mhmr: 5'-CTG GCA CAT AGT TWG CTG TCA CTT A-3'; the fluorogenic probes (reporter/ quencher dye) was: 5'-6-FAM-AAG GCT TAA TCA TTT CCT-MGB-3'.

The assay for *Anaplasma marginale* amplified specifically a 65 bp fragment of the MSP4 gene of *Anaplasma marginale* (M.L. Meli, unpublished data). The PCR reactions contained a final concentration of 0.9  $\mu$ M of the primers AmarMSP4.520f (5'-TGA CGT GCT GCA CAC AGA TTT-3') and AmarMSP4.586r (5'-AAC AAA GCT TGC GCC TAT CC-3') for forward and reverse primers respectively, and 0.25  $\mu$ M of the probe MSP4.542p (5'-6FAM-CCT GTG TCC CCG TAT GTA TGT GCC G-3'), 12.5  $\mu$ l of the 2x qPCR Master Mix (Eurogentec) and 5  $\mu$ l of template TNA in a final volume of 25  $\mu$ l. The assay was performed using the ABI PRISM 7700 Sequence Detection System (Applied Biosystems) with an initial step of 50°C for 2 min and a denaturation at 95°C for 10 min followed by 45 cycles at 95°C for 15 sec and 60°C for 1 min.

### 3.2.1.9 Conventional PCR amplification (paper V)

Conventional PCR was used in the amplification of the gene fragments of interest for *Anaplasma*, *Babesia* and *Theileria* species. A total of 118 tick-DNA pools were amplified for *Anaplasma* species using conventional PCR targeting a gene fragment of 452bp of the 16S rRNA gene as previously described (Goodman et al. 1996). Briefly, the reaction volume was 25 $\mu$ l, consisting of 2.5 $\mu$ l of reaction buffer (10x), 2.5 $\mu$ l of MgCl<sub>2</sub> (25mM), 0.5 $\mu$ l of dNTPs (10 mM each), 0.625 $\mu$ l each of the forward and reverse primers (20  $\mu$ M of Ehr1:5'-TTT ATC GCT ATT AGA TGA GCC TATG-3' and 20 $\mu$ M of Ehr2:5'-CTC TAC ACT AGG AGG AAT TCC GCT AT-3' respectively), 0.5 $\mu$ l of Taq Polymerase (5U/ $\mu$ l, Sigma-Aldrich Chemie GmbH, Buchs, Switzerland), 15.25 $\mu$ l of distilled water and 2.5 $\mu$ l of DNA sample. The PCR reaction was performed using a Tpersonal 48 Thermocycler (Biometra

GmbH, Gottingen, Germany). Cycling conditions were: 95°C for 5 min, followed by 35 cycles of 95°C for 30 s, 45°C for 30 sec, 72°C for 45 sec and finally 72°C for 10 min.

For *Babesia* species 102 tick-DNA pools were amplified using conventional PCR and the primers used in the analysis were able to detect multi species DNA sequences of 18S rRNA gene fragment of size of about 500bp. The primers used were broad and are able to amplify *Babesia* species and some *Theileria* species (M.L.Meli unpublished data). The reaction mixture for each sample was 25µl consisting of 2.5µl reaction buffer (10x), 1.5µl MgCl<sub>2</sub> (25mM), 0.5µl dNTPs (10mM), 0.625µl each of forward and reverse primers (20µM of Bbovis.1066f:5'-AAV CTC ACC AGG TCS RGA CA-3' and 20µM of Bbovis.1528r:5'-GGA TCA CTC GAT CGG TAG GA-3' respectively), 0.5µl of Sigma Taq Polymerase (2.5U/µl, Sigma-Aldrich Chemie GmbH), 2.5µl of DNA template and 16.25µl of distilled water. The temperature profile was 95°C for 5 min, followed by 35 cycles of 95°C for 30 s, 62°C for 30 sec, 72°C for 45 sec and finally 72°C for 10 min.

The amplification of 115 pools for *Theileria* species was conducted targeting a fragment of 250bp of the 18S rRNA gene. The primers used are also broad and able to amplify *Theileria* species and some *Babesia* species (M.L.Meli unpublished data). The total volume of the reaction mixture of 25µl consisted of 2.5µl reaction buffer (10x), 2.5µl MgCl<sub>2</sub> (25 mM), 0.5µl of dNTPs (10mM), 0.625µl of forward and reverse primers (20µM of Theilsp.f:5'-AAT GAT GGG AAT TTA AAC CYC TTC-3' and 20µM of Theilsp.r:5'-AAG GCA AAA GCC TGC TTK RAGC-3' respectively), 2.5µl of DNA template, 0.5µl Sigma Taq Polymerase (2.5U/µl, Sigma-Aldrich Chemie GmbH), and 15.25µl of distilled water. The thermal profile was 95°C for 5 min, followed by 35 cycles of 95°C for 30 s, 54.4°C for 30 sec, 72°C for 45 sec and finally 72°C for 10 min. PCR products were gel-purified and stored at -20°C for subsequent cloning and sequencing. Negative and positive controls were incorporated in the reaction. The PCR product was visualized in 1.5% agarose gel stained with ethidium bromide after electrophoresis. The DNA pools which yielded PCR products with fragment of

interest were gel-purified and stored at  $-20^{\circ}\text{C}$  for subsequent cloning and sequencing.

#### **3.2.1.10 Cloning and sequencing of tick-borne pathogens (paper V)**

The cloning and sequencing was performed from selected PCR positive samples. The cloned and sequenced tick-borne pathogens included *Anaplasma* species, *Babesia* species and *Theileria* species. The purification of DNA fragments was performed using the MinElute Gel Extraction Kit (Qiagen). Purified PCR products were cloned into the vector pCR®II-TOPO® (TOPO TA Cloning® Kit, Invitrogen). Purified plasmid DNA (QIAprep Spin Minprep Kit, Qiagen) was checked for insert by restriction digestion with *EcoRI* and 10 clones from each positive PCR product were then sequenced from both sides.

Cycle sequencing was performed with approximately 10ng of DNA and 3.3pmol plasmid-specific primers (M13 forward, M13 reverse) using the BigDye Terminator Cycle Sequencing Ready Reaction Kit v1.1 (Applied Biosystems, Rotkreuz, Switzerland). Cycling conditions were as follows: 1min at  $96^{\circ}\text{C}$ , then 25 cycles at  $96^{\circ}\text{C}$  for 10sec and  $50^{\circ}\text{C}$  for 5sec, followed by  $60^{\circ}\text{C}$  for 4min. Products were purified using the DyeEx Spin column (Qiagen), and analyzed on the ABI Prism 310 Genetic Analyzer (Applied Biosystems). The sequences were aligned to one consensus sequence by SeqScape (Version 1.1, Applied Biosystems) and then compared to reference sequences deposited in the Genbank.

#### **3.2.1.11 Histopathology and bacteriological analyses of hyena samples (paper VI)**

Following the disease outbreak, systematic investigation was conducted beginning with histopathological examination and bacteriological analyses of tissue samples and swabs. Tissue samples were obtained from one hyena that died with severe clinical signs in 2002, seven hyenas that died without clinical signs between 1997 and 2004, and a swab from a minor skin wound of a zebra that was anaesthetised in 2004 for other purposes. Samples for histopathology were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned at  $4\mu\text{m}$  and stained with haematoxylin-eosin and Giemsa. Samples for bacteriological culture were

stored and transported at  $-80^{\circ}\text{C}$ , and cultivated on Columbia sheep blood agar (5% blood), Chocolate agar, Gassner medium, and McConkey II agar (Oxoid, Wesel, Germany). Agar plates were incubated aerobically (5%  $\text{CO}_2$ ) and anaerobically (AnaeroJar 2.5l, Oxoid) at  $37^{\circ}\text{C}$  for 48-72 h. In addition, all samples were incubated in nutrient broth I (Sifin, Berlin, Germany) at  $37^{\circ}\text{C}$  for 24 h. Isolates were characterised on the basis of Gram staining, cellular morphology, and catalase and oxidase reaction. The Lancefield serological group reaction was determined using the Streptococcal Grouping Kit (Oxoid) according to the manufacturer's instructions.

#### **3.2.1.12 Sequencing and phylogenetic analyses (paper VI)**

The phylogenetic relationship of bacterial isolates was investigated by sequencing a 1396 bp segment of their 16S rRNA gene, and comparing the sequences with *Streptococcus* sequences entered in the GenBank. Cycling conditions were: 3 min  $94^{\circ}\text{C}$ , 35x (15s  $94^{\circ}\text{C}$ , 30s  $55^{\circ}\text{C}$ , 90s  $72^{\circ}\text{C}$ ), 7 min  $72^{\circ}\text{C}$ . PCR reaction mixtures (25 $\mu\text{l}$ ) contained 0.5 U AmpliTaq DNA polymerase (Applied Biosystems, Darmstadt, Germany), 2.5 $\mu\text{l}$  10x PCR-buffer, 1.5mM  $\text{MgCl}_2$ , 200 $\mu\text{M}$  dNTPs, 10pmol of each primer (TPU1 and RTU8; Wyss et al., 1996) and ~50ng of DNA. PCR products were purified with ExoSAP-IT<sup>TM</sup> (Amersham Bioscience, Freiburg, Germany), sequenced bidirectionally using the BigDye<sup>TM</sup> cycle sequencing kit (Applied Biosystems), and visualized on an ABI A3100 automated sequencer. The following sequencing primers were used: Strep2F 5'-TAA CTA ACC AGA AAG GGA CG-3', Strep3R 5'-CAC GAG CTG ACG ACA ACC-3', Strep4F 5'-AGA ACC TTA CCA GGT CTT GAC-3' and Strep5R 5'-TCA GAC TTA TTA AAC CGC CTG-3'. The phylogenetic tree was constructed by applying the neighbor-joining algorithm and Kimura-2 parameter distances using MEGA 2.1 (Kumar et al., 2001). We compared these results with those produced by maximum parsimony and maximum likelihood analyses using PAUP\* v.4.0b10 (Swofford 2002), and found that all analyses revealed trees with similar topologies.

#### **3.2.1.13 Blood sample collection from buffalo and wildebeest (paper VII)**

The study to establish the sero-prevalence to brucellosis was initiated following the observation of brucellosis in agro-pastoral and pastoral livestock in rural areas surrounding the Serengeti ecosystem. The hypothesis was that wildlife was at risk of contracting the infection from livestock due to free ranging condition that exists. The wild herbivores serum samples, which were used in the study, included archived sera previously collected from buffalo and wildebeest. The samples were retrieved from the serum and tissue bank at the Serengeti Wildlife Research Center (SWRC) Veterinary laboratory, which were collected during rinderpest sero-surveillance (2001 to 2004), wildebeest migration and reproductive physiology studies (2001 to 2003) and from tick ecology and tick borne pathogens study in Ngorongoro Crater (2004). A total of 205 serum samples were used in the serological analysis to determine the exposure to brucellosis. Serum samples included 103 buffalo and 102 wildebeest. For buffalo 27 serum samples were from NCA and 76 serum samples were from SNP and for wildebeest 31 serum samples were from NCA and 71 serum samples were from SNP respectively.

#### **3.2.1.14 Sero-survey for *Brucella abortus* in buffalo and wildebeest (paper VII)**

Brucellosis is an endemic disease among pastoral communities. Due to free ranging condition of wildlife and livestock in the Serengeti ecosystem it was decided to conduct a sero-survey to establish the exposure status to the micro-organism. To evaluate for exposure to *Brucella* organisms among buffaloes and wildebeests serum collected from these wildlife species in the Serengeti ecosystem (SNP & NCA) were used for two serial serological tests. The Rose Bengal Plate Agglutination Test (Standardized *B. abortus* Rose Bengal Plate Test Antigen, PA0060 Batch 266, Veterinary Laboratories Agency, New Haw, Addlestone, Surrey KT15 3NB, UK) a qualitative procedure involving visual assessment of agglutination of serum on glass slides was conducted at SWRC veterinary laboratory and tests were interpreted as positive or negative (Chernysheva et al. 1980). Subsequent to the qualitative test the competitive ELISA (BRUCELLA 400 Veterinary Laboratories Agency, Weybridge UK) was conducted at Central

Veterinary Laboratory (CVL) in Dar es Salaam by a procedure described by Harlow and Lane (1996).

#### **3.2.1.15 Disease outbreak in wild dogs (paper VIII)**

In October 2007, 23 (nine adults, six yearlings and eight pups) of approximately 38 members of one pack of African wild dogs (the Ololosokwan pack) were found dead or dying during a period of 8 days in the Loliondo Game Controlled Area (LGCA), located approximately 12 kms from the eastern boundary of the Serengeti National Park. Affected animals showed clinical signs of ataxia, weakness, soiling of the perineum and dehydration. Some carcasses were markedly decomposed at the time of sampling.

#### **3.2.1.16 Post mortem examination of wild dogs and sample collection (paper VIII)**

Post mortem examination of dead wild dogs was suggestive of infectious disease; therefore, tissue samples were collected for subsequent laboratory analysis. Histopathological analysis was performed from internal organs and brain. Tissue samples from six relatively fresh carcasses (four pups, two adults) including liver, spleen, lung, heart, brain and kidney were stored in 10% neutral buffered formalin for histopathological examination. Tissue samples for histopathological examination were processed routinely, embedded in paraffin, sectioned at 4µm and stained with hematoxylin eosin.

#### **3.2.1.17 RT-PCR and CDV sequence analysis (paper VIII)**

The histopathological examination was suggestive of canine distemper virus (CDV), therefore, molecular analysis was performed to establish the CDV strain involved. Internal organs from two pup carcasses previously preserved in RNAlater® (Sigma-Aldrich Co, St. Louis, MO, USA) and transported at -10°C and then stored at -80°C for virus screening were used in the molecular analysis. Viral RNA and DNA were isolated from samples initially stored in RNAlater simultaneously using the MinElute virus spin Kit (Qiagen, Hilden, Germany) following the user manuals instructions. Previously published primer pairs were

used for the amplification of the target and used for RNA isolation and reverse transcriptase-polymerase chain reaction (RT-PCR) with *Morbillivirus*-specific primers that amplify a region of the P gene fragment P1: 5'-ATGTTTATGATCACAGCGGT-3' and P2: 5'-ATTGGGTTGCACCACTTGTC-3' as described by Barret et al. (1993) as well as primers homologous to sequences of the F gene fragment FC1: 5'-GGACTGATAATGTCCATTA-3' and FC2: 5'-ATAGCTTTGTTAGACTGTT-3' as described by Liermann et al. (1998). PCR products were processed using Big Dye X Terminator solution (Applied Biosystems Inc. [ABI], Foster City, California) and sequences were determined using the sequence analyser ABI 3100 Genetic Analyser (ABI). Editing and alignment of the sequences was done with BioEdit 7.0.9.0 (Hall 1999). A neighbour-joining tree of the P-gene fragment was generated using the maximum composite likelihood model with 1000 bootstrap pseudoreplications as implemented in Mega 4 (Tamura et al., 2007). Tissue samples were also screened using established PCR protocols for several other known pathogens of wild carnivores in the Serengeti ecosystem to investigate whether the pack was co-infected with more than one pathogen.

### **3.2.1.18 Diseases of economic and conservation significance (paper IX)**

A review was conducted to compile a list of known diseases that have been reported in livestock-wildlife interface in Tanzania. The source of information was from peer reviewed journals, conference proceedings, unpublished scientific reports and personal communication with scientists who had information on diseases in livestock-wildlife interface.

### **3.3.1 Data Analyses**

#### **3.3.1.1 Tick density, vegetation and tick-borne pathogens (papers II, III, IV, V)**

A linear regression was used to convert the mean canopy height of the grass sward into the corresponding mean disc height and therefore mean grass fuel load in kg/ha as described by W.S.W. Trollope & L.A. Trollope (2001, unpublished

data) and the relationship between tick density and grass height was investigated using a linear regression analysis. Associations between the number of adult ticks in tall grass ( $\geq 50$  cm) and in short grass ( $< 50$  cm) during the wet season, or the number of immature ticks in less grazed ( $> 20$  cm) and heavily grazed ( $< 20$  cm) vegetation in the dry season, were tested by means of a Student t-test. A non-parametric Wilcoxon paired-sample test statistic (T) was used to compare the associations between tick density and grass height in burned and unburned areas. The criterion for judging the level of significance in both analyses was as described by Fowler et al. (1998) and the statistical significance was set at 0.05.

When a PCR product was obtained from a pool of five ticks, only one tick in the pool was assumed to be infected and the maximum likelihood estimation of the infection (MLE) was determined using the formula:  $MLE = 1 - (1 - Y/X)^{1/m}$ , as previously described (Walter et al. 1980); thus may have an underestimate by a factor of at least 4. Where Y = number of positive pools; X = number of pools and m = number of organisms per pool. Chi-squared test ( $\chi^2$ ) was used to compare the rate of detection of tick-borne haemoparasites in tick species (Petrie & Watson 2004).

### **3.3.1.2 Sero-survey for brucellosis in buffalo and wildebeest (paper VII)**

The sero-survey results for brucellosis in buffalo and wildebeest were presented as an overall prevalence for the Serengeti ecosystem (NCA and SNP) and separately from each protected area. The two protected areas have different level of interaction between wildlife and livestock and different management systems with the former having high interaction with Maasai livestock. The Odds Ratio (OR) and Relative Risk (RR) were established to understand whether there is an association between location and prevalence of the infection and Chi-squared test ( $\chi^2$ ) was used to compare the statistical significance in prevalence between the two protected areas (Petrie & Watson 2004).



## 4.0 Results

### 4.1 Diagnosis of dead black rhinos and treatment (paper I)

The blood smear samples that were submitted to the Central Veterinary Laboratory (CVL) in Dar es Salaam and those examined on site revealed numerous intra-erythrocytic parasites resembling *Babesia* spp. PCR analysis confirmed that the rhinos were infected with a blood parasite, *Babesia bicornis* (Nijhof et al. 2003). The parasite is tick-borne, but the possible tick species that transmit it are not yet known. Concurrent with the rhino deaths, high tick infestation with a number of different tick species was observed on the crater grassland. Following the diagnosis of babesiosis, it was decided in January 2001 to provide therapeutic cover for the remaining rhinos in the crater as an immediate temporary solution to the problem. Diminazine aceturate (Berenil<sup>®</sup> Hoechst) was used to treat the rhinos. Berenil has the advantage of being stable, has a wide safety margin and can be concentrated into a small volume to fit in the 3-ml projectile darts. It has also been administered to black rhino in South Africa that had babesiosis without obvious side effects (P. Morkel 2001, personal observation). For this purpose, 2.36 g of Berenil powder was mixed with 1.3 ml sterile water (3 Berenil sachets made up 5 ml of the suspension) and a maximum of 3 ml Berenil suspension was injected per animal. The rhinos received a dose of 2–3 mg kg<sup>-1</sup> intramuscularly. Remote injection avoided the physiological stress that results from the process of chemical immobilization and hand-injection of the drug when they were possibly recovering from drought-related stress. At the end of the disease outbreak in 2002 31.5% (n=6) of Crater rhinos had died from the episode and only 13 rhinos remained including one of the two individuals which were translocated from South Africa in 1997. The post-treatment response was good and no reactions were seen at the injection sites except that one rhino developed an abscess. Fortunately the abscess, in the neck, drained by itself and within two months it was healed.

#### 4.2 Seasonal tick density in the crater grassland (paper II)

The study found that immature ticks were far more numerous than adult ticks and each life stage varied greatly with season. Before burning in 2001, most adult ticks were present in the wet season between March and June and most immature ticks were present during the peak dry season in September and October. Using the same methodology of removal and drag sampling, the tick score from the Crater rim and from the neighbouring Serengeti plains was zero. On the rim near Maasai residences, grass cover is often overgrazed, trampled or burnt, while the plains are arid with very short grasses.

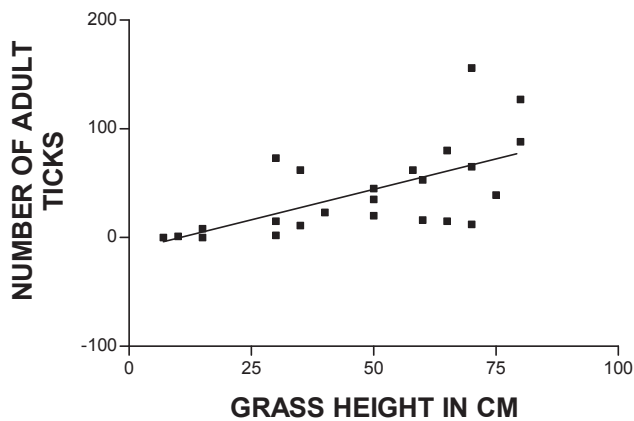
The difference in immature tick densities in both habitats between dry and wet season was highly significant ( $p < 0.001$ ), with more immature ticks in the dry season. In areas with tall grass in the preceding wet season, larger numbers of immature ticks were collected in the dry season in lightly grazed compared to heavily grazed areas. The higher density of adult ticks in areas with tall grass ( $\geq 50$  cm) compared to those with short grass ( $< 50$  cm) during the wet season was statistically significant ( $p < 0.05$ ).

#### 4.3 Identified tick species (papers I, II, III, IV, V)

Adult ticks belonging to 15 species were identified and comprised of *Amblyomma gemma*, *A. sparsum*, *A. tholoni*, *A. variegatum*, *A. cohaerens*, *Rhipicephalus (Boophilus) decoloratus*, *R. appendiculatus*, *R. compositus*, *R. evertsi*, *R. longus*, *R. praetextatus*, *R. pravus*, *R. Pulchellus*, *R. sanguineus* and *Hyalomma truncatum*. Of the 15 tick species present on hosts, the highest number occurred on buffalo and cattle. All the adult questing ticks collected belonged to the genus *Rhipicephalus*. *Rhipicephalus appendiculatus* was the most abundant species by far, for example comprising 98 % of the 1'039 ticks recovered from the vegetation in the middle of the wet season in 2001.

#### 4.4 Plant species (paper II)

The predominant grass species on the Crater floor were *Chloris gayana*, *Cynodon* spp., *Digitaria* spp., *Pennisetum* spp., *Hyperrhenia* spp., *Sporobolus* spp., *Eragrostis* spp., Nut grass (*Cyperus rotundus*), *Themeda* spp., *Setaria* spp. and *Panicum maximum*. Herbaceous plants in the Crater that are favoured by herbivores include *Amaranthus spinosus*, *Amaranthus hybridus*, *Achelanthes aspera*, *Justicia betonica* and *Trifolium masaiense*. The mean grass height and the corresponding grass fuel load in the 24 transects were, as expected, highly significant between wet and dry seasons ( $p < 0.001$ ). High tick density and the presence of *Chloris gayana*, *Cynodon dactylon* and *Pennisetum clandestinum* were correlated ( $p < 0.05$ ; Figure 2). These grass species and herbs like *Amaranthus spinosus*, *A. hybridus*, *Achelanthes aspera*, *Justicia betonica* and *Trifolium masaiense* are favoured by large herbivores, including buffalo. Regardless of height, few ticks were observed in areas where the following grasses predominated: *Eragrostis* spp., *Hyperrhenia rufa*, Nut grass, *Panicum maximum*, *Pennisetum mezianum*, *Setaria* spp., and *Themeda triandra*.



**Figure 2:** Grass height in relation to adult ticks during wet season in Ngorongoro Crater in 2001.

#### 4.5 Effects of prescribed burning (paper II)

The effects of fire on the grass sward virtually eliminated adult ticks and reduced immature ticks significantly. The effect was rapid: one year after the start of burning (2002) unburned areas had immature tick densities comparable prior to burning ( $372\pm 50$ ) but burned areas showed a decline of immature ticks ( $23\pm 17$ ) at the peak of dry season in September.

The mean 'peak' tick density ( $\pm$ SE) before burning was  $42\pm 7$  per square metre (adults, wet season) and  $819\pm 153$  per square metre (immature ticks, dry season). After 27 months post burning, these values had fallen to  $0\pm 0.08$  (adults, wet season) and  $33\pm 41$  (immature ticks, dry season) respectively.

In Ngorongoro Crater, grass height of less than 10cm with a fuel load below 4'000 kg/ha appears to present an environment unfavourable to tick survival.

#### 4.6 PCR amplification and infected tick species (papers III, IV, V)

##### 4.6.1 Haemotropic *Mycoplasma* species (paper III)

*Rhipicephalus sanguineus* was found to contain DNA of haemotropic *Mycoplasma* species. Four out of six *R. sanguineus* pools tested positive for *M. haemofelis* and three out of six were positive for 'Candidatus *Mycoplasma haemominutum*'. All positive pools from *R. sanguineus* were from ticks collected from immobilized lions in the Crater during a disease outbreak in 2001. The infection rate of *R. sanguineus* tick species was 19.7% and 12.9% for *M. haemofelis* and 'Candidatus *M. haemominutum*', respectively. Two positive pools from *R. sanguineus* (7.8%) tested PCR-positive for both *M. haemofelis* and 'Candidatus *M. haemominutum*'. Although more ticks were found to have *M. haemofelis* DNA than 'Candidatus *M. haemominutum*', the difference was statistically not significant ( $\chi^2 = 1.43$ ;  $df = 1$ ;  $P > 0.05$ ). Some of the real-time PCR amplification positive pools are shown in Figure 3 and example of haemotropic *Mycoplasma* species in infected susceptible host is shown in Figure 4.

#### 4.6.2 *Anaplasma* species (papers IV & V)

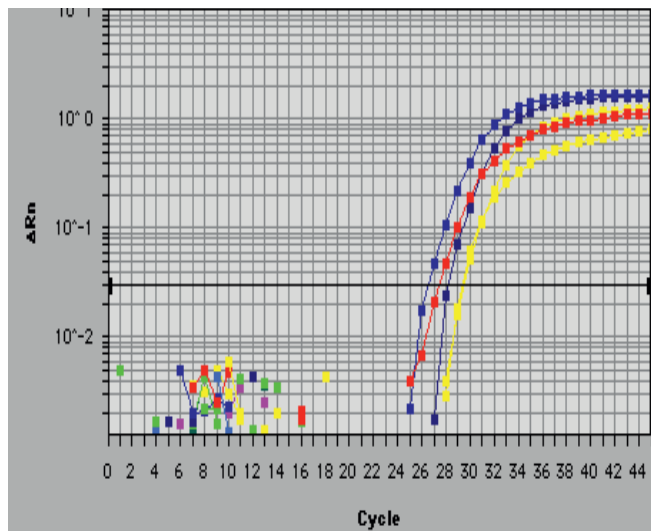
Six tick species contained *Anaplasma marginale* DNA and 29 out of 527 pools tested positive for *A. marginale*. The infected tick pools included *Amblyomma gemma*, *Rhipicephalus appendiculatus*, *R. compositus*, *R. decoloratus*, *R. praetextatus* and *R. pulchellus*. Four *R. pulchellus*, seven *R. appendiculatus*, four *R. compositus* and one *R. decoloratus* positive pools were from buffalo; one *R. praetextatus* positive pool was from wildebeest; one *R. appendiculatus* positive pool was from zebra; one *A. gemma* positive pool from cattle. Five *R. appendiculatus*, two *R. compositus*, one *R. praetextatus* and two *R. pulchellus* positive pools were from questing ticks. *R. appendiculatus* had the least detection rate out of six tick species detected with *A. marginale*. Sequencing of gene fragments (452bp) of *Anaplasma* positive pools from *R. praetextatus* based on 16S rRNA gene revealed presence of *Anaplasma bovis* (GenBank accession numbers GU361777-GU361780). One of the resolved PCR products in 1.5% agarose gel is shown in Figure 5.

#### 4.6.3 *Babesia* species (paper V)

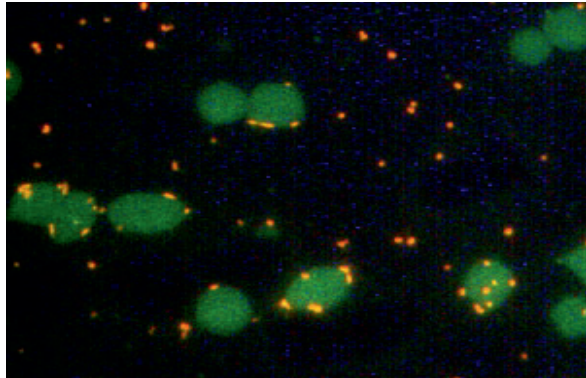
In the PCR amplification of 102 DNA pools, 37 pools from five tick species tested positive for *Babesia* species including *A. gemma*, *Rhipicephalus appendiculatus*, *R. compositus*, *R. decoloratus* and *R. evertsi evertsi*. One pool of *A. gemma*, one pool of *R. compositus* from grass cover and questing respectively; and two *R. decoloratus* pools from buffalo tested positive for *Babesia* species. Two out of 37 *Babesia* species positive pools from *R. evertsi evertsi* had mixed infection with *Anaplasma* species which were not identified as *A. marginale* or *A. phagocytophilum* by real-time PCR (Fyumagwa et al. 2009a). Sequencing of amplified 18S rRNA fragments (500bp) of *Babesia* species positive pools from *R. appendiculatus* and *R. evertsi evertsi* detected *Babesia equi* (GenBank accession numbers GU361784- GU361791).

#### 4.6.4 *Theileria* species (paper V)

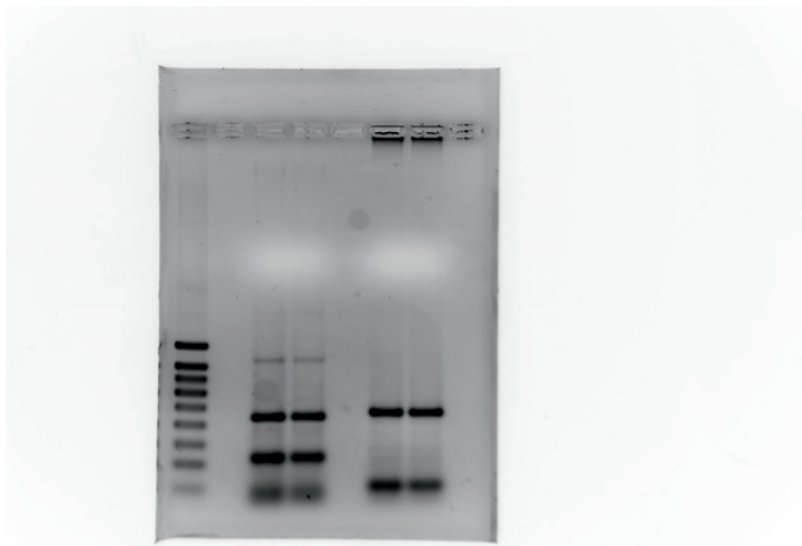
Thirteen out of 115 DNA pools comprising of *R. appendiculatus* and *R. compositus* tested positive for *Theileria* species. The sequence analysis of selected amplified 18S rRNA fragments (250bp) from *R. appendiculatus* revealed *T. parva* (GenBank accession numbers GU361781-GU361783; GU361792-GU361794) and *T. buffeli* (GenBank accession numbers GU361795-GU361799).



**Figure 3:** Curves generated by TaqMan real-time PCR amplification indicating four positive pools with cycle threshold (CT) values falling between a range of CT 26 and 30.



**Figure 4:** Haematropic *Mycoplasma* species on the surface of erythrocytes and in the cytoplasm of an infected domestic cat (Adopted from Dr. Barbara Willi).



**Figure 5:** PCR products from *Anaplasma* species resolved in 1.5% agarose gel showing positive bands in lane 3, 4, 6 and 7 (from left) of size of 452bp lying between a band of 400 bp and 500 bp on the ladder in lane 1.

## **4.7 Disease outbreak in spotted hyenas (paper VI)**

### **4.7.1 Clinical signs of infected hyenas**

During the outbreak, 21 hyenas out of a mean total population of 301 animals were observed to express external signs of bacterial infection. The first hyena with external signs was observed in August 2001. Nineteen individuals had external signs on one occasion; one hyena expressed and cleared signs in three different years (2001, 2002 and 2004). Hyenas with external signs were mostly observed in 65% of cases ( $n=23$ ) during a period of six months between September 2002 and February 2003. After this outbreak, animals developed less severe swellings and abscesses than during the outbreak and hyenas with signs after the outbreak did not express apathy or marked ataxia.

Signs were pronounced and included a diffuse, unilateral swelling of the head, respiratory distress, serous ocular discharge, mild ataxia, and lethargy. Subsequently, swellings became localized at the submandibular region, where abscesses were observed in 13 (72.2%) of the affected animals (Figure 6a). Abscesses from 11 animals (84.6%) ruptured and drained externally, resulting in the clearance of external signs (Figure 6b). Clinical signs were expressed and cleared within 7 to 24 days, but in one case the abscess was visible for 9 months. Three hyenas expressed acute signs characterized by severe swelling of head and neck, apathy and marked ataxia. While one of these individuals recovered, the two others died within days of developing acute signs. Most hyenas (94%,  $n = 15$ ) that showed clinical signs were observed between September 2002 and February 2003, suggesting an outbreak of infection during this period. Only one further hyena expressed clinical signs after this period, in December 2004. For further details of the impact of this outbreak of bacterial disease on the spotted hyena population in the Crater see Höner et al. (2011).





**Figure 6a:** One of the affected spotted hyenas showing excessive salivation and swelling of the submandibular region (Photo by Oliver Höner & Betina Wachter).



**Figure 6b:** The photo shows a spotted hyena with ruptured submandibular region leaving an open septic wound (Photo by Oliver Höner & Betina Wachter).

#### **4.7.2 Histopathological and bacteriological examination of hyena samples (paper VI)**

Histopathological examination of tissue from one hyena that died with acute signs revealed extensive suppurative inflammation and oedema with numerous bacterial colonies within the soft tissue around the mandibular angle. Additionally, there was severe suppurative necrotizing pneumonia intermixed with similar bacteria, and marked intraalveolar haemorrhage. Three phenotypically similar bacterial isolates were cultured from lung, mandibular lymph node, and tissue from the mandibular angle from the hyena that died with clinical signs. Two further phenotypically similar isolates were cultured from the tonsil from one hyena without clinical signs at death and from a minor skin wound of a zebra. Two

isolates were obtained from the tonsil from a second hyena without signs at death; one isolate was phenotypically similar to and the other different from the previous cultures. All seven isolates were Gram-positive, catalase-negative, and beta-hemolytic with colony morphology suggestive of *Streptococcus*, and were classified as *Streptococcus* of Lancefield's serogroup C. Samples from an additional five hyenas that died without signs did not reveal streptococci of Lancefield's serogroup C.

#### **4.7.3 Molecular analysis of bacterial isolates from hyenas (paper VI)**

One of three phenotypically similar isolates from the hyena that died with clinical signs (Hyena M149), the two similar isolates from the two hyenas without signs (Hyena A084, Hyena E118-1), the dissimilar isolate from one of the hyenas without signs (Hyena E118-2), and the isolate from the zebra (Zebra EQ003) were sequenced (paper VI). All five isolates were genetically highly similar (in group mean distance =  $0.001 \pm 0.001$ , overall mean distance =  $0.045 \pm 0.004$ ), and the isolate from one hyena (Hyena A084) was identical to the zebra isolate. The *Streptococcus* isolates from the Crater were identical or highly similar to the *S. equi* subsp. *ruminatorum* type strain CECT 5772<sup>T</sup> (AJ605748). The five Crater isolates plus the *S. equi* subsp. *ruminatorum* type strain formed a sister clade to the clade containing *S. equi* subsp. *equi* and *S. equi* subsp. *zooepidemicus* as supported by high bootstrap values. The detailed characterization of the *Streptococcus equi ruminatorum* in spotted hyena and zebra in the Crater is described by Speck et al. (2008).

### **4.8 Sero-survey in buffalo and wildebeest against brucellosis (papers VII & IX)**

#### **4.8.1 Prevalence of brucellosis in buffalo and wildebeest**

In buffalo the overall prevalence was 24% (n= 103) sero-positive for brucellosis in the Serengeti ecosystem. However, the prevalence of exposure in the two different locations for buffalo was 30% in NCA (n= 27) and 22% in SNP (n= 76). In wildebeest the overall prevalence was 17% (n= 102) seropositive for brucellosis

in the Serengeti ecosystem and the location prevalence was 19% in NCA (n= 31) and 15% in SNP (n= 71). Abortion with retained placenta has been observed in Ngorongoro Crater buffalo population (Figure 7).



**Figure 7:** Aborted buffalo with a retained placenta in Ngorongoro Crater (Photo by S. Kuya of NCAA)

#### 4.8.2 Risk of exposure to *Brucella* microorganism

The NCA is a multiple land use area and is considered to have the highest livestock- wildlife interaction than any other protected area in Tanzania. The measure of biological association between locations as a contributing factor to the difference in the rate of exposure to the *Brucella* microorganisms in the two protected areas was determined using Odds ratio (OR) and Relative Risk (RR). The OR for buffalo and wildebeest in NCA and SNP populations were 1.46, and 1.3 respectively and the RR for both species was 1.3.

The 95% confidence interval (CI) for buffalo and wildebeest in the two protected areas were 3.065 to 6.634 and 11.176 to 18.887 respectively. Because the OR for both buffalo and wildebeest are not contained within the CI and the RR is the same for both animal species, it is likely that herbivores in NCA and SNP are at

equal risk of exposure to the microorganisms. Statistically the difference in the rate of exposure to *Brucella* organism between the two protected areas was not significant for either buffalo or wildebeest ( $\chi^2=0.245$ ;  $df=1$ ;  $P>0.05$  and  $\chi^2=0.037$ ;  $df=1$ ;  $P>0.05$  respectively).

#### **4.9 Laboratory result of wild dog samples (paper VIII)**

##### **4.9.1 Histopathological examination of wild dog samples**

Histopathological examination of the organs from all six pack members revealed that the main pathological changes occurred within the lungs. Moderate to severe multilobular suppurative to necrotizing bronchopneumonia was found with extensive intra-alveolar and interstitial infiltration with mononuclear inflammatory cells and some neutrophilic granulocytes, as well as marked (secondary) bacterial colonisation. Epithelial lining cells of bronchi and bronchioli contained clearly visible eosinophilic intracytoplasmic inclusion bodies and some animals had additional formation of multiple syncytial cells within the parenchymal tissue. The severity of the findings is consistent with fatal canine distemper virus infection. Further pathological changes of other organs were considered side findings and mostly confined to generalised congestion.

##### **4.9.2 RT- PCR and CDV P and F gene fragment sequences**

Two sequences were obtained for a 388nt fragment of the phosphoprotein (P) gene (GenBank accession numbers: EU481827, EU 481828) and a phylogenetic comparison of sequence data from these fragments with published information revealed that the African wild dog pack in 2007 was infected with a CDV variant closely related to a CDV variant described from a captive African wild dogs in northeast Tanzania in 2001 (Van de Bildt et al. 2002), and to CDV variants previously described from lions, spotted hyenas and a bat-eared fox (*Otocyon megalotis*) in Serengeti National Park in 1994 (Haas et al., 1996; Roelke-Parker et al., 1996; Carpenter et al., 1998).

A sequence from one 335 nt fragment of the fusion (F) gene was obtained from one pup and was submitted to GenBank (GenBank accession number: EU481829). This fragment was most closely related (99% homology) to the only available F gene fragment from Tanzania (Liermann et al., 1998; GenBank accession number: AF026233) which was obtained from a variant that infected a spotted hyena host during an outbreak of CDV infection among wild carnivores in the SNP in 1993 and 1994.

#### **4.10 Diseases of economic and conservation significance (paper IX)**

About 20 diseases of economic and conservation significance that affected wildlife or have a wildlife reservoir were described. Some of which have been relatively recently introduced to Tanzania or their range within Tanzania has changed due to anthropogenic influence whereas others can be considered to be native to Tanzania. The diseases include rinderpest, foot and mouth disease, canine distemper, rabies, rift valley fever, malignant catarrhal fever, Newcastle disease, brucellosis, tuberculosis, anthrax, tick-borne diseases, *Treponema* infection in olive baboons (*Papio anubis*) and poisoning caused by cyanobacteria toxins.

## **5.0 Discussion**

Tanzania contains several areas that are internationally important for their biodiversity and has the largest wild herbivore population in Africa which can interact freely with livestock; as a result there is high risk of infectious agents to spillover and spillback between livestock and wildlife. A good example is the largest population of migratory wildebeest and zebra from the Serengeti ecosystem in Tanzania to Maasai Mara National Reserve in Kenya and back. African buffaloes are known to migrate long distances across international boundaries from northern Serengeti and Mkomazi National Park to Kenya (Fyumagwa 2010). Together with this migration, large carnivores like spotted hyenas do commute from their clan territories for long distances (40-70 km) to forage in areas containing high concentrations of migratory herbivores which are their preferred prey (East et al. 2001; 2005). These migratory animals know nothing of international boundaries, neither do their diseases. Also the movement of Maasai pastoralists with livestock between Kenya and Tanzania is another possible way of animal disease transmission. Due to high diversity of wildlife and large animal populations, the wildlife veterinarians in Tanzania are not able to conduct disease surveillance in all protected areas in the country (30% of land cover). As a result, little information is available regarding the prevalence of many wildlife diseases.

### **5.1 Tick infestation and tick-borne diseases**

Tick-borne diseases account for more than 72% of livestock loss in Tanzania and east coast fever (ECF) alone decimate 12% of indigenous cattle and mortality can be up to 90% in exotic cattle breeds if not treated (Fyumagwa et al. 2009a; Fyumagwa 2010). Unusual mortality among herbivores in the Crater was noted from the first half of 2000 and by February 2001 six black rhinos more than 1000 African buffalo and hundreds of other herbivore species had died and their carcasses were heavily infested with ticks (Fyumagwa et al. 2004; 2007; 2009a). Two female black rhinos were diagnosed by PCR to have died from babesiosis caused by *Babesia bicornis*, previously described in South Africa (Nijhof et al. 2003). In addition, about 20 lions in the Crater died from multiple tick-borne infections in an outbreak of February 2001 exacerbated by stress from swarms of

biting flies and mechanical transmission of haemoparasites (Fyumagwa et al. 2008). Long term weather changes with cycles of severe drought followed by wet years and lack of prescribed burning exacerbated tick infestation in the Crater grassland. Severe drought caused nutritional stress to herbivores which is immunosuppressive and hence wildlife succumbed to clinical infections from multiple infectious agents (Fyumagwa et al. 2007).

The Ngorongoro Crater experienced considerable vegetation changes which were vividly observed in the crater grassland and along the tourist roads. However, wilting of the acacia trees in the Lerei forest in the Crater and the consequent decrease in the tree canopy favouring growth of unpalatable herbs is perhaps more serious. Interestingly, according to Joe Ole Kuwai (2001, pers. comm.) the decline in the Lerei forest and the tendency for it to re-establish up towards the Lerei Ranger Post is a natural cyclic phenomenon. Unpalatable herbs and alien toxic plants such as *Guatenbelgia cordifolia* and *Datura stromonium* invaded a large portion of the crater grassland (Fyumagwa & Nyahongo 2010). Most of these invading species, which occur throughout the Crater, particularly along the roads, were not there three decades ago, therefore might have been brought into the Crater by vehicles or road maintenance trucks. These plants are also seen along the main road towards Serengeti short grass plains and seem to be spreading rapidly. If a thorough assessment was done to quantify the standing unpalatable weed in the Crater in 2001, probably as much as one quarter of the Crater would have been found to be occupied by these notorious plant species. The invasion of large areas of the Crater grassland by the alien toxic plants forced animals to concentrate in certain areas only thus probably leading to increased grazing competition among herbivores. The Ngorongoro Conservation Area Authority (NCAA) intervened in 2002 by manual uprooting and occasionally mowing the toxic plants which restored the even distribution of herbivores.

The reintroduction of controlled burning in the Crater reduced the area covered with coarse and tall grass thereby creating unfavourable habitat for ticks and buffalo. Fire destroys moribund grass and stimulates growth of new and palatable sprouts. Extensive areas with short grass are unsuitable for buffalo, the maintenance host for a diversity of tick species (Fyumagwa et al. 2007). Moreover

controlled burning influenced the distribution of other herbivores in the Crater grasslands. All grazers favoured burned areas with short grass except buffalo, which prefer coarse tall grass. Therefore, prescribed burning should be maintained by the NCAA as a rangeland management tool for tick control and enhancement of vegetation structure, composition and quality, even though this is likely to place buffalos under nutritional stress during the dry season and may result in a decline in the buffalo population on the Crater floor. This expected decrease in buffalo numbers is likely to be associated with increase in medium sized (wildebeest) and small (e.g. Thomson gazelles) herbivores. In view of the fact that buffalo is currently an ungulate species being sought after for viewing by tourists, the aim should not be eviction but rather reducing the population size.

Vector-borne diseases outbreak in free ranging wildlife with noticeable mortality is a sign of imbalance in the ecosystem components (hosts, parasites and habitat), suggesting that the ecology of the Crater had reached a point whereby it was experiencing a “Sick Habitat or Ecosystem Distress Syndrome” (Aguirre & Gomez 2009).

## **5.2. Infectious diseases**

Disease pathogens that have co-evolved with wildlife for millions of years do not necessarily cause devastating pathological effects in wildlife. These pathogens may cause clinical diseases to susceptible livestock populations that are often bred for desired traits other than disease resistance as opposed to wildlife which is a result of natural selection. Individuals in wildlife populations can succumb to infection for example immunologically naïve young animals or animals suffering immunosuppression caused by poor nutrition for example during a drought or other causes (Fyumagwa et al. 2004; Nijhoff et al. 2005). Here I discuss some infectious diseases (those that are influenced by anthropogenic factor and those that are considered to occur naturally) including canine distemper virus (CDV), *Streptococcus* infection in spotted hyena, brucellosis in wildlife, rinderpest, foot and mouth disease (FMD), rabies, malignant catarrhal fever (MCF), Newcastle disease, rift valley fever (RVF), tuberculosis, anthrax, *Treponema* infection in olive baboons and cyanobacteria toxins in lesser flamingoes.



Since disappearance of wild dogs in Serengeti National Park (SNP) in 1991 most probably due to rabies despite individuals in all study packs being vaccinated against rabies (Burrows et al. 1994; Woodroffe 2001). Wild dog packs have continued to persist outside the SNP, particularly on its eastern side. An epidemic of canine distemper that increased mortality in lions and spotted hyenas occurred in 1993/ 1994 in the Serengeti National Park (Haas et al. 1996; Roelke-Parker et al. 1996; Carpenter et al. 1998). Since 1995 what have been termed 'silent' outbreaks of CDV have occurred in the Serengeti ecosystem in both lions and spotted hyenas (Harrison et al. 2004; Munson et al. 2008) based on serological data. Despite this evidence of continued exposure to CDV there is no evidence that CDV infection has resulted in clinical disease in lions or spotted hyenas in the SNP. In 2001, 94% (n=52) of wild dogs (*Lycoan pictus*) bred in captivity in Mkomazi National Park, in the northeast of Tanzania died from CDV, interestingly, many of these captive animals were vaccinated with an attenuated CDV vaccine before they died of CDV. Genetic analysis of the CDV strain from these captive wild dogs demonstrated that they did not die of a vaccine strain but rather from a CDV strain circulating in unknown reservoir (van de Bildt et al. 2002).

Against the backdrop of a lack of any evidence of fatal CDV infection in any carnivore species in the SNP, the occurrence of clinical CDV infection in 2007 in a wild dog pack in the LGCA close to the eastern boundary of the SNP is interesting. CDV caused approximately 66% (n=38) mortality in the pack (Goller et al. 2010). Large home range of wild dogs (>5000 km<sup>2</sup>) is a risk factor for exposure to infection. The wild dogs in the 2007 outbreak contracted the infection from undescribed reservoir host. However, phylogenetic analyses revealed that the pack was infected with CDV variants most closely related to those that caused CDV epidemic in lions in Serengeti ecosystem in 1993/1994 (Goller et al. 2010). Thus it is worth asking why only one African wild dog pack of several present in the area died of CDV infection in 2007. One possible explanation could be the synergistic effects of co-infection with several pathogens. Pathogen screening of members of the pack revealed co-infection with parvovirus and a tick-borne blood parasite (*Hepatozoon* sp.). Parvovirus, like CDV is an aggressive virus that causes severe

immunosuppression. Goller et al. (2010) suggested that the reason why this particular pack died of CDV infection may have been the synergistic effect of co-infection with two virulent viruses. Goller et al. (2010) following both Creel et al. (1997) and Alexander et al. (2010) commented that there is little evidence that CDV is a threat to healthy African wild dog populations. This is strongly implicated by the large number of long-term studies on different African wild dog populations throughout their range and the general lack of evidence that CDV caused mortality to any study population. Indeed, the diagnosis of CDV induced mortality in the pack in the Serengeti ecosystem in 2007 reported by Goller et al (2010) was only the second laboratory confirmed case from free-ranging African wild dogs packs, although it should be noted that George Schaller reported a possible case of CDV in wild dog pups in the Serengeti based on clinical signs in 1968 (Goller et al. 2010). The other being from a pack in Botswana (Alexander et al. 1996)

Spotted hyenas are hardy animals, do not succumb easily to infectious agents with overt clinical diseases and are regarded as ecosystem cleaners. However, consumption of carcasses with infectious agents can have a serious pathological effect to these carnivores. In 2001 spotted hyenas in the Ngorongoro Crater succumbed to clinical disease consistent with severe bacterial infection (Höner et al. 2006). On sequence analysis it was revealed to be a Lancefield group C *Streptococcus* homologous to *S. equi* subsp. *ruminatorum*, recently described in mastitis cases in domestic sheep and goats in Spain (Höner et al. 2006; 2011). Strains similar to this bacterium were also isolated from a sympatric Burchell's zebra (*Equus burchelli*) a herbivore species often consumed by hyenas suggesting that inter-specific transmission may occur when hyenas consume infected carcasses. The highest mortality in the Crater hyena population during the outbreak of bacterial infection was in the age / sex categories most likely to suffer nutritional stress (Höner et al. 2011). It is very unfortunate that no study has been conducted to investigate on the prevalence of the bacterium in Maasai sheep and goats in Ngorongoro since spotted hyenas have high interaction with livestock in the area.

Brucellosis causes infertility to animals and is endemic among nomadic pastoral communities with a prevalence of 3.5 to 16% in pastoral and agro-pastoralists in different zones in Tanzania (Kiputa et al. 2008; Bugwesa et al. 2009). In agro-pastoral communities in western Serengeti where the livestock-wildlife interaction is not very high compared to NCA, the sero-prevalence is about 14% in cattle (Bugwesa et al. 2009). High prevalence of exposure to *Brucella abortus* in buffalo and wildebeest populations of 24% and 17% respectively that have been reported in the Serengeti ecosystem was an unexpected observation (Fyumagwa et al. 2009b). Abortions in buffalo and wildebeest observed in the past in the Serengeti ecosystem are likely to be associated with brucellosis. The prevalence of brucellosis is higher in buffalo and wildebeest compared to livestock in the interface suggesting that livestock and humans are at risk of contracting the infection from wildlife and the infection is probably sustained in wildlife population.

Historically, rinderpest (*Morbillivirus*) pandemic decimated over 95% of cattle and wild ungulates in Africa from Cairo to Cape Town. Until early 1950's the wild ungulate population in the Serengeti ecosystem remained very low, for example the wildebeest population was between 200,000 and 300,000 only (Sinclair 1973; 1995). The virus spread from Eastern Asia to Africa due to anthropogenic influence during military operations around the turn of the last century. However, after a successful ring vaccination of agro-pastoral and pastoral cattle around the ecosystem the wildebeest population increased exponentially to 1.5 million within only a decade (Sinclair 1995). Disease control through vaccination of cattle (the reservoir) made it possible to eradicate the virus in Tanzania and by 2005 the country declared freedom from rinderpest (Fyumagwa 2010).

Foot and mouth disease (FMD) caused by *Picornavirus* is a transboundary disease of cloven-hoofed livestock and wild animals. In Tanzania five serotypes have been reported to affect susceptible animals including serotype A, O, SAT1, 2, & 3 (Fyumagwa 2010). The former two serotypes are exotic in Africa and were brought with infected exotic cattle breeds from Europe and the latter three serotypes are indigenous in Africa. Susceptible wildlife including eland, impala and

kudu show clinical symptoms similar to cattle, sheep, goats and pigs, while buffalo is the natural reservoir of SAT1, 2 & 3 (Fyumagwa 2010).

Rabies (*Lyssavirus*) has a global distribution but is a zoonotic disease of the poor people in developing countries mainly in sub-Saharan Africa (Fyumagwa et al. 2002; Kitala et al. 2002; East et al. 2005; Cleaveland et al. 2006; Talbi et al. 2009). In Tanzania rabies has reached an epidemic proportion after leaving the control program into private hands. Three rabies strains have been described in the Serengeti ecosystem; a hyenid strain which is 8.5% sequence divergence to the canid strain found in spotted hyenas, a canid strain found in domestic dogs, bat-eared foxes, white tailed mongooses (East et al. 2001; 2005) and a suspected bat strain (Ikoma virus) detected in African civet (*Civettictis civetta*) in SNP (Marson et al. 2012). Recently, the most extensive rabies surveillance has been conducted in South Africa where canid rabies strains have been found in several jackal species, some mongoose species (although a specific yellow mongoose strain also occurs), bat-eared foxes (*Otocyon megalotis*) and spotted hyenas (Swanepoel 1993; Bourhy et al. 2008; Talbi et al. 2009). The large domestic dog is the main reservoir of 'canid' rabies in Africa (Kitala et al. 2002; Talbi et al. 2009) and other wild carnivores have been described to contribute to the reservoir (Bourhy et al. 2008; Talbi et al. 2009). This is supported from the fact that cases of rabies that occur in species such as bat-eared foxes in the centre of the SNP during years when the level of rabies in domestic dogs surrounding the park is endemic are not easily explained in the absence of rabies maintenance in a wildlife species. However, the current knowledge of rabies in wildlife is insufficient to draw such a conclusion.

Malignant catarrhal fever (Alcelaphine herpes virus-1) is a fatal disease of cattle and in Africa is wildebeest associated. The virus is shed in nasal discharges of new born wildebeest calves and fetal membranes (Grootenhuis 2000). Malignant catarrhal fever is a major problem in years of drought because wildebeest are forced to go to pastoral grazing areas and contaminate the pasture for livestock (Fyumagwa 2010). In such years the short grass plains have shortage of forage, thus wildebeests are forced to extend their grazing range into highlands that would usually be utilized by Maasai cattle only. In 2000 for instance, 3'000 heads of cattle are estimated to have died from MCF (Fyumagwa 2010). Affected cattle is a dead

end host because the virus is not capable of cattle to cattle transmission only wildebeest to cattle transmission. The disease outbreak occurs when cattle come in contact with calving wildebeests an event, which happen when the migration is in Serengeti short grass plains, Maasai steppe in Simanjiro and other adjacent dispersal areas. However, Maasai pastoralists have learned to live with the disease; during wildebeest calving Maasai cattle are moved into highlands and abandon short grass plains despite their high nutritive value.

Local chicken is the main source of animal protein in majority of rural people; however, the poultry industry is faced with a highly fatal new-castle disease (*Paramyxovirus*). The disease decimates 45% of the poultry population (57 million) annually in Tanzania and viscerotropic form of the disease dominates followed by velogenic-viscerotropic form (Fyumagwa 2010). In 2006, thousands of free ranging laughing doves (*Streptopelia senegalensis*) died from *Paramyxovirus* in northern Tanzania and affected wild birds exhibited clinical symptoms similar to new-castle disease in poultry (Fyumagwa 2010). The disease was drought associated because many birds were congregating in small water sources and it declined following the onset of short rains that began in November 2006.

Rift Valley Fever (RVF) is an arthropod borne zoonotic viral disease affecting livestock (cattle, sheep, goats and camels), wildlife and humans caused by *Phlebovirus* (Evans et al. 2008). It is a transboundary disease and occurs in periodic cycles of 4-15 years associated with flooding from unusually high precipitations in many flood-prone drylands (Amwanyi et al. 2010; Munyua et al. 2010). *Aedes* and *Culex* spp and other mosquito species are important epidemic vectors (Sang et al. 2010). Because of poor living conditions and lack of knowledge on the pathogenesis of RVF, nomadic pastoralists and agro-pastoralists are at high risk of contracting the disease during epidemics (Munyua et al. 2010). RVF is a professional hazard for health and livestock workers because of poor biosafety measures in routine activities including lack of proper Personal Protective Equipment (PPE). Despite technological advancement and knowledge on the epidemiology of RVF and presence of enough trained manpower, 50 years after independence Tanzania like many at risk African countries is unable to control the disease in the event of an outbreak (Fyumagwa et al. 2011). The episodic nature of

the disease creates special challenges for its mitigation and control and many of the epidemics happen when the governments are not prepared and have limited resource to contain the disease at source.

Tuberculosis (TB) is an insidious zoonotic disease affecting livestock, wildlife and humans. *Mycobacterium bovis* has been reported in diary cattle, wildlife and humans in Tanzania (Cleaveland et al. 2005). *M. tuberculosis* has only so far been reported in human, however, with the global upsurge of TB in humans it is possible that *M. tuberculosis* can spill over from human to animals (Aranaz et al. 2004; Figuero-Munoz & Ramon-Pardo 2008). Screening of live animals can be performed using Gamma interferon assay in livestock and wildlife but the Intradermal Tuberculin test is only possible in confined animals not in free ranging wildlife. Because of its insidious nature, most TB cases in animals are detected at slaughter slabs during meat inspection or post mortem examination. Similarly, sporadic cases have been detected in wildlife including eland, buffalo, giraffe, wildebeest and lions (Cleaveland et al. 2005; Fyumagwa 2010).

Anthrax pathogen (*Bacillus anthracis*) is one of the ancient zoonotic diseases in the history of mankind that has no reservoir animal hosts rather is a free living micro-organism in the environment in the natural ecosystem in low land areas or under river beds or swamp base. The animals are infected when they stir up the mud or overgraze and ingest the exposed bacteria spores affecting domestic, wild animals and humans. Herbivores are more susceptible than carnivores that have high degree of resistance (Lembo et al. 2011). Among herbivores in the Serengeti ecosystem, zebra and impala are more susceptible while buffalo and wildebeest have shown some antibodies against anthrax suggesting that not all infected wild herbivores die from the disease (Fyumagwa 2010; Lembo et al. 2011). Anthrax does not penetrate intact skin; it is only a danger to those eating undercooked meat from dead animals a situation which is not uncommon among pastoral communities who have the culture of not disposing dead animals including anthrax cases. In many protected areas in Africa the disease is drought associated and the epidemics wane-off with the onset of rainfall. The wide variation in anthrax patterns suggest that several possible environmental, genetic and host related factors could be important determinants of infection (Lembo et al. 2011).

A number of other diseases have been reported in recent years in the Serengeti ecosystem including genital infection in olive baboons (*Papio anubis*) caused by *Treponema pallidum* (Knauf et al. 2011) and cyanobacteria toxins (anatoxin-a & microcystins) complicated by secondary bacterial infections leading to mass die-offs of lesser flamingoes in soda lakes in northern Tanzania (Fyumagwa 2010; Nonga 2011).

### **5.3 Conclusion**

The Serengeti ecosystem has the highest density of wild herbivores of any protected area in the country. High animal population in conjunction with the existing communal land tenure system is a constraint to prevention and control efforts of infectious diseases. Uncontrolled movements of livestock and wildlife exacerbate transmission of pathogens including bacterial, viral and vector-borne haemoparasites to new areas and to susceptible populations. This makes it difficult to effectively control disease spill-over and spill-back between livestock and wild animals.

The conservation strategy for this ecologically important ecosystem should focus on changes in habitat structure and land use; emergence and re-emergence of pathogens and the effects of environmental contaminants; maintenance of biodiversity and ecosystem function and the effect of diseases on rare or endangered species. However, many disease outbreaks occur in remote areas that are inaccessible. Lack of enough trained manpower to maintain vigilance in disease monitoring and surveillance is a constraint. Availability of reliable source of funding and equipment to conduct disease monitoring and surveillance is another obstacle. Some of the disease agents are living freely in the environment and many pathogens have reservoirs in species which are either ubiquitous or low in numbers making it difficult to understand their epidemiology. With such a vast protected area of about 370,000 km<sup>2</sup> (30% of the country's land cover) it is impossible to eradicate these diseases and they will remain a source of infection to susceptible animal populations. As wildlife populations become denser from habitat restriction and decline in resource availability, the risk of a catastrophic epidemic increases, which results in a decrease of population size.

#### **5.4. Recommendations**

- In order to minimize the risk for livestock and wildlife to succumb to unusual disease outbreaks in the interface from imbalanced ecosystem components, the establishment of a long-term ecological monitoring strategy for the ecosystem is strongly recommended.
- Prescribed burning should be maintained by the NCAA as a rangeland management tool for tick control and enhancement of vegetation structure, composition and quality.
- In order to understand the epidemiology of *S. equi subsp ruminatorum* it would be important to conduct a study to establish whether some of these domestic animals are infected or are free from the described bacterium in Maasai pastoralists.
- It will be necessary to study in future the question to what degree the disease control measures affect the prevalence of tick species and tick-borne haemoparasites as well as other infectious agents in the Serengeti ecosystem.



## 6.0 References

- Aguirre, A.A., & Gomez, A. 2009. Essential veterinary education in conservation medicine and ecosystem health: a global perspective. *Review Scientifique Technologique Office Internationale Epizootique* 28(2): 597-603.
- Alekseev, A.N. 1998. The possible consequences of the probable global warming of the climate for the spread of blood-sucking ectoparasites and the pathogens they transmit. *Medical Parasitology* 4: 3-8.
- Alexander, K.A., Kat, P.W., Munson, L.A., Kalake, A., Appel, M.J.G., 1996. Canine distemper-related mortality among wild dogs (*Lycaon pictus*) in Chobe National Park, Botswana. *Journal of Zoo Wildlife Medicine* 27: 426-427.
- Alexander, K.A., McNutt, J.W., Briggs, M.B., Stander, P.E., Funston, P., Hemson, G., Keet, D., van Vuuren, M., 2010. Multi-host pathogens and carnivore management in southern Africa. *Comparative Immunology and Microbiology of Infectious Disease* 33: 249–265.
- Amwanyi, A.S., Gould, H.L., Sharif, S.K., Nguku, P.M., Omolo, J.O., Mutonga, D., Rao, C.Y., Lenderman, E.R., Schnabel, D., Paweska, J.T., Katz, M., Hightower, A., Kariuki Njenga, M., Feikin, D.R., & Breiman, R.F. 2010. Risk Factors for Severe Rift Valley fever infection in Kenya, 2007. *American Journal of Tropical Medicine and Hygiene* 83(suppl 2): 14-21.
- Appel, M.J.G., Summers, B.A. 1995. Pathogenicity of morbilliviruses for terrestrial carnivores. *Veterinary Microbiology* 44: 187–191.
- Aranaz, A., Juan, L., Montero, N., Sa'nchez, C., Galka, M., Delso, C., lvarez, J.A., Romero, B., Bezos, J., Vela, A.I., Briones, V., Mateos, A. & Domínguez, L. 2004. Bovine tuberculosis (*Mycobacterium bovis*) in wildlife in Spain. *Journal of Clinical Microbiology* 42(6): 2602–2608.
- Barret T., Visser, I.K.G., Mamaev, L., Goatley, L., Van Bresseem, M.F., Osterhaus, A.D.M.E., 1993. Dolphin and porpoise morbilliviruses are genetically distinct from Phocine distemper virus. *Virology* 193: 1010-1012.

- Bourhy, H., Reynes, J.M., Dunham, E. J., Dacheux, L., Larrous, F., Huong, V.T.Q., Xu, G., Yan, J., Miranda, M.E.G., & Holmes, E.C. 2008. The origin and phylogeography of dog rabies virus. *Journal of General Virology* 89:2673-2681.
- Bugwesa, Z.K., Fyumagwa, R.D., Mdaki, M.R., Kuya, S., & Hoare, R. 2009. Seroprevalence of *Brucella abortus* in livestock-wildlife interface in Serengeti ecosystem. In: Proceedings of the 7<sup>th</sup> TAWIRI Scientific conference held on 2<sup>nd</sup> to 4<sup>th</sup> December, 2009 in Arusha, Tanzania. Pp. 54-62.
- Burrows, R., Hofer, H., & East, M.L., 1994. Demography, extinction and intervention in a small population: the case of the Serengeti wild dogs. *Proceedings of Royal Society of London, Series B* 256: 281-292.
- Carpenter, M.A., Appel, M.J.G., Roelke-Parker, M.E., Munson, L., Hofer, H., East, M.L., O'Brien, S.J., 1998 Genetic characterization of canine distemper virus in Serengeti carnivores. *Veterinary Immunology and Immunopathology* 65: 259-266.
- Chernysheva, M.I., Gubina, F.A., Zheludkov, M.M. & Perekopskaia, T.I. 1980. Use of acidic rose Bengal antigen in the plate agglutination test for brucellosis in humans. *Journal of Microbiology, Epidemiology and Immunology*.6: 84-88.
- Cleaveland, S., Kaare, M., Knobel, D., & Laurensen, K. 2006. Canine vaccination-providing broader benefits for disease control. *Veterinary Microbiology* 117: 43-50.
- Cleaveland, S., Mlengeya, T., Kazwala, R.R., Michel, A., Kaare, M.T., Jones, S.L., Eblate, E., Shirima, G.M., and Packer, C. 2005. Tuberculosis in Tanzanian Wildlife. *Journal of Wildlife Diseases* 41(2):446-453.
- Creel, S., Creel, N.M., Munson, L., Sanderlin, D., & Appel, M.J.G. 1997. Serosurvey for selected viral diseases and demography of African wild dogs in Tanzania. *Journal of Wildlife Disease* 33: 823-832.
- Daszak, P., Cunningham, A.A. & Hyatt, A.D. 2000. Emerging Infectious Diseases of Wildlife-Threats to Biodiversity and Human Health. *Review: Wildlife Ecology, Science Compass* 287: 443- 449.
- Deem, S.L., Karesh, W.B., & Weisman, W. 2001. Putting theory into practice: wildlife health in conservation. *Conservation Biology* 15(5): 1224-1233.

- Dobson, A.P., & Hudson, P.J. 1995. Microparasites; Observed patterns in wild animal populations. In: B.T. Grenfell & Dobson, A.P. (Eds). *Ecology of infectious diseases in natural populations*. Publications of the Newton Institute, Cambridge University Press. pp 52-89.
- East, M.L., Bassano, B., & Ytrehus, B. 2011. The role of pathogens in the population dynamics of European ungulates. *Ungulate Management in Europe: Problems and practices*. Eds. R. Putman, M. Apollonio & R. Anderson. Cambridge University Press 2011. Pp 319-348.
- East, M. L., Hofer, H., Cox, J. H., Wulle, U., Wiik, H., & Pitra, C. 2001. Regular exposure to rabies virus and lack of symptomatic disease in Serengeti spotted hyenas. *Proceedings of the National Academy of Science* 98: 15026-15031.
- East, M.L., Muller, T., Fyumagwa, R., Pitra, C. & Hofer, H. 2005. A rabies epizootic in rural domestic dog population close to the Serengeti national park, Tanzania, does not lead to an increase in the incidence of rabies in wild carnivores within the park. In: *Proceedings of the Institute for Zoo and Wildlife Research, Berlin*, 4-8 May 2005 Prague, Czech Republic.
- Estes, R.D., Attwood J.L., & Estes, A.B. 2006. Downward trends in Ngorongoro Crater ungulate populations: Conservation concerns and the need for ecological research *Biological Conservation* 106: 106-120.
- Evans, A., Gakuya, F., Paweska, J.T., Rostal, M., Akoolo, L., Van Vure, P.J., Manyibe, T., Macharia, J.M., Ksiazek, T.G., Feikin, D.R., Breiman, R.F., & Kariuki Njenga, M. 2008. Prevalence of antibodies against Rift Valley Fever Virus in Kenyan wildlife. *Epidemiology and Infection* 136: 1261-1269.
- Figuero-Munoz J. & Ramon-Pardo, P. 2008, Tuberculosis control in vulnerable groups. *Bulletin of the World Health Organization*, 86(9): 657-736.
- Fowler, J., Cohen, L. & Jarvis, P. 1998. *The Normal distribution and Regression Analysis. Practical statistics for field biology*, 2<sup>nd</sup> Edition. John Wiley and Sons, New York. Pp 74-82; 142-163.

- Fyumagwa, R.D. 2010. Diseases of economic and conservation significance in the livestock-wildlife interface in Tanzania. In: E.J.Gereta and E. Roskraft (Eds). Conservation of Natural Resources. Some African and Asian examples. Tapir academic press, Trondheim, Norway. Chapter 22, pp 419-444.
- Fyumagwa, R.D., Ezekiel, M.J., Nyaki, A., Mdaki, M.L., Katale, Z.B., Moshiro, C., & Keyyu, J.D. 2011. Response to Rift Valley Fever in Tanzania: Challenges and Opportunities. Tanzania Journal of Health Research 13(suppl.1), December 2011. DOI: [HTTP://dx.doi.org/10.4314/thrb.v13i5.3](http://dx.doi.org/10.4314/thrb.v13i5.3).
- Fyumagwa, R.D., Mkumbo, S. S. & Morkel, P. B. 2004. Remote treatment of black rhinos against babesiosis in Ngorongoro Crater, Tanzania. *Pachyderm* 37: 80- 83.
- Fyumagwa, R.D., & Nyahongo, J.W. 2010. Black rhino conservation in Tanzania: Translocation efforts and further challenges. *Pachyderm* 47(1): 59-65
- Fyumagwa, R.D., Wiik, H., & Kilewo, M. 2002. Rabies in Moshi rural district; an example of a disease conflict in the interface; In: Proceedings of the third TAWIRI Scientific conference, 2002, Arusha, Tanzania. Pp 90-95.
- Fyumagwa, R.D., Runyoro, V., Horak, I.G., & Hoare, R. 2007. Ecology and control of ticks as disease vectors in wildlife of the Ngorongoro Crater, Tanzania. *South African Journal of Wildlife Research* 37(1): 79-90.
- Fyumagwa, R.D., Simmler, P., Meli, M.L., Hoare, R., Hofmann-Lehmann, R. & Lutz, H. 2009a. Prevalence of *Anaplasma marginale* in different tick species from Ngorongoro Crater, Tanzania. *Veterinary Parasitology* 161: 154-157.
- Fyumagwa, R.D., Simmler, P., Willi, B., Meli, M.L., Sutter, A., Hoare, R., Dasen, G., Hofmann-Lehmann, R., & Lutz, H. 2008. Molecular detection of haemotropic *Mycoplasma* species in *Rhipicephalus sanguineus* tick species collected on lions (*Panthera leo*) from Ngorongoro Crater, Tanzania. *South African Journal of Wildlife Research* 38(2): 117-122.
- Fyumagwa, R.D., Wambura, P.N., Mellau, L.S.B. & Hoare, R. 2009b. Sero-prevalence of *Brucella abortus* in buffalo and wildebeest in the Serengeti ecosystem: a threat to human and animal health. *Tanzania Veterinary Journal* 26(2): 62-67.

- Goller, K.V., Fyumagwa, R.D., Nikolin, V., East, M.L., Kilewo, M., Speck, S., Müller, T., Matzke, M. & Wibbelt, G. 2010. Fatal canine distemper infection in a pack of African wild dogs in the Serengeti ecosystem, Tanzania. *Veterinary Microbiology* 146: 245-252.
- Goodman, J.L., Nelson, C., Vitale, B., Madigan, J.E., Dumler, J.S., Kurtti, T.J., Munderloh, U.G., 1996. Direct cultivation of the causative agent of human granulocytic ehrlichiosis. *New England Journal of Medicine* 334: 209-215.
- Grootenhuis, J.G. 2000. Wildlife, Livestock and Animal Disease Reservouirs, In: H.H.T. Prins, Grootenhuis, J.G. & Dolan, T.T. (Eds). *Wildlife Research for Sustainable Use*. Kluwer Academic Publishers, Dordrecht, The Netherlands. Pp 81-113.
- Gulland, F.M.D. 1995. The impact of infectious diseases on wild animal populations-a Review. In: B.T. Grenfell & Dobson, A.P. (Eds). *Ecology of infectious diseases in natural populations*. Publications of the Newton Institute. Cambridge University Press. Pp 20-51.
- Haas, L., Hofer, H., East, M., Wohlsein, P., Liess, B., Barrett, T. 1996. Canine distemper virus infection in Serengeti spotted hyaenas. *Veterinary Microbiology* 49: 147-152.
- Hall, T.A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series* 41: 95-98.
- Harlow, E. & Lane, D. (Eds.). 1996. *Antibodies- A Laboratory Manual*. Cold Spring Harbor Laboratory.
- Harrison, T.M., Mazet, J.K., Holekamp, K.E., Dubovi, E., Engh, A.L., Nelson, K., Van Horn, R.C., & Munson, L. 2004. Antibodies to canine and feline viruses in spotted hyenas (*Crocuta crocuta*) in the Masai Mara National Reserve. *Journal of Wildlife Disease* 40: 1-10.
- Hofer, H., & East, M.L. 2010. Impact of global climate change on wildlife hosts and their pathogens. *Nova Acta Leopoldina NF*. 111(381): 105-112.

- Höner, O.P., Wachter, B., Speck, S., Wibbelt, G., Ludwig, A., Fyumagwa, R.D., Wohlsein, P., Lieckfeldt, D., Hofer, H., East, M.L., 2006. Severe *Streptococcus* infection in spotted hyenas in the Ngorongoro Crater, Tanzania. *Veterinary Microbiology* 115: 223–228.
- Höner, O.P., Wachter, B., Goller, K.V., Hofer, H., Runyoro, V., Thierer, D., Fyumagwa, R.D., Muller, T., & East, M.L. 2011. The impact of a pathogenic bacterium on a social carnivore population. *Journal of Animal Ecology* DOI: 10.1111/j.1365-2656.2011.01873.x.
- Kennedy-Stoskopf, S. 1998. Carnivores. Emerging Viral Infections in Large Cats. In: M. E. Fowler and R. E. Miller (Eds). *Zoo and Wild Animal Medicine. Current Therapy* Vol. 4. W. B. Saunders Company, Philadelphia, USA. Pp 401- 410.
- Kiputa, V.P.S., Kimera, S.I. & Wambura, P.N. 2008. Studies on the role of trade cattle in the transmission of brucellosis in Karagwe district, Tanzania. *Tanzania Veterinary Journal* 25(1): 48-59.
- Kitala, P.M., McDermott, J.J., Colman, P.G., & Dye, C. 2002. Comparison of vaccination strategies for the control of dograbies in Machakos District, Kenya. *Epidemiology and Infection* 129:215-222.
- Knauf, S., Batamuzi, E.K., Mlengeya, T., Kilewo, M., Lejora, I.A.V., Nordhoff, M., Ehlers, B., Harper, K.N., Fyumagwa, R., Hoare, R., Failing, K., Wehrend, A., Kaup, F.J., Leendertz, F.H., & Ma'tz-Rensing, K. 2011. Treponema Infection Associated With Genital Ulceration in Wild Baboons. *Veterinary Pathology*. DOI: 10.1177/0300985811402839.
- Kumar, S., Tamura, K., Jakobsen, I.B., Nei, M., 2001. MEGA 2: Molecular Evolutionary Genetics Analysis Software. Arizona State University, Tempe, USA.
- Lembo, T., Hampton, K., Auty, H., Beasley, C.A., Bessell, P., Packer, C., Haliday, J., Fyumagwa, R., Hoare, R., Ernest, E., Mentzel, C., Mlengeya, T., Stamey, K., Wilkins, P.P., & Cleaveland, S. 2011. Serologic surveillance of Anthrax, Tanzania, 1996-2009. *Emerging Infectious Diseases* 17(3): 387-394.
- Liermann, H., Harder, T.C., Löchelt, M., von Messling, V., Baumgärtner, W., Moennig, V., Haas, L., 1998. Genetic analysis of the central untranslated genome

- region and the proximal coding part of the F gene of wildtype and vaccine distemper morbilliviruses. *Virus Genes* 17: 259-270.
- Marson, D.A., Horton, D.L., Ngeleja, C., Hampton, K., McElhinney, L.M., Banyard, A.C., Haydon, D., Cleaveland, S., Rupprecht, C.E., Bigambo, M., Fooks, A.R., & Lembo, T. 2012. Ikoma Lyssavirus, Highly Divergent Novel Lyssavirus in an African Civet. *Emerging Infectious Diseases* 18(4): 664-667.
- Mfunda, I.M. & Roskaft, E. 2011. Wildlife or crop production: the dilemma of conservation and human livelihoods in Serengeti, Tanzania. *International Journal of Biodiversity Science, Ecosystem Services & Management*. DOI:10.1080/21513732.2011.602028.
- Munson, L., Terio, K.A., Kock, R., Mlengeya, T., Roelke, M.E., Dubovi, E., Summers, B., Sinclair, A.R.E & Packer, C. (2008) Climate extremes promote fatal co-infections in African lions. *Plos One* 6, e2545. Doi: 10.1371/journal.pone.0002545.
- Munyua, P., Murithi, R.M., Wainwright, S., Githinji, J., Hightower, A., Mutonga, D., Macharia, J., Ithondeka, P.M., Musaa, J., Breiman, R.F., Bloland, P., Njenga, M.K. 2010. Rift Valley fever outbreak in livestock in Kenya, 2006-2007. *American Journal of Tropical Medicine and Hygiene* 83(suppl 2):58-64.
- Nielsen, N.O. 1995. Ecosystems health: application of the concept and wildlife as indicators. Proceedings of the Joint conference of the American Association of Zoo Veterinarians, Wildlife Diseases Association and American Association of Wildlife veterinarians, East Lansing, Michigan, USA, August 12-17, 1995.
- Nijhof, A.M., Penzhorn, B.L., Lynen, G., Mollé, J.O., Morkel, P., Bekker, C.P.J., & Jongejan, F. 2003. *Babesia bicornis* sp. Nov. and *Theileria bicornis* sp. Nov.: Tick-borne parasites associated with mortality in the black Rhinoceros (*Diceros bicornis*). *Journal of Clinical Microbiology* 41(5): 2249-2254.
- Nijhof, A.M., Pillay, V., Steyl, J., Prozesky, L., Stoltsz, W.H., Lawrence, J.A., Penzhorn, B.L., & Jongejan, F. 2005. Molecular characterization of *Theileria* species associated with mortality in four species of African antelopes. *Journal of Clinical Microbiology* 43: 5907-5911.

- Nonga, H.E. 2011. Impact of human activities in selected lake ecosystems in Tanzania and occurrences of microcystins and potential microcystins producing cyanobacteria. PhD Thesis. NVH, Oslo, Norway, 2011.
- Peterson, M.J. 1991. Wildlife parasitism, science and management policy. *Journal of Wildlife Management* 55: 782-789.
- Petrie, A., & Watson, P. 2004. The Chi-squared test comparing proportions, In: *Statistics for Veterinary and Animal science*. Blackwell Science Ltd, UK. Pp 101-113, 202.
- Pfützer, S., Last, R., & De Waal, D.T. 2004. Death of a buffalo calf (*Syncerus caffer*) due to suspected heart water (*Ehrlichia ruminantium*): Clinical communication. *Journal of South African Veterinary Association* 75 (1): 54-57.
- Pomeroy, L., Björnstad, O., Holmes, E. 2008. The evolutionary and epidemiological dynamics of the paramyxoviridae. *Journal of Molecular Evolution* 66: 98–106.
- Roelke-Parker, M.E., Munson, L., Packer, C., Kock, R., Cleaveland, S., Carpenter, M., O'Brien, S.J., Popischil, A., Hofmann-Lehmann, R., Lutz, H., Mwamengele, G.L.M., Mgasia, M.N., Machange, G.A., Summers, B., & Appel, M.J.G. 1996. A canine distemper virus epidemic in Serengeti lions (*Panthera leo*). *Nature* 379: 441-445.
- Runyoro, V.A., Hofer, H., Chausi, E.B., & Moehlman, P.D. 1995. Long-term trends in the herbivore populations of the Ngorongoro Crater, Tanzania. In: A.R.E. Sinclair and Peter Arcese (Eds.). *Serengeti II, Dynamics, management and conservation of an ecosystem*. The University of Chicago Press, Chicago, USA. Pp 146-68.
- Sang, R., Kioko, E., Lutomiah, J., Warigia, M., Ochieng, C., O'Guinn, M., Lee, J.S., Koka, H., Godsey, M., Hoel, D., Hanafi, H., Miller, B., Schnabel, D., Breiman, R.F., & Richardson, J. 2010. Rift Valley fever virus epidemic in Kenya, 2006/2007: the entomological investigations. *American Journal of Tropical Medicine and Hygiene* 83(suppl 2): 28-37.
- Schaller, G.B., 1972. *The Serengeti Lion*. Chicago University Press, Chicago. pp. 327.
- Short, N.J. & Norval, R.A.I. 1981. The seasonal activity of *Rhipicephalus appendiculatus* Neum. 1901 (*Acarina: Ixodidae*) in the highveld of Zimbabwe Rhodesia. *Journal of Parasitology* 67: 77-84.



- Sinclair, A.R.E. 1973. Population increase of buffalo and wildebeests in the Serengeti. *East African Wildlife Journal* 11: 93-107.
- Sinclair, A.R.E. 1995. Serengeti past and present, In: A.R.E Sinclair & Peter Arcese (Eds). *Serengeti II, Dynamics, management and conservation of an ecosystem*. The University of Chicago Press, Chicago, USA. pp 3-30.
- Skinner, D. 2010. Report: Seventh meeting of the IUCN/SSC African Elephant Specialist Group. Climate change and payments for ecosystem services. *Pachyderm* 47(1): 90-92.
- Speck, S., Höner, O.P., Wachter, B.& Fickel, J. 2008. Characterization of *Streptococcus equi* subsp. *ruminatorum* isolated from spotted hyenas (*Crocuta crocuta*) and plains zebras (*Equus burchelli*), and identification of a M-like protein (SrM) encoding gene. *Veterinary Microbiology* 128: 148-159.
- Swanepoel, R., Barnard, B. J., Meredith, C. D., Bishop, G. C., Bruckner, G. K., Foggin, C. M. & Hubschle, O. J. 1993. Rabies in southern Africa. *Onderstepoort J Vet Res* 60, 325–346.
- Swofford, D.L. 2002. PAUP\*: Phylogenetic Analysis Using Parsimony (\* and Other Methods). Version 4.0b.10. Sinauer Associates, Sunderland, USA.
- Sykes, J.E., Ball, L.M., Bailiff, N.L. & Fry, M.M. 2005. ‘*Candidatus Mycoplasma haematoparvum*’ a novel small haemotropic *Mycoplasma* from a dog. *International Journal of Systematic Evolution and Microbiology* 55: 27-30.
- Talbi, C., Holmes, E.C., de Benedicts, P., Faye, O., Nakuone, E., Gamatie, D., Diarra, A., Elmamy, B.O., Sow, A., Adjogoua, E.V., Sangare, O., Dundon, W.G., Capua, I., Sall, A.A. & Bourhy, H. 2009. Evolutionary history and dynamics of dog rabies virus in western and central Africa. *Journal of General Virology* 90: 783-791.
- Tamura, K., Dudley, J., Nei, M., Kumar, S., 2007. MEGA4: Molecular evolutionary genetics analysis (MEGA) software version 4.0. *Molecular Biology and Evolution* 24: 1596-1599.
- Thomson, L.G., Mosely-Thomson, E., Davis, M.E., Hardman, K.A., Brecher, H.L., Zagorodnov, V.S., Mashiotto, T.A., Ping-Nan, L., Mikhalenko, V.N., Hardy, D.R. & Beer, J. 2002. Kilimanjaro ice core research: Evidence of Holocene climate change in tropical Africa. *Science* 298: 589-593.

- Trollope, W.S.W. & Potgieter, A.L.F. 1986. Estimating grass fuel loads with a disc pasture meter in Kruger National Park. *Journal of Grassland Society of South Africa* 3: 148-152.
- Ubukata, H. 2000. The impact of global warming on insects. A threat to life. In: Domoto, A., Iwatsuki, K., Kawamichi, T., & McNeely, J.A. (Eds). *The impact of climate change on Japan's Biodiversity*. Tsukiji-Shokan Publishing Co. & IUCN, Tokyo & Cambridge, UK. Pp 61-70.
- Van de Bildt, M.W.G., Kuiken, T., Visee, A.M., Lema, S., Fitzjohn, T.R., Osterhaus, A.D.M.E., 2002. Distemper outbreak and its effect on African wild dog conservation. *Emerging and Infectious Diseases* 8: 211-213.
- Walker, A.R., Bouattour, A., Camicas, J.L., Estrada-Pena, A., Horak, I.G., Latif, A.A., Pegram, R.G., & Preston, P.M. 2003. *Ticks of Domestic Animals in Africa: a Guide to Identification of Species*. Bioscience Reports, 42 Comiston Drive, Edinburgh EH10 5QR, Scotland, U.K. Pp 1-221.
- Walter, S.D., Hildreth, S.W. & Beaty, B.J. 1980. Estimation of infection rates in populations of organisms using pools of variable size. *American Journal of Epidemiology* 112: 124-128.
- Willi, B., Boretti, F.S., Baumgartner, C., Tasker, S., Wenger, B., Cattori, V., Meli, M.L., Reusch, C.E., Lutz, H., & Hofmann-Lehmann, R. 2006. Prevalence, risk factor analysis and follow-up of infections caused by three feline haemoplasma species in cats in Switzerland. *Journal of Clinical Microbiology* 44: 961-969.
- Willi, B., Boretti, F.S., Cattori, V., Tasker, S., Meli, M.L., Reusch, C.E., Lutz, H., & Hofmann-Lehmann, R. 2005. Identification, molecular characterization and experimental transmission of a new haemoplasma isolate from a cat with haemolytic anaemia in Switzerland. *Journal of Clinical Microbiology* 43(6): 2581-2585.
- Wilson, M.E., 2000. Environmental change and infectious diseases. *Ecosystem health* 6: 7-11.
- Woodroffe, R. 2001. Assessing the risk of intervention: immobilization, radio-collaring and vaccination of African wild dogs. *Oryx* 35: 234-244.

Wyss, C., Choi, B.K., Schupbach, P., Guggenheim, B., Göbel, U.B., 1996.  
Treponema maltophilum sp. nov., a small oral spirochete isolated from human  
periodontal lesions. International Journal of Systematic Bacteriology 46: 745–752.



Paper *i*



## FIELD NOTES

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### Remote treatment of black rhinos against babesiosis in Ngorongoro Crater, Tanzania

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#### Abstract

Higher than usual mortality among herbivores in the Ngorongoro Crater was noted beginning in March 2000. In August 2000 a year-old female black rhino (*Diceros bicornis*) was found dead in the crater floor. The carcass was heavily infested with ticks. On 5 and 14 January 2001 two adult resident female rhinos died. The cause of death was suspected to be babesiosis, a tick-borne disease. Microscopic examination of blood smears from the dead rhinos revealed intra-erythrocytic parasites resembling *Babesia* sp. Tissue samples from dead animals analysed using polymerase chain reaction identified *Babesia bicornis*, a previously unknown blood parasite. The life cycle of the parasite is not yet known. The death of the rhinos from tick-borne disease was associated with stress from starvation due to severe drought in the preceding months concurrent with high tick infestation. Two rhinos died from the same disease syndrome. Heavy tick burden at that time was noticeable in both wild animals and Maasai livestock, which are permitted to graze in the crater. Therapeutic treatment of rhinos was undertaken by remote means (darting) using diminazine aceturate (Berenil) to apparent good effect.

#### Résumé

Au début de mars 2000, on a noté une mortalité plus élevée que d'habitude parmi les herbivores du Cratère du Ngorongoro. En août 2000, on a trouvé le cadavre d'un rhino noir (*Diceros bicornis*) femelle âgé d'un an dans le cratère. La carcasse était très infestée de tiques. Le 5 et le 14 janvier 2001, deux rhinos femelles adultes résidentes sont mortes. On suspecta que la cause de ces décès était la babésiose, une maladie transmise par les tiques. L'examen microscopique de prélèvements sanguins effectués chez les rhinos morts a révélé des parasites des globules rouges ressemblant à *Babesia* sp. L'analyse de tissus des animaux morts par une réaction de polymérisation en chaîne a permis d'identifier *Babesia bicornis*, un parasite du sang inconnu jusqu'alors. Le cycle de reproduction du parasite n'est pas encore connu. La mort des rhinos à cause d'une maladie due aux tiques fut liée au stress de la faim causée par la sécheresse sévère des mois précédents, associée à une forte infestation de tiques. Deux rhinos sont morts de la même maladie. Une forte infestation de tiques était visible à cette époque aussi bien chez les animaux sauvages que chez le bétail des Masai qui peut paître dans le cratère. Le traitement thérapeutique des rhinos entrepris par des moyens utilisés à distance (fléchettes) comprenait du Diminazène (Bérénil) et semble avoir eu un effet bénéfique.

## Introduction

Reports of mortality among buffalo and other herbivores in Ngorongoro Crater began in March 2000 (Fyumagwa 2001). The dead animals were heavily infested with ticks but investigators initially suspected rinderpest as the killer due to past experience in the crater. Laboratory analysis performed at the Animal Disease Research Institute (ADRI), Dar es Salaam, Tanzania, and Pirbright, UK, of serum samples collected from 10 immobilized buffalo in the crater ruled out rinderpest (Wiik 2000). Since it is believed that wildlife is fairly resistant to tick-borne diseases (Grootenhuys 2000), ticks were not initially suspected to be a contributing factor in the massive die-off. However, it is known that vector-borne disease epidemics can occur when vector numbers increase due to environmentally favourable conditions or the presence of alternative host species, which amplify vector abundance (Dobson and Hudson 1995).

In 2000 rainfall was below average, resulting in a severe drought in the entire northern zone of Tanzania. Therefore when rinderpest was ruled out, efforts to continue looking for the cause of deaths ceased because it was thought that mortality was a result of starvation. In May 2000 lions took a black rhino calf that was born in the crater floor after her mother (Zakia) failed to protect it. Little attention was paid to the mother's health because the predation was thought to be attributable to the naivety of the mother, who came from Addo Elephant National Park in South Africa where there are no lions.

In early August 2000 a year-old female rhino (Papageno's calf) was found dead with heavy tick infestation. The veterinary staff at Ngorongoro Conservation Area Authority (NCAA) conducted a post-mortem but did not take tissue specimens or blood smears. Meanwhile buffalo and other herbivores continued to die and many carcasses were seen stuck in mud near water sources. This observation led to the suspicion that starvation was the sole cause of the wildlife mortalities. Surprisingly Serengeti National Park, which is adjacent to NCAA, did not record any high mortality among herbivores. In mid-August 2000 an adult female rhino (Zakia, who had just lost her calf) was found dead in the thick bush with her horns intact, thus ruling out poaching as the cause. Unfortunately the carcass was seen when it was at an advanced stage of decomposition and diagnostic samples were not collected.

In early December 2000, two adult resident female rhinos (Fausta and Vicky) exhibited symptoms of red urine and anorexia. In view of the recent rhino mortality over the last few months, NCAA requested a thorough investigation into the condition. Tanzania Wildlife Research Institute (TAWIRI) and NCAA veterinarians went to assess the condition of the two rhinos. This paper discusses the clinical post-mortem examination, results from two animals and treatment that the authorities took to safeguard the remaining small population of rhinos in the crater.

## Materials and methods

### *Clinical examination*

The two sick rhinos were seen to be lethargic and anorexic. They passed frothy red urine, and their faeces were unusually dark, almost black. The two affected animals spent an unusual amount of time lying down. After showing these signs for almost two weeks these two rhinos recovered spontaneously. In early January 2001 two adult female rhinos who were original crater residents (Maggie and Bahati) died nine days apart, on 5 and 14 January. Both exhibited similar symptoms of lethargy, anorexia and red urine. These animals both died the same day that the game rangers noticed the symptoms.

### *Post-mortem examination*

The gross pathology in the necropsied rhino carcasses included jaundice in the liver and other visceral organs, frothing in the trachea, bronchi and bronchioles, anaemia, enlarged spleen, haemoglobinuria and dark intestinal contents (melena). Blood smears, brain specimens and lymph node biopsies were collected. Ticks collected from the dead animals were identified as *Amblyomma gemma*, *A. sparsum*, *A. tholoni*, *A. variegatum* and *Rhipicephalus compositus* (Horak 2001; Nijhof et al. 2003). To identify the *Babesia* species involved, blood smears and brain and spleen impression smears were sent to ADRI in Dar es Salaam and duplicates to Utrecht University (Netherlands) for diagnosis using polymerase chain reaction (PCR), a technique that amplifies genetic material enabling identification of the parasites.

### *Treatment*

Following the provisional diagnosis, it was decided in January 2001 to provide therapeutic cover for the re-



maining rhinos in the crater as an immediate temporary solution to the problem. Diminazine aceturate (Berenil™ Hoechst) was used to treat the rhinos. Berenil has the advantage of being stable, has a wide safety margin and can be concentrated into a small volume to fit in the 3-ml darts. It has also been administered to black rhino in South Africa without obvious side effects (P. Morkel 2001, pers. obs.). For the injections, 2.36 g Berenil powder was mixed with 1.3 ml sterile water (3 Berenil sachets made up 5 ml of the suspension) and a maximum of 3 ml Berenil suspension was injected per animal. The rhinos received a dose of 2–3 mg kg<sup>-1</sup> intramuscularly in the neck region. By this time it had started raining and parts of the crater were flooded. Remote injection avoided the stress that might have resulted from immobilizing the rhinos and hand-injecting the drug when they were possibly recovering from drought-related stress.

## Results

### Diagnosis

The blood smear samples that NCAA veterinary staff submitted to ADRI and those examined on site by NCAA and TAWIRI veterinarians revealed numerous intra-erythrocytic parasites resembling *Babesia* spp. Results confirmed by PCR revealed that the rhinos were infected with a blood parasite, *Babesia bicornis* (Nijhof et al. 2003). The parasite is tick-borne, but the tick species that transmit it are not yet known. Concurrent with the rhino deaths, high tick infestation with a number of different tick species (Fyumagwa and Wiik 2001) was recorded on the crater grassland.

### Treated animals

The crater rhinos were treated from 24 to 29 January 2001, with 10 out of 13 animals treated in the operation. Berenil suspension was administered from a distance of approximately 40 to 50 m in nine rhinos; one juvenile rhino was remotely treated at a distance of approximately 15 m while it was moving. The 3-ml darts used had uncollared 40-mm needles as this type of dart falls out easily after its contents are discharged. A Dan-Inject dart gun (JM model) was used; it is quiet and fires relatively light and atraumatic darts.

The post-treatment response was good and no reactions were seen at the injection sites except that

one rhino developed an abscess. Fortunately the abscess, in the neck, drained by itself and within two months it was healed.

The three animals that were not treated were orphan calves that were wary and could not be approached, possibly due to the recent loss of their mothers. It was recommended that they be monitored from a distance to see if they formed associations with adults, which would protect them from predators. As their stress levels were probably very high it was also important to monitor their condition in general and watch for clinical symptoms of disease.

## Discussion

The disease outbreak among the rhino population in NCA is a newly reported phenomenon. The death of the rhinos was most probably a result of infection with *Babesia bicornis* (Nijhof et al. 2003), which was opportunistic as a result of immunosuppression brought on by stress from severe drought in the preceding months and concurrent with a high level of tick infestation in the crater grassland (Fischer-Tenhagen et al. 2000). Thus the evidence suggests that latent infection with a stress trigger is more likely than sudden exposure to a naïve infection due to poor immune response (Gulland 1995).

It can be argued that conditions of high tick density in the crater and large populations of almost sedentary wild animals in a small area (260 km<sup>2</sup>) led to an increase in the prevalence of ticks acting as vectors for blood parasites. Exposure to infection probably increased in rhinos due to sharing the habitat with other wildlife and domestic animals (Fischer-Tenhagen et al. 2000). The total number of rhinos that died from babesiosis is most likely two: the adult cows that died in January 2001. The death of the year-old female calf that died in August 2000 remains unexplained.

A build-up of tick numbers in the crater has been observed since the *El Niño* phenomenon of 1997/98 (R.D. Fyumagwa 2001, unpublished data; Cosmas Soombe 2002, pers. comm.). The tick burden was noticeable on animals and in grassland in the crater, where ideal conditions apparently exist for vector-borne diseases (Dobson and Hudson 1995). There was a concurrent increase in tick-borne diseases, especially East Coast fever, anaplasmosis and babesiosis, in cattle that were brought into the crater to water, salt lick and graze (Fyumagwa 2001). The disease called ‘ormilo’ by the

Maasai (cerebral theileriosis) emerged in cattle at the same time that wild herbivores were dying in large numbers (J.O. Mollel 2002, pers. comm.).

## Recommendation

As prophylactic veterinary treatment does not allow immunity to develop it is not usually recommended in wildlife. But the course of action decided upon and taken in this case was to counter the disease threat to a small population of endangered and very valuable animals. It is therefore important in future to actively monitor conditions that might predispose to a recurrence of this problem: poor nutrition, induced by drought followed by heavy rains, and the associated high levels of ticks.

## Acknowledgement

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## References

- Dobson, A.P., and Hudson, P.J. 1995. Microparasites: observed patterns in wild animal populations. In: Grenfell, B.T., and Dobson, A.P., eds., *Ecology of infectious diseases in natural populations*. Cambridge University Press, Cambridge. p. 52–89.
- Fischer-Tenhagen, C., Hamblin, C., Quandt, S., and Folich, K. 2000. Serosurvey for selected infectious disease agents in free-ranging black and white rhinos in Africa. *Journal of Wildlife Diseases* 36(2):316–323.
- Fyumagwa, R.D. 2001. Investigation into the cause of disease outbreaks and wildlife mortalities in the Ngorongoro Conservation Area. Report submitted to the Ngorongoro Conservation Area Authority. Unpublished.
- Fyumagwa, R.D., and Wiik, H. 2001. Tick infestation in the Ngorongoro Crater grassland, In: Proceedings of the TAWIRI Annual Scientific Conference held at Impala Hotel, Arusha, 4–6 December 2001. p. 205–211. Unpublished.
- Grootenhuis, J.G. 2000. Wildlife, livestock and animal disease reservoirs. In: Prins, H.H.T., Grootenhuis, J.G., and Dolan, T.T., eds., *Wildlife conservation by sustainable use*. Kluwer Academic Publishers, Dordrecht, The Netherlands. p. 81–113.
- Gulland, F.M.D. 1995. Impact of infectious diseases on wild animal populations: a review. In: Grenfell, B.T., and Dobson, A.P., eds., *Ecology of infectious diseases in natural populations*. Cambridge University Press, Cambridge. p. 20–51.
- Horak, I.G. 2001. Free-living ticks collected from the vegetation in the Ngorongoro Crater, Tanzania. Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, University of Pretoria, Onderstepoort 0110 South Africa. p. 1–8. Unpublished report.
- Nijhof, A.M., Penzhorn, B.L., Lynen, G., Mollel, J.O., Morkel, P., Bekker, C.P.J., and Jongejan, F. 2003. *Babesia bicornis* sp. Nov. and *Theileria bicornis* sp. Nov.: Tick-borne parasites associated with mortality in the black rhinoceros (*Diceros bicornis*). *Journal of Clinical Microbiology* 41(5):2249–2254.
- Wiik, H. 2000. TAWIRI veterinary programme. annual report, 2000. Tanzania Wildlife Research Institute, Arusha. Unpublished report.

Paper ii



# Ecology and control of ticks as disease vectors in wildlife of the Ngorongoro Crater, Tanzania

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**Wild mammals in Africa mostly have high levels of innate resistance to haemoparasites and the tick vectors that transmit them. Occasionally though, biotic and abiotic factors combine to alter this relationship and tick-borne disease is diagnosed in wildlife. We postulate an inter-relationship between anthropogenic and natural factors that resulted in wildlife mortality, attributable to disease transmission associated with a gradual build-up of large numbers of ticks. Suppression of grassland fire for 27 years in a distinct ecological unit promoted a gradual expansion of areas covered by tall grass. Changes in composition of the pasture led to improved tick survival, which was further boosted by the availability of increasing numbers of a coarse-grazing species and preferred tick host, African buffalo. Alternating climatic cycles then appeared to precipitate an outbreak of tick-borne haemolytic disease by subjecting ticks and their herbivore hosts to ideal conditions (in wet years) followed by starvation and immune suppression (in dry years). Evidence supporting the hypothesis was gathered retrospectively in the present study through systematic sampling of tick density and correlating life stages of ticks to season, grass species and height of the grass sward. Tick host preference was noted by collection from immobilized wild animals and sympatric livestock. A long series of census data confirmed the changing composition of resident wild herbivores in the Ngorongoro Crater. To reduce the tick challenge, prescribed burning of the crater grassland was reintroduced; tick numbers fell rapidly and three years of subsequent monitoring confirmed the success of this strategy.**

**Keywords:** tick density, buffalo, climate variation, fire, wildlife diseases.

## INTRODUCTION

Ngorongoro Crater has very high diversity, densities (>100 ungulates/km<sup>2</sup>) and biomass (11 000 kg/km<sup>2</sup>) of African wildlife (Runyoro *et al.* 1995; Estes *et al.* 2006). This combined with easy accessibility, small size, good visibility and the presence of rare species makes it a prime African game-viewing destination, thereby earning enormous revenues from tourism. Any health problems in wildlife species are likely to be visible and of great concern to management authorities because of the extreme sensitivity of the high fee-paying tourism industry.

Severe infestations of ticks and biting flies on wild animals were noticed in the Ngorongoro Crater during 2000 and 2001 (Fyumagwa *et al.* 2004). Animals in very poor condition died in large numbers – an estimated 1500 African buffalo

(*Syncerus caffer*); hundreds of wildebeest (*Connochaetes taurinus*); smaller numbers of other grazers (Estes *et al.* 2006) – but due to a lack of on-site veterinary capacity, disease diagnosis went unconfirmed. Once rinderpest virus was ruled out, deaths of common herbivores were assumed to be from starvation during a drought, so tick-borne disease was largely overlooked (Fyumagwa *et al.* 2004).

In 2001 the small population of lions (*Panthera leo*) in the crater declined markedly; they were plagued by biting flies and appeared unhealthy. Some lion deaths in 2001 were investigated and revealed tick-borne haemoparasites (*Ehrlichia* spp., *Babesia* and *Theileria* spp.; M. Meli *et al.*, pers. comm., 2002), but since contagious canine distemper virus was also implicated (Kissui & Packer 2004) there appeared to be co-infection, producing a situation where interpretation of mortality causes remains unresolved.

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One of Tanzania's four small populations of black rhinoceros (*Diceros bicornis*) occurs in Ngorongoro Crater where, unusually for this species, they frequent open grassland on the crater floor. Once illness and mortality was suddenly noticed in this extremely rare and valuable species in 2001, the alarm was raised. In total four rhinos died, of which two were observed sick and fully investigated post mortem. A severe haemolytic syndrome was seen and subsequently a new species of intra-erythrocytic protozoan parasite (*Babesia bicornis*) was identified and implicated as the tick-borne cause of death (Nijhof *et al.* 2003). This situation prompted a very unusual veterinary intervention, in which the remainder of the small rhino population (10) was treated with a curative (babesicidal) drug, delivered remotely via darts (Fyumagwa *et al.* 2004). The intervention was successful but a debate ensued as to whether these micro-parasites were indigenous to Ngorongoro or introduced, as two rhinos had previously been translocated from South Africa. Rhino deaths from tick-borne disease were the main catalyst for the present study of tick ecology.

The present study (i) identified ticks, (ii) quantified tick density and distribution in relation to grassland composition and structure, and (iii) examined tick/grass/host associations. Ecological factors preceding and predisposing to the deaths of rhinoceros, lion, buffalo and other herbivores were wide-ranging and disparate. We propose a slow inter-relationship between suppression of fire, changes in pasture composition, changes in resident herbivore populations and climatic variation that progressed for nearly 30 years until it precipitated an eruption of tick-borne disease.

Once mortality from tick-borne disease was confirmed in one species and suspected in others, grassland ecologists assessed the condition of the pasture in 2001 and recommended reintroducing controlled burning to reduce the tick challenge (W.S.W. Trollope & L.A. Trollope, pers. comm, 2001). The present study concluded after evaluating suppressant effects of the prescribed burning regime on tick populations.

## MATERIALS AND METHODS

### Study area

The Ngorongoro Conservation Area (NCA) encompasses a multiple land use area of about 8300 km<sup>2</sup> lying at the western edge of the Great Rift Valley in the Arusha region, northern Tanzania,

and is populated by wildlife, livestock and people, the latter mostly Maasai pastoralists. The Ngorongoro Crater (03°10'S, 35°35'E), the world's largest intact, inactive caldera occupies approximately 300 km<sup>2</sup> (4%) of the total NCA. The rim is 2200m above sea level and the crater floor (250 km<sup>2</sup>) is about 600 m below, composed predominantly of grassland with small patches of swamp and *Acacia* riverine forest. Although a geographically distinct unit, it is part of the greater Serengeti ecosystem (25 000 km<sup>2</sup>) and regarded 'to some extent' as an ecologically distinct unit (Runyoro *et al.* 1995).

### Tick sampling

To quantify the tick population in its different habitats, 24 initial transects were established using GPS coordinates and local names. Each transect had a diameter of 200 m. Transects were in all types of grassland, including areas where the Maasai graze their cattle, areas with tall grass mainly preferred by buffalo, areas with short grass dominated by smaller herbivores, tall grass near swamps and preferred by buffalo and elephants, and in patches of grass in the *Acacia* forest which is a refuge for black rhino and elephants (locations in Appendix 1). After prescribed burning commenced in late 2001, six additional transects were selected where tick density could be compared in adjacent burned and unburned treatments.

Tick collection was carried out in all study transects over 2–3 days every month. Removal sampling for adult ticks and drag sampling for immature ticks (larvae and nymphs) as previously described by Short & Norval (1981), were used with some modifications (below). Data collection was continued for a total of 34 months in the initial 24 transects (from March 2001 to January 2004) and for 27 months in the additional six transects (from October 2001 to January 2004).

### Adult tick collection

*Questing ticks.* Removal sampling of adult ticks was done by throwing a metre square steel quadrant at random within each of the 30 transects of 200 m diameter and the ticks within the square were collected. This procedure was repeated nine times in each transect, and the ticks from each collection were stored separately in 70% ethanol in 10 ml labelled silicon tubes. Adult tick density was calculated by dividing the total number of ticks collected each month by the number of quadrants thrown. Adult *Amblyomma* and *Hyalomma* species

usually quest for hosts from the ground, and ticks of both genera are apparently more active at night (Walker *et al.* 2003). Consequently these ticks were collected from grass litter or from the road surfaces in the early morning, or from immobilized animals.

*Non-questing ticks.* Adult *Rhipicephalus (Boophilus)* species and those of *Rhipicephalus evertsi evertsi* were collected from immobilized animals in 2004.

#### *Immature tick collection*

The larvae of and nymphs several tick species quest for hosts from the vegetation, and can be collected by 'drag-sampling' – dragging a flannel cloth at a constant speed over the pasture. The drag apparatus used in the present study consisted of five white flannel strips, each 10 cm wide and 100 cm long adjacent to one another and attached at one end to a wooden spar. A dragging cord was attached to the ends of the spar. Each drag of 50 m length was repeated nine times in each of the 30 transects of 200 m diameter. Immature ticks were recovered from the flannel strips by means of jeweller's forceps, counted and the tick density per square metre of vegetation calculated by dividing the total number of immature ticks collected by the total surface area covered by the 0.5 × 50 m long drag-samples.

#### *Tick collection from hosts*

In 2004/05 direct tick collection from hosts was carried out on 47 animals of five herbivore species using chemical immobilization (buffalo, wildebeest, zebra, Grant's and Thomson's gazelle). In addition, four domestic species (cattle, sheep goat and donkey;  $n = 125$ ) were similarly sampled in those Maasai 'bomas' from which cattle are still herded into the crater. Ticks were also collected during post mortem of two dead black rhinos in 2001 and two dead elephants in 2005.

#### *Tick identification*

The adult ticks in each silicone tube were poured separately into a Petri dish and with the help of a dissecting microscope, counted and identified, using the descriptions of Walker *et al.* (2003). No attempt was made to identify the immature ticks (Appendix 2).

#### *Vegetation structure*

In March 2001, at the commencement of the study, the canopy height of the grass sward and the corresponding quantity of grass material

(grass fuel load) in kg/ha was determined in each transect by means of a tape measure and Disc Pasture Meter (DPM), respectively (Trollope & Potgieter 1986). The predominant grass and herb species within the sampling locality were also recorded. During subsequent monthly data collections only the canopy height was measured and linear regression was used to establish the corresponding grass fuel load (W.S.W. Trollope and L.A. Trollope, pers. comm., 2001).

#### *Prescribed burning*

The management authority followed a recommendation to burn areas of grassland with a fuel load of >4000kg/ha (W.S.W. Trollope and L.A. Trollope, pers. comm., 2001; W.S.W. Trollope *et al.*, pers. comm., 2002) starting from September 2001 and repeated annually in September/October, the late dry season. Around 10–20% of the area of the crater floor is burnt on a rotational basis every year in separate blocks, situated between tourist roads acting as firebreaks.

#### *Statistical analysis*

A linear regression was used to convert the mean canopy height of the grass sward into the corresponding mean disc height and therefore mean grass fuel load in kg/ha as described by (W.S.W. Trollope and L.A. Trollope, pers. comm., 2001). Associations between the number of adult ticks in tall grass ( $\geq 50$  cm) and in short grass (<50 cm) during the wet season, or the number of immature ticks in less grazed (>20 cm) and heavily grazed (<20 cm) vegetation in the dry season, were tested by means of a Student's *t*-test. A non-parametric Wilcoxon test statistic (*T*) was used to compare the associations between tick density and grass height in burned and unburned areas. The criterion for judging the level of significance in both analyses was a 95% confidence interval (CI) (Fowler *et al.* 1998).

## RESULTS

### **Seasonal tick density in the unburned grassland**

As expected, immature ticks were far more numerous than adults and each life stage varied greatly with season. Before burning in 2001, most adult ticks were present in the wet season between March and June and most immature ticks were present during the peak dry season in September and October (Fig. 1). The mean tick counts from various localities in the crater in relation to season



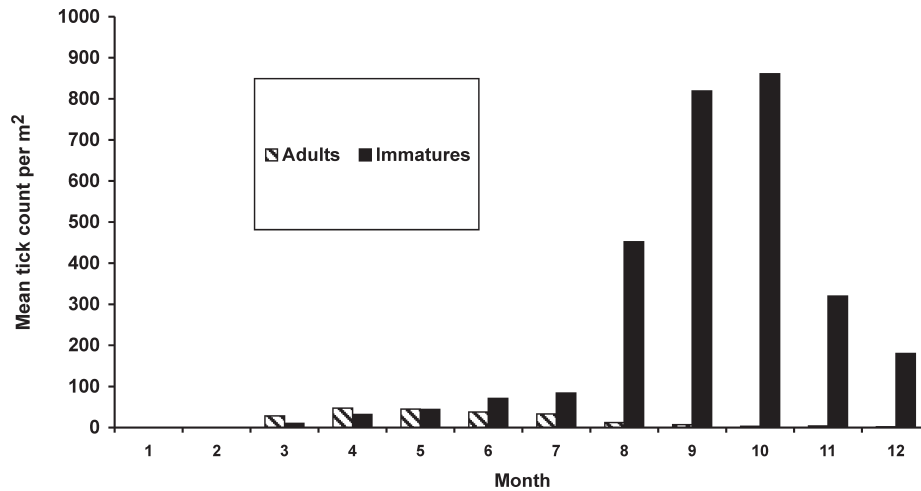


Fig. 1. Monthly mean density of adult and immature ticks in the unburned grassland of the Ngorongoro Crater during 2001 (*i.e.* before prescribed burning).

and grass height are summarized (Table 1). Using the same method of removal and drag sampling, the tick score from the crater rim and from the neighbouring plains was zero. On the rim near habitation, grass cover is often overgrazed, trampled or burnt by Maasai, while the plains are arid with very short grasses.

The difference in immature tick densities in both habitats between dry and wet season was highly significant ( $P < 0.001$ ), with more immature ticks in the dry season. In areas with tall grass in the preceding wet season, larger numbers of immature ticks were collected in the dry season in lightly grazed than in heavily grazed areas. The difference in adult tick density during the wet season between areas with tall grass ( $\geq 50$  cm) and those with short grass ( $< 50$  cm) was statistically significant ( $P < 0.05$ ).

#### Tick species

Adult ticks belonging to 15 species were collected and identified, namely *Amblyomma gemma*,

*A. sparsum*, *A. tholoni*, *A. variegatum*, *A. cohaerens*, *Rhipicephalus (Boophilus) decoloratus*, *Rh. appendiculatus*, *Rh. compositus*, *Rh. evertsi evertsi*, *Rh. longus*, *Rh. praetextatus*, *Rh. pravus*, *Rh. pulchellus*, *Rh. sanguineus* and *Hyalomma truncatum*.

Of the 15 tick species present on hosts, the highest number occurred on buffalo and cattle (Table 2). All the adult questing ticks collected belonged to the genus *Rhipicephalus*. *Rhipicephalus appendiculatus* was the most abundant species by far, for example comprising 98% of the 1039 ticks recovered from the vegetation in the middle of the wet season immediately following the 2001 disease outbreak (Table 3).

#### Plant species

The predominant grass species on the crater floor were *Chloris gayana*, *Cynodon* sp., *Digitaria* sp., *Pennisetum* sp., *Hyperthenea* sp., *Sporobolus* sp., *Eragrostis* sp., Nut grass, *Themeda* sp., *Setaria* sp. and *Panicum maximum*. Herbaceous

Table 1. Mean tick density (ticks/m<sup>2</sup> ± S.E.) during wet and dry season in the Ngorongoro Crater grassland.

Season	Grassland status	Transects (n)	Adult ticks	Immature
<b>Wet season</b> (March–June 2001)	Tall grass (≥50 cm)	15	57 ± 6.93	7 ± 2.08
	Short grass (<50 cm)	9	7 ± 2.08	6 ± 1.34
	All transects combined	24	42 ± 7.4	85 ± 37.24
<b>Dry season</b> (July–December 2001)	Less grazed (>20 cm)	18	28 ± 5.63	961 ± 146
	Heavily grazed (<20 cm)	6	0	771 ± 109.95
	All transects combined	24	7 ± 4.41	819 ± 152.95



**Table 2.** Tick species distribution on wild and domestic herbivore hosts in the Ngorongoro Crater.

Herbivore species	Tick species	Infestation level	No. of tick spp. on host
<b>Wild</b>			
Buffalo	<i>Amblyomma gemma</i> , <i>A. variegatum</i> , <i>A. sparsum</i> , <i>A. coherens</i> , <i>Boophilus decoloratus</i> , <i>Hyalomma truncutum</i> , <i>H. marginatum rufipes</i> , <i>Rhipicephalus appendiculatus</i> , <i>Rh. compositus</i> , <i>Rh. evertsi</i> , <i>Rh. longus</i> , <i>Rh. praetextatus</i> , <i>Rh. pulchellus</i>	Abundant	13
Black rhinoceros	<i>A. gemma</i> , <i>A. sparsum</i> , <i>A. tholoni</i> , <i>A. variegatum</i> , <i>Rh. compositus</i>	Moderate	5
Zebra	<i>Hyalomma truncutum</i> , <i>Rh. appendiculatus</i> , <i>Rh. evertsi</i> , <i>Rh. compositus</i> , <i>Rh. pulchellus</i>	Moderate	5
Wildebeest	<i>Rh. appendiculatus</i> , <i>Rh. evertsi</i> , <i>Rh. compositus</i>	Very low	3
Thomson's gazelle	<i>Rh. appendiculatus</i> , <i>Rh. evertsi</i> , <i>Rh. compositus</i>	Very low	3
Grant's gazelle	<i>Rh. appendiculatus</i> , <i>Rh. evertsi</i> , <i>Rh. compositus</i>	Very low	3
Elephant	<i>Amblyomma tholoni</i> ; <i>Rh. praetextatus</i>	Very low	2
<b>Domestic</b>			
Cattle	<i>A. gemma</i> , <i>A. variegatum</i> , <i>B. decoloratus</i> , <i>Rh. appendiculatus</i> , <i>Rh. evertsi</i> , <i>Rh. praetextatus</i> , <i>Rh. compositus</i>	Moderate	7
Donkey	<i>Rh. appendiculatus</i> , <i>Rh. evertsi</i> , <i>Rh. compositus</i>	Very low	3
Sheep	<i>Rh. appendiculatus</i> , <i>Rh. evertsi</i> , <i>Rh. compositus</i>	Very low	3
Goat	<i>Rh. appendiculatus</i> , <i>Rh. evertsi</i>	Very low	2

plants in the crater that are favoured by herbivores include *Amaranthus spinosus*, *Amaranthus hybridus*, *Achelanthes aspera*, *Justicia betonica* and *Trifolium masaiense*. The mean grass height and the corresponding grass fuel load in the 24 transects were, as expected, significantly different between wet and dry seasons ( $P < 0.001$ ). High tick density and the presence of *Chloris gayana*, *Cynodon dactylon* and *Pennisetum clandestinum* were correlated ( $P < 0.05$ ). These grass species and herbs like *Amaranthus spinosus*, *A. hybridus*, *Achelanthes aspera*, *Justicia betonica* and *Trifolium masaiense* are favoured by large herbivores, including buffalo. Regardless of height, few ticks

were observed in areas where the following grasses predominated: *Eragrostis* sp., *Hyper-rhenia rufa*, Nut grass, *Panicum maximum*, *Pennisetum mezianum*, *Setaria* sp. and *Themeda triandra*.

#### Effects of prescribed burning

The effects of fire on the grass sward virtually eliminated adult ticks and reduced immature ticks significantly (Table 4). The effect was rapid: one year after the start of burning (2002), unburned areas had tick densities similar to before burning (Fig. 2a) but burned areas showed a build-up of immature ticks occurring that declined precipitously at

**Table 3.** Predominance of *Rhipicephalus appendiculatus* among ticks collected in the Ngorongoro Crater grassland during one wet season month prior to prescribed burning (May 2001).

Collection method (No. with ticks)	Tick species	Stage of development and sex				Total
		Larvae	Nymphs	Males	Females	
Quadrant × 13 (9)	<i>Rhipicephalus appendiculatus</i>	–	–	150	154	304
50 m Drag × 14 (13)	<i>Amblyomma variegatum</i>	18	0	0	0	11
	<i>Rhipicephalus appendiculatus</i>	383	162	61	116	722
	<i>Rhipicephalus praetextatus</i>	0	0	1	0	1
	<i>Rhipicephalus</i> sp.	0	0	1	0	1

**Table 4.** Mean tick density (ticks/m<sup>2</sup>) and mean grass height (cm) and corresponding grass fuel load (kg/ha) ( $\pm$  S.E.) on unburned and burned areas in the Ngorongoro Crater grassland.

Location	Adult ticks		Immature ticks		Mean grass height and (fuel load)	
	Unburned	Burned	Unburned	Burned	Unburned	Burned
Daraja 3	11 $\pm$ 6.35	0	63 $\pm$ 36.18	2 $\pm$ 1.83	31 $\pm$ 4.39 (9594 $\pm$ 453)	10 $\pm$ 1.65 (4006 $\pm$ 356)
M/morum	0.4 $\pm$ 0.36	0	188 $\pm$ 85.0	12 $\pm$ 8.78	23 $\pm$ 1.20 (7801 $\pm$ 795)	6 $\pm$ 0.80 (2350 $\pm$ 1262)
Msalabani	7 $\pm$ 3.83	0	452 $\pm$ 232.82	50 $\pm$ 34.97	35 $\pm$ 4.16 (10403 $\pm$ 140)	7 $\pm$ 1.14 (2806 $\pm$ 860)
Munge	2 $\pm$ 1.25	0.2 $\pm$ 0.18	663 $\pm$ 376.97	64 $\pm$ 51.04	36 $\pm$ 2.63 (10598 $\pm$ 427)	10 $\pm$ 2.13 (4006 $\pm$ 50)
Ngoitokitok	11 $\pm$ 5.2	0	738 $\pm$ 349.9	5 $\pm$ 2.61	40 $\pm$ 3.76 (11352 $\pm$ 116)	9 $\pm$ 1.45 (3629 $\pm$ 543)
S_corner	1 $\pm$ 0.18	0	130 $\pm$ 83.80	5 $\pm$ 2.47	18 $\pm$ 1.14 (6516 $\pm$ 860)	5 $\pm$ 0.89 (1854 $\pm$ 1148)
Wilcoxon <i>P</i> -value	<i>P</i> < 0.0001		<i>P</i> < 0.001		<i>P</i> < 0.0001	

the peak of dry season in September (Fig. 2b).

The mean 'peak' tick density  $\pm$  S.E. before burning was 42  $\pm$  7.4 per m<sup>2</sup> (adults, wet season) and 819  $\pm$  152.95 per m<sup>2</sup> (immature ticks, dry season) (Fig. 1; Table 1). Twenty-seven months post-burning, these values had fallen to 0  $\pm$  0.08 (adults, wet season) and 33  $\pm$  40.67 (immature ticks, dry season), respectively (Table 4). In Ngorongoro Crater, a grass height of less than 10 cm with a fuel load below 4000 kg/ha appears to present an environment unfavourable to tick survival.

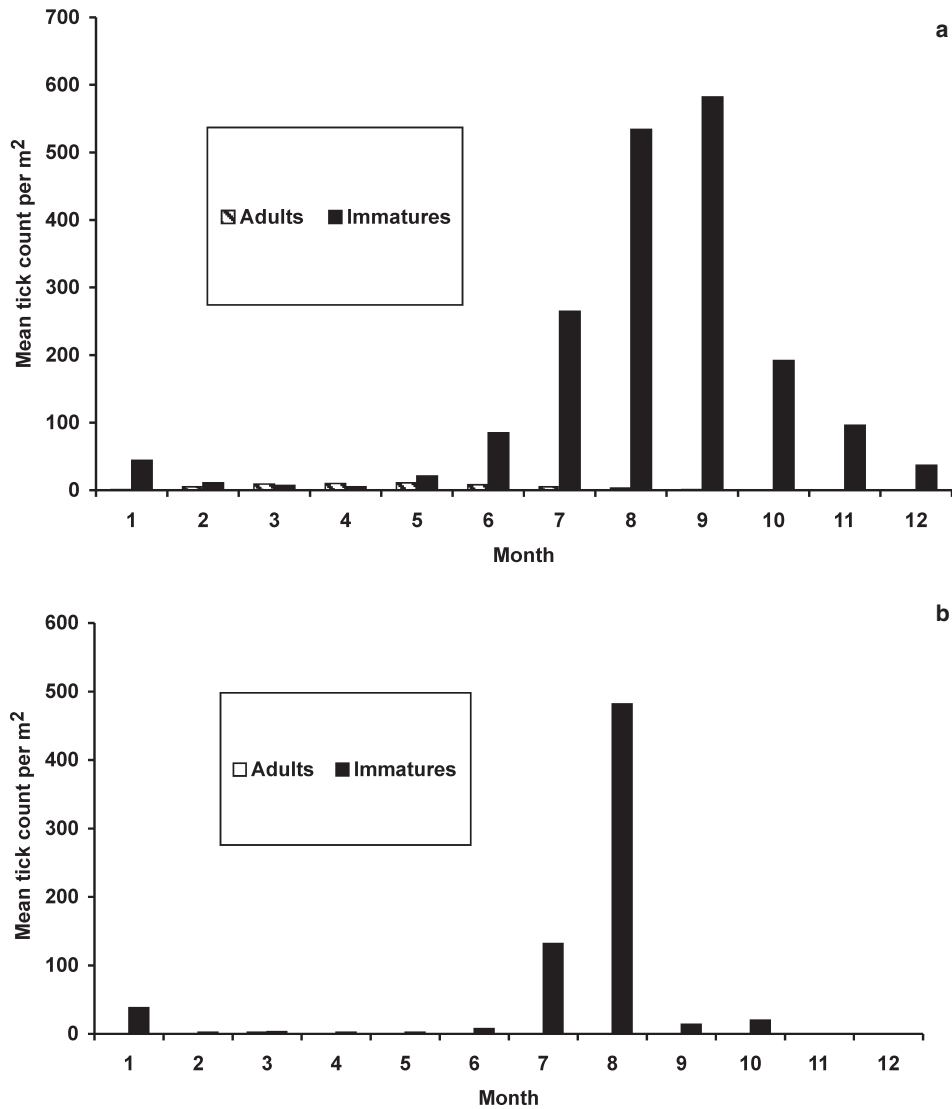
## DISCUSSION

### Tick populations and disease

Widely varying associations between tick density and different grass/host localities in this study were of course expected. The value of these findings is that they can be combined with other evidence to construct a plausible, retrospective picture of the interplay of factors leading to the emergence of tick-borne disease in Ngorongoro Crater wildlife. A sequence of disparate events over 30 years steadily produced positive cumulative effects on tick vector numbers until a threshold was reached at which clinical disease erupted. Interestingly, the process involved both abiotic factors (fire frequency and climate) and biotic factors (changes in pasture composition and herbivore abundance), both having strong anthropogenic and natural components.

Maasai pastoralists historically lived in the Ngorongoro Crater alongside high densities of wildlife. Traditional range management by Maasai involving cattle grazing and the liberal use of fire favours a short grass sward with a larger proportion of palatable species, occupied by pure grazers (Pratt 1967). In 1974 the wildlife authority (NCAA) relocated these Maasai out of the crater, believing their presence was incompatible with rapidly growing wildlife tourism, and subsequently carried out a 'no burning policy' of the crater grassland.

The complete suppression of fire for 27 years (1974–2001) caused gradual changes in composition of the grassland that promoted the expansion of areas covered by tall grass, which in turn led to changes in grazing species abundance. A census data series now spanning 40 years demonstrates how the crater retained very high and fairly constant biomass of wild herbivores (around 11 000 kg/km<sup>2</sup>) but the species composition had changed. Runyoro *et al.* (1995) first proposed a strong link between the range management effects of pasto-



**Fig. 2.** a, Monthly mean density of adult and immature ticks on unburned areas during 2002 (*i.e.* after starting prescribed burning); b, Monthly mean density of adult and immature ticks on burned areas during 2002 (*i.e.* after starting prescribed burning).

ralists and the increasing buffalo population, dividing their analysis into periods pre- and post-removal of pastoralists. Other authors have concurred (Estes *et al.* 2006). The post-pastoralist period is characterized by declines in five species of grazers [wildebeest, hartebeest (*Alcephalus busephalus*), eland (*Taurotragus oryx*), Thomson's gazelle (*Gazella thomsoni*) and Grant's gazelle (*Gazella granti*)] and especially marked in wildebeest and Thomson's gazelle, the most abundant

short grass grazers (Estes & Small 1981; Estes *et al.* 2006). Conversely, the rate of increase of buffalo, a coarse grazer preferring taller, lower quality grasses (Vesey-Fitzgerald 1965) and a superb host for ticks (Horak *et al.* 1983b), significantly accelerated after removal of the crater Maasai in 1974, and by 1992 this was the dominant species in terms of herbivore biomass (Runyoro *et al.* 1995).

Expansion of tall grass pasture and a build-up of

moribund grass material from lack of fire provide an ideal habitat for the survival of all free-living life stages of ticks. By contrast, short grass areas are unfavourable for tick survival (due to high desiccation and ultraviolet light) and furthermore are predominantly occupied by hosts with relatively high innate resistance to ticks, like wildebeest, gazelles and hartebeest (Horak *et al.* 1983a; Bigalke 1994; Gallivan & Horak 1997; Horak 1998; Grootenhuys 2000). We propose that completion of tick life cycles in the contrasting grass/host localities thus became strongly and increasingly skewed towards tall grass/buffalo areas.

The probability of clinical disease due to tick-borne haemoparasites is directly linked to abundance of their vectors (Bester 2002; Latif *et al.* 2002). Measured density of grass-questing ticks was extremely high compared to other conservation areas (Spickett *et al.* 1992, 1995; Horak *et al.* 1995; Zieger *et al.* 1998) and although ground-questing ticks (*Amblyomma*, *Hyalomma*) and some *Rhipicephalus* species were not quantified, large burdens on immobilized buffalo (consisting of 13 of the 15 tick spp.) and cattle (7 of 15 spp.), suggest that these were also abundant.

Climatic variation in the form of alternating wet and dry cycles appeared to be the factor that finally precipitated an outbreak of tick-borne disease in 2001. Mean annual rainfall is around 500 mm (Estes *et al.* 2006) but the 1990s showed the highest variation on record (NCAA unpubl. data). The 1997/98 wet season was characterized by an *El Niño* effect, in which the highest rainfall on record (1642 mm) further boosted tick survival after the already long build-up in tick numbers ascribed to changes in both the pasture and tick hosts. In 2000 one of the worst recorded droughts struck the region (the two years following *El Niño* recorded <300 mm rainfall) and individuals of the more common species, especially coarse-grazing buffalo, that were starving and immunosuppressed very likely succumbed to tick toxicosis or tick-borne pathogens that are normally harmless at low vector densities. The predominance of *R. appendiculatus* would have increased the likelihood of such toxicoses (Thomas & Neitz 1958; Norval *et al.* 1988, 1989; Norval & Horak 1994). Despite a drought, an already high tick population could be maintained by high densities of immunosuppressed hosts (FAO 1984; Gulland 1995).

The question of the origin of the tick-borne *Babesia bicornis* organism implicated in the rhino deaths in 2001 was settled by investigations of

rhinos in South Africa, where it was found in both dead and healthy animals (Nijhof *et al.* 2003), and by closer examination of earlier veterinary records from East Africa (P. Morkel, pers. comm., 2006). So latent infection with a stress trigger (starvation) was considered far more likely than sudden exposure to naïve infection due to poor immune response in rhinos (Nijhof *et al.* 2003; Fyumagwa *et al.* 2004).

It would be difficult to show whether livestock-wildlife proximity had any additional effect on the crater's wildlife tick problem. Since eviction, previously resident pastoralists have been permitted to take their cattle herds into the crater for daytime grazing, salt licks and watering. Superficially this is unobtrusive to tourism and wildlife. Structured interviews with Maasai from crater-grazing bomas provided additional anecdotal evidence about the prevalence of tick-borne disease (a separate study). These Maasai stated that far fewer livestock in the same herds were affected in the past when both tick density and the buffalo population in the crater were lower, while increased cattle mortality from tick-borne East coast fever, cerebral theileriosis (locally called *ormilo*) and babesiosis coincided with conditions prevailing in 2000/01 (Fyumagwa *et al.* 2004).

#### Control of ticks

By 2001, the high tick population had demonstrated resilience to climatic variation. Management intervention was recommended by grassland scientists in the wake of assessment of poor range condition and the rhino deaths (W.S.W. Trollope and L.A. Trollope, pers. comm, 2001). A strategically-timed, sectional burning regime of the crater floor was designed in order to control ticks. Annual late dry season burns probably combine several likely effects to reduce on tick numbers. One is direct killing of immature ticks that are abundant during the dry season. Secondly, fire exposes grassland habitat to desiccation and ultraviolet light making it less favourable for oviposition, tick survival or questing. These burns soon become 'green bite' areas frequented by short grazers (Pratt 1967; Wisley 1996) that are poorer hosts for ticks. Minshull & Norval (1982) propose that attraction of 'burn grazer' hosts may negate the effects of fire on ticks, but we note that their study area was a largely woodland ecosystem with neither buffalo nor gazelles. Lastly, an indirect effect of burns might be to deny buffalo some grazing on taller grasses and thus force them

to leave the crater temporarily.

The present study monitored the subsequent effects of prescribed burning on tick densities for two and a half years. The burning regime was demonstrably successful in significantly lowering tick numbers initially, and ecological factors resulting from burns maintained them at low levels thereafter. If this effect is negatively cumulative it should adequately control tick populations and therefore tick-borne disease in the crater despite wide fluctuations in climate. Burning might also help dampen down the changes in herbivore species abundance and the census trend in recent years is indeed suggestive that buffalo are no longer increasing (Estes *et al.* 2006).

### CONCLUSION

The unique grassland ecosystem of the Ngorongoro Crater – a UNESCO World Heritage Site and International Biosphere Reserve – suffers from many ecological problems (for example changes in pasture composition, animal species abundance and hydrological flows; weed infestations; high human impacts) that should be addressed by applied research (Estes *et al.* 2006). This study, demonstrating how research can benefit management, hopefully fulfils a small part of that objective. Ultimately the study may also add further evidence to suggest that anthropogenic fire has had an important role in the evolution of African ecosystems.

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### REFERENCES

- BESTER, P. 2002. Disease prevention and management on game ranches. In: B.L. Penzhorn (Ed.), Game ranch planning and management. Proceedings of a symposium on game ranch planning and management (pp. 54–59). 1–2 November 2002, Onderstepoort, South Africa.
- BIGALKE, R. D. 1994. The important role of wildlife in the occurrence of livestock diseases in southern Africa. In: J.A.W. Coetzer, G.R. Thomson & R.C. Tustin (Eds), Infectious diseases of livestock with special reference to southern Africa, Vol. 1 (pp.152–163). Oxford University Press, Cape Town.
- ESTES, R.D. & SMALL, R. 1981. The large herbivore populations of the Ngorongoro crater. *Afr. J. Ecol.* 19: 175–185.
- ESTES, R. D., ATTWOOD J.L., & ESTES, A.B. 2006. Downward trends in Ngorongoro Crater ungulate populations: conservation concerns and the need for ecological research. *Biol. Conserv.* 106: 106–120
- FAO 1984. Ecological principles in tick control; Biological data on ticks, In: Ticks and tick-borne disease control. A practical field manual, Vol. 1 (pp. 188–199). Tick control. Food and Agriculture Organization of the United Nations, Rome.
- FOWLER, J., COHEN, L. & JARVIS, P. 1998. The normal distribution and regression analysis. Practical statistics for field biology, 2nd edn (pp. 74–82; 142–163). John Wiley and Sons, New York.
- FYUMAGWA, R.D., MKUMBO, S. S. & MORKEL, P. B. 2004. Remote treatment of black rhinos against babesiosis in Ngorongoro Crater, Tanzania. *Pachy.* 37: 80–83.
- GALLIVAN, G.J. & HORAK, I.G. 1997. Body size and habitat as determinants of tick infestations of wild ungulates in South Africa. *S. Afr. J. Wildl. Res.* 27: 63–70.
- GROOTENHUIS, J.G. 2000. Wildlife, livestock and animal disease reservoirs. In: H.H.T. Prins, J.G. Grooten-huis & T.T. Dolan (Eds), Wildlife conservation by sustainable use (pp. 81–113). Kluwer Academic Publishers, Netherlands.
- GULLAND, F.M.D. 1995. Impact of infectious diseases on wild animal populations: a review. In: B.T. Grenfell & A.P. Dobson (Eds), Ecology of infectious diseases in natural populations (pp. 20–51). Cambridge University Press, Cambridge.
- HORAK, I.G. 1998. The relationship between ticks, hosts and the environment in the Kruger National Park, South Africa. In: Proceedings and abstracts of the second international conference on tick-borne pathogens at the host-vector interface: a global perspective (pp. 413–426). 28 August – 1 September 1995, Kruger National Park.
- HORAK, I.G., DE VOS, V. & BROWN-MOIRA, R. 1983a. Parasites of domestic and wild animals in South Africa. XVI. Helminth and arthropod parasites of blue and black wildebeest (*Connochaetes taurinus* and *Connochaetes gnou*). *Onderstepoort J. Vet. Res.* 50: 243–255.
- HORAK, I.G., POTGIETER, F.T., WALKER, J.B., DE VOS, V. & BOOMKER, J. 1983b. The ixodid tick burdens of various large ruminant species in South

- African nature reserves. *Onderstepoort J. Vet. Res.* 50: 221–228.
- HORAK, I.G., DE VOS, V. & BRAACK, L.E.O. 1995. Arthropod burdens of impalas in the Skukuza region during two droughts in the Kruger National Park. *Koedoe* 38: 65–71.
- KISSUI, B.M. & PACKER, C. 2004 Top-down regulation of a top predator: lions in the Ngorongoro Crater. *Proc. Roy. Soci. Lond. B* 271: 1867–1874.
- LATIF, A.A., HOVE, T., KANHAI, G.K. & MASAKA, S. 2002. Buffalo-associated *Theileria parva*: the risk to cattle of buffalo translocation into the Highveld of Zimbabwe. *Ann. N. Y. Acad. Sci.* 969: 275–279.
- LIGHTFOOT, C.J. & NORVAL, R.A.I. 1981. Tick problems in wildlife in Zimbabwe. I. The effects of tick parasitism on wild ungulates. *S. Afr. J. Wildl. Res.* 11: 41–45.
- MINSHULL, J.I. & NORVAL, R.A.I. 1982. Factors influencing the spatial distribution of *Rhipicephalus appendiculatus* in the Kyle Recreational Park, Zimbabwe. *S. Afr. J. Wildl. Res.* 12: 118–123.
- NIJHOF, A.M., PENZHORN, B.L., LYNNEN, G., MOLLEL, J.O., MORKEL, P., BEKKER, C.P.J. & JONGEJAN, F. 2003. *Babesia bicornis* sp. nov., and *Theileria bicornis* sp. nov.: tick-borne parasites associated with mortality in the black rhinoceros (*Diceros bicornis*). *J. Clin. Microbiol.* 41: 2249–2254.
- NORVAL, R.A.I., SUTHERST, R.W., KURKI, J., GIBSON, J.D. & KERR, J.D. 1988. The effect of the brown ear-tick *Rhipicephalus appendiculatus* on the growth of Sanga and European breed cattle. *Vet. Parasitol.* 30: 149–164.
- NORVAL, R.A.I., SUTHERST, R.W., JORGENSON, O.G., GIBSON, J.D. & KERR, J.D. 1989. The effect of the bont tick (*Amblyomma hebraeum*) on the weight gain of Africander steers. *Vet. Parasitol.* 33: 329–341.
- NORVAL, R.A.I. & HORAK, I.G. 1994. Vectors: Ticks, In: J.A.W. Coetzer & R.C. Tustin (Eds), *Infectious diseases of livestock* Vol. 1 (pp. 3–42). Oxford University Press, Cape Town.
- PRATT, D.J. 1967. A note on the vegetation of burnt grassland by wildfire. *E. Afr. Wildl. J.* 5: 178–179.
- RUNYORO, V.A., HOFER, H., CHAUSI, E.B. & MOEHL-MAN, P.D. 1995. Long-term trends in the herbivore populations of the Ngorongoro Crater, Tanzania. In: A.R.E. Sinclair & P. Arcese (Eds), *Serengeti II, Dynamics, management and conservation of an ecosystem* (pp. 146–168). University of Chicago Press, Chicago.
- SHORT, N.J. & NORVAL, R.A.I. 1981. The seasonal activity of *Rhipicephalus appendiculatus* Neum. 1901 (Acarina: Ixodidae) in the highveld of Zimbabwe Rhodesia. *J. Parasitol.* 67: 77–84.
- SPICKETT, A.M., HORAK, I.G., VAN NIEKERK, A. & BRAACK, L.E.O. 1992. The effect of veld-burning on the seasonal abundance of free-living ixodid ticks as determined by drag-sampling. *Onderstepoort J. Vet. Res.* 59: 285–292.
- SPICKETT, A.M., HORAK, I.G., HEYNE, H. & BRAACK, L.E.O. 1995. The effect of severe drought on the abundance of ticks on vegetation and on scrub hares in the Kruger National Park. *Koedoe* 38: 59–64.
- THOMAS, A.D. & NEITZ, W.O. 1958. Rhipicephaline toxicosis in cattle: its possible aggravating effects on certain diseases. *J. S. Afr. Vet. Med. Assoc.* 29: 39–50.
- TROLLOPE, W.S.W. & POTGIETER, A.L.F. 1986. Estimating grass fuel loads with a disc pasture meter in Kruger National Park. *J. Grassl. Soc. S. Afr.* 3: 148–152.
- VESEY-FITZGERALD, D.F. 1965. The utilization of natural pastures by wild animals in the Rukwa valley, Tanganyika. *E. Afr. Wildl. J.* 3: 38–48.
- WALKER, A.R., BOUATTOR, A., CAMICAS, J-L., ESTRADA-PENA, A., HORAK, I.G., LATIF, A.A., PEGRAM, R.G. & PRESTON, P.M. 2003. Ticks of domestic animals in Africa: a guide to identification of species (pp. 1–221). Bioscience Reports, Edinburgh, Scotland, U.K.
- WILSEY, B.J. 1996. Variation in use of green flushes following burns among African ungulate species: the importance of body size. *Afr. J. Ecol.* 34: 32–38.
- ZIEGER, U., HORAK, I.G., CAULDWELL, A.E. 1998. Dynamics of free-living ixodid ticks on a game ranch in the Central Province, Zambia. *Onderstepoort J. Vet. Res.* 65: 49–59.

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#### Appendix 1. Sampling locations in different transects in the Ngorongoro Crater.

S/n.	Local name	GPS coordinates	Adult ticks	Immature ticks
<b>(a) Sampling locations and mean tick density (ticks/m<sup>2</sup>) during one wet season (March to June 2001).</b>				
1	Ascend road	35.52023 E, 3.23535 S	21	74
2	S/ faru_1	35.53013 E, 3.22194 S	62	97
3	Ndulele	35.53804 E, 3.22699 S	02	00
4	S/ faru_2	35.52790 E, 3.21367 S	65	78
5	Gorigori	35.52445 E, 3.23879 S	66	36
6	Ranger post	35.50200 E, 3.23036 S	11	57
7	Water springs	35.50319 E, 3.21136 S	12	24
8	Boma utafiti	35.50741 E, 3.20480 S	15	72
9	Miti_3	35.51033 E, 3.19663 S	49	51
10	Seneto	35.50687 E, 3.17716 S	00	00
11	Jackal hill	35.52322 E, 3.15186 S	33	57



**Appendix 1** (continued)

S/n.	Local name	GPS coordinates	Adult ticks	Immature ticks
12	Foot_engitati	35.53585 E, 3.14560 S	127	83
13	Engitati	35.54996 E, 3.13370 S	54	34
14	Daraja_3	35.58308 E, 3.20172 S	67	88
15	Mlima morum	35.61374 E, 3.17546 S	19	00
16	S_corner	35.60833 E, 3.18414 S	08	28
17	Rumbe hill	35.62676 E, 3.17496 S	01	00
18	Msalabani	35.58989 E, 3.18464 S	22	928
19	Munge	35.58573 E, 3.13738 S	76	56
20	Ngoitokitok	35.58340 E, 3.20566 S	36	35
21	Lerai West	35.51908 E, 3.23165 S	60	89
22	Lerai_north	35.51083 E, 3.20413 S	130	72
23	Mti_1	35.57567 E, 3.18808 S	0	18
24	West_mandusi	35.52744 E, 3.14842 S	78	62
<b>(b) Sampling locations and mean tick density (ticks/m<sup>2</sup>) during one dry season (July to December 2001)</b>				
1	Ascend road	35.52023 E, 3.23535 S	00	1770
2	S/ faru_1	35.53013 E, 3.22194 S	00	375
3	Ndulele	35.53804 E, 3.22699 S	00	22
4	S/ faru_2	35.52790 E, 3.21367 S	22	260
5	Gorigori	35.52445 E, 3.23879 S	00	1695
6	Ranger post	35.50200 E, 3.23036 S	00	1780
7	Water springs	35.50319 E, 3.21136 S	00	2200
8	Boma utafiti	35.50741 E, 3.20480 S	00	965
9	Miti_3	35.51033 E, 3.19663 S	00	90
10	Seneto	35.50687 E, 3.17716 S	00	357
11	Jackal hill	35.52322 E, 3.15186 S	00	135
12	Foot_engitati	35.53585 E, 3.14560 S	26	118
13	Engitati	35.54996 E, 3.13370 S	00	814
14a	Daraja_3 unburned	35.58308 E, 3.20172 S	00	200
14b	Daraja_3 burned*		00	20
15a	Mlima morum unburned	35.61374 E, 3.17546 S	00	478
15b	Mlima_morum burned*		00	28
16a	S_corner unburned	35.60833 E, 3.18414 S	00	460
16b	S_corner burned*		00	40
17	Rumbe hill	35.62676 E, 3.17496 S	00	125
18a	Msalabani unburned	35.58989 E, 3.18464 S	04	1370
18b	Msalabani burned*		00	228
19a	Munge unburned	35.58573 E, 3.13738 S	02	2190
19b	Munge burned*		00	462
20a	Ngoitokitok unburned	35.58340 E, 3.20566 S	00	1954
20b	Ngoitokitok burned*		00	05
21	Lerai_west	35.51908 E, 3.23165 S	82	1052
22	Lerai_north	35.51083 E, 3.20413 S	08	249
23	Mti_1	35.57567 E, 3.18808 S	00	07
24	West_mandusi	35.52744 E, 3.14842 S	24	985

\*Oct-Dec in burned areas.

**Appendix 2.** Density (per square metre) of identified tick species in different transects during wet season in Ngorongoro Crater in 2001.

Location	Tick species													Total		
	<i>A. gamma</i>	<i>A. cohaer.</i>	<i>A. sparsum</i>	<i>A. tholoni</i>	<i>A. variegat.</i>	<i>H. m. rufipes</i>	<i>H. truncat.</i>	<i>Rh. appendic.</i>	<i>Rh. composit.</i>	<i>Rh. decolor.</i>	<i>Rh. evertsi</i>	<i>Rh. longus</i>	<i>Rh. praetext.</i>		<i>Rh. pulchell.</i>	<i>Rh. sanguin.</i>
Ascend road	0	*	*	*	0	*	*	15	3	*	*	1	1	1	*	21
S/Faru_1	0.5	*	*	*	0.6	*	*	46	8	*	*	2	3	3	*	62
Ndulele	0	*	*	*	0	*	*	2	0	*	*	0	0	0	*	2
S/Faru_2	1	*	*	*	1.4	*	*	47	8	*	*	2	4	3	*	65
Gorogori	0	*	*	*	0	*	*	48	10	*	*	2	3	3	*	66
Ranger post	0	*	*	*	0	*	*	6	3	*	*	0.4	1	1	*	11
Water springs	0	*	*	*	0	*	*	8	1	*	*	0	2	1	*	12
Boma utafiti	0	*	*	*	0	*	*	11	2	*	*	0	1	1	*	15
Miti_3	0.2	*	*	*	0.4	*	*	36	6	*	*	2	3	2	*	49
Seneto	0	*	*	*	0	*	*	0	0	*	*	0	0	0	*	0
Jackal hill	0	*	*	*	0	*	*	25	4	*	*	1	2	1	*	33
Foot_engitlati	1	*	*	*	0.5	*	*	90	19	*	*	4	8	5	*	127
Engitlati	0	*	*	*	0	*	*	38	9	*	*	2	3	2	*	54
Daraja_3	0	*	*	*	0	*	*	47	11	*	*	2	4	3	*	67
Mlima morum	0	*	*	*	0	*	*	14	2	*	*	1	1	1	*	19
S_corner	0	*	*	*	0	*	*	6	1	*	*	0	1	0	*	8
Rumbe hill	0	*	*	*	0	*	*	1	0	*	*	0	0	0	*	1
Msalabani	0	*	*	*	0	*	*	15	3	*	*	1	2	1	*	22
Munge	0.4	*	*	*	0.1	*	*	55	11	*	*	2	4	3	*	76
Ngoitokitok	0.4	*	*	*	0.3	*	*	26	4	*	*	1	2	2	*	56
Lerai west	1	*	*	*	1	*	*	42	8	*	*	2	3	3	*	60
Lerai north	0	*	*	*	0.6	*	*	93	20	*	*	4	7	5	*	130
Miti_1	0	*	*	*	0	*	*	0	0	*	*	0	0	0	*	0
West-Mandusi	0.2	*	*	*	1	*	*	58	10	*	*	2	4	3	*	78

\*Tick species collected on immobilized animals not quantified per unit area.



## Paper iii



# Molecular detection of haemotropic *Mycoplasma* species in *Rhipicephalus sanguineus* tick species collected on lions (*Panthera leo*) from Ngorongoro Crater, Tanzania

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Haemotropic *Mycoplasma* species are pathogens that can cause haemolytic anaemia in susceptible mammalian species worldwide. The cause of haemolysis is due to membrane damage through stimulation of IgM cold agglutinins production, which induces autoimmune haemolysis of infected erythrocytes. A study was conducted to establish the prevalence of *Mycoplasma haemofelis*, 'Candidatus *Mycoplasma haemominutum*' and 'Candidatus *M. turicensis*' in ticks and the diversity of tick species that are possible vectors of the pathogens that can transmit the infection to wildlife in Ngorongoro Crater. Three real-time PCR assays were used for the analysis of DNA pools ( $n = 507$ ) derived from 11 tick species. *Mycoplasma haemofelis* and 'Candidatus *M. haemominutum*' were detected in *Rhipicephalus sanguineus*. On average 19.7% and 12.9% of *R. sanguineus* were PCR-positive for *M. haemofelis* and 'Candidatus *M. haemominutum*', respectively. This tick species therefore represent an important reservoir for feline haemotropic *Mycoplasma* species in the crater. These organisms with their known pathological effects are probably one of the factors potentially exacerbating the severity of infection during the disease outbreak in wildlife and can have undesirable outcome to wild cats like lions when under nutritional stress or in case of concurrent infection.

**Key words:** haemotropic *Mycoplasma*, lions, Ngorongoro Crater, real-time PCR, ticks.

## INTRODUCTION

*Haemobartonella* and *Eperythrozoon* species have been renamed as haemotropic *Mycoplasma* species (haemoplasmas) based on strong phylogenetic evidence and 16S ribosomal RNA gene sequence (Neimark *et al.* 2002; Tasker *et al.* 2003a). These microorganisms have a wide host range infecting carnivores, herbivores and omnivores worldwide (Lobetti & Tasker 2004; Messick 2004; Neimark *et al.* 2004; Willi *et al.* 2006; 2007a) and are thought to be transmitted by arthropod vectors and through mechanical means (Shaw *et al.* 2004; Dean *et al.* 2005; Woods *et al.* 2005). In 2001 about 20 lions (*Panthera leo*) died in the Ngorongoro Crater from suspected tick-borne

diseases and probably other causes (Kissui & Packer 2004; Fyumagwa *et al.* 2007). The unusual mortality prompted the investigation of various tick-borne haemoparasites including haemotropic *Mycoplasma* species. Organisms resembling haemoplasmas were microscopically observed in Giemsa stained blood smears, but it was difficult to differentiate these from background debris (Bobade *et al.* 1988; Inokuma *et al.* 2004). Retrospective records show that the Ngorongoro Crater has experienced four disease epidemics in its lion population since its establishment in 1959 (Kissui & Packer 2004). In 1962 the crater was infested with swarms of biting flies (*Stomoxys calcitrans*) which caused severe bites to lions with noticeable lion mortality (Kingdon 1977; Kissui & Packer 2004). In 1994 and 1997 there were two epidemics

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shortly after the Serengeti Canine Distemper Virus (CDV) epidemic of 1993/94 (Roelke-Parker *et al.* 1996; Kissui & Packer 2004).

There are many haemoplasma species (Neimark *et al.* 2001; Neimark *et al.* 2002), but *M. haemofelis*, 'Candidatus *M. haemominutum*' and the recently discovered 'Candidatus *M. turicensis*' have been described as important in causing clinical disease in domestic cats and *M. haemocanis* and 'Candidatus *M. haematoparvum*' cause clinical disease in domestic dogs (Inokuma *et al.* 2004; Kenny *et al.* 2004; Sykes *et al.* 2005; Willi *et al.* 2005; 2006). However, the mode of transmission between cats is still under discussion. The morphology of these organisms has been previously described (Norval & Horak 1994; Neimark *et al.* 2004; Willi *et al.* 2007a). *M. haemofelis*, *M. haemocanis* and 'Candidatus *M. haemominutum*' are morphologically indistinguishable but 'Candidatus *M. haemominutum*' seems to be smaller in size (Kewish *et al.* 2004; Messick 2004; Sykes *et al.* 2005). *M. haemofelis* has been reported to be responsible for a severe, often fatal haemolytic anaemia in acutely infected domestic cats (Foley & Pedersen 2001; Kewish *et al.* 2004; Messick 2004; Willi *et al.* 2006). Immune suppression exacerbates multiplication of the organisms and development of clinical symptoms (Sykes *et al.* 2003; Kenny *et al.* 2004; Sykes *et al.* 2005). In the acute infection there is intermittent fever with progressive anaemia corresponding to the level of microorganisms in blood circulation (Sykes *et al.* 2003; Kenny *et al.* 2004).

The microorganisms may act as cofactors in the progression of retroviral, neoplastic and immune-mediated diseases (Bobade *et al.* 1988; Messick 2004; Willi *et al.* 2006). Intimate contact of haemoplasmas with erythrocytes to lead to direct cell damage and through autoimmune-mediated mechanisms (Hoelzle 2008). The cat flea *Ctenocephalides felis* and dog tick *Rhipicephalus sanguineus* have been found to harbour the pathogens (Inokuma *et al.* 2004; Shaw *et al.* 2004; Woods *et al.* 2005). Recently, *M. haemofelis*, 'Candidatus *M. haemominutum*' and 'Candidatus *M. turicensis*' DNA have been amplified in domestic and wild cats using polymerase chain reaction (PCR) and there is a growing interest in investigating the epidemiology, especially pathogenicity and transmission of these pathogens (Criado-Fornelio *et al.* 2003; Tasker *et al.* 2003b; Inokuma *et al.* 2004; Lobetti & Tasker 2004; Willi *et al.* 2005, 2006, 2007a).

PCR amplification of 16S rRNA gene fragments

is routinely used in the diagnosis of infections or epidemiological studies of haemoparasites. Here we report on the molecular detection of haemoplasma species DNA from ticks collected in Ngorongoro Crater, Tanzania using TaqMan real-time PCR.

## MATERIALS AND METHODS

### Study area

The Ngorongoro Conservation Area (NCA) encompasses a multiple land use area of about 8300 km<sup>2</sup> lying at the western edge of the Great Rift Valley in the Arusha region, northern Tanzania and is populated by wildlife, livestock and people, the latter mostly Maasai pastoralists. The NCA is managed by the Ngorongoro Conservation Area Authority. The Ngorongoro Crater (03°10'S, 35°35'E), the world's largest intact inactive caldera occupies approximately 300 km<sup>2</sup> (4%) of the total NCA. The rim is 2200 m above sea level and the crater floor (250 km<sup>2</sup>) is about 600 m below, composed predominantly of grassland with small patches of swamp and *Acacia* riverine forest. Although a geographically distinct unit, it is part of the greater Serengeti ecosystem (25 000 km<sup>2</sup>) and regarded 'to some extent' as an ecologically distinct unit (Runyoro *et al.* 1995).

### Sample collection

Tick species were collected from ten immobilized lions during a disease outbreak in the Ngorongoro Crater lion population in February 2001 and from immobilized wild herbivores (African buffalo, *Syncerus caffer*; blue wildebeest, *Connochaetes taurinus*; plains zebra, *Equus burchelli*) and cattle (*Bos indicus*) in 2004 and 2005. Systematic tick sampling in the crater grassland was performed in 2002 and 2003 by drag and removal sampling (Fyumagwa *et al.* 2007). Opportunistic tick sampling was carried out on two dead African elephants (*Loxodonta africana*) in 2002 and 2004, respectively. Collected ticks were preserved in liquid nitrogen and transported in dry shipper (-196°C) to the Centre for Clinical Studies of the Vetsuisse Faculty of the University of Zurich, Switzerland.

### Identification of tick species

All ticks were identified to the species level using a dissecting microscope (Wild M38, Heerbrugg, Switzerland) and tick identification manual (Walker *et al.* 2003). Pools of five ticks of the same

species and collected in the same location or from the same animal species were prepared and preserved at  $-80^{\circ}\text{C}$  for subsequent laboratory procedures.

#### Nucleic acids extraction

The frozen tick pools were thawed and placed on biochemically clean parafilm previously fitted on a sterile glass frame. One tick pool at a time was mechanically disrupted using sterile scalpel blades followed by decontamination of the glass frame and scalpel with DNA-EX (DNA contaminate removal solution, Inno-Train Diagnostic GmbH, Kronberg, Germany), 70% ethanol and rinsed with distilled water prior to processing the next pool. The mechanically disrupted ticks were transferred into 2 ml microcentrifuge tubes and to each of the macerated pools, 250  $\mu\text{l}$  of phosphate buffered saline (PBS 1x, without  $\text{MgCl}_2$  and  $\text{CaCl}_2$ , Invitrogen, Basel, Switzerland) and 375  $\mu\text{l}$  of external lysis buffer (Guanidinium thiocyanate Triton X-100, MagNA Pure LC Total Nucleic acid isolation kit, Roche Diagnostics, Rotkreuz, Switzerland) were added together with a 5 mm stainless steel bead. The samples were electro-homogenized at 30 000 Hz for 2 minutes in a Mixer Mill device (Retsch GmbH, Haan, Germany), cooled in ice for 15 minutes, electro-homogenized again for 2 minutes and cooled for 15 minutes then centrifuged at 8000 rpm. From each sample, 500  $\mu\text{l}$  of the lysate was used for nucleic acid extraction using the MagNA Pure LC automated system as previously described (Exner & Lewinski 2003). Sterile aerosol-barrier tips were used during all procedures. At the end of the extraction procedure 90  $\mu\text{l}$  of the eluate was transferred and preserved at  $-80^{\circ}\text{C}$  for subsequent molecular analysis. Nucleic acids extraction and PCR reactions were performed in separate laboratories. Extraction controls using distilled water were included in each extraction process to monitor absence of cross-contamination.

#### PCR amplification

TaqMan real-time PCR was used for the amplification of the haemotropic *Mycoplasma* species with the ABI PRISM™ 7700 Sequence Detector System (Applied Biosystems, Rotkreuz, Switzerland) using fluorogenic probe and laser detection system as previously described (Willi *et al.* 2005; 2006). The ABI PRISM™ 7700 Sequence detector provides cycle by cycle measurement of the fluorescence emission from each PCR reaction.

Positive, negative and extraction controls were included in each PCR reaction. Haemotropic *Mycoplasma* species amplified in the tick DNA samples included *M. haemofelis*, 'Candidatus *M. haemominutum*' and 'Candidatus *M. turicensis*'. The number of DNA pools tested for each haemoplasma species was 415, 507 and 440, respectively. Of note is that the *M. haemofelis* assay also amplifies *M. haemocanis* (Willi *et al.* 2005; 2006) due to the high degree of sequence similarity between the two microorganisms (>99% sequence identity across the 16S rRNA gene sequence) (Sykes *et al.* 2005).

#### Data analysis

For statistical purposes, when a PCR product was obtained from a pool of five ticks, only one tick in the pool was assumed to be infected and the maximum likelihood estimation of the infection (MLE) was determined using the formula:  $MLE = 1 - (1 - Y/X)^{1/m}$ , as previously described (Walter *et al.* 1980). Where  $Y$  = number of positive pools;  $X$  = number of pools and  $m$  = number of organisms per pool. Chi-square test ( $\chi^2$ ) was used to compare the rate of detection in ticks between *M. haemofelis* and 'Candidatus *M. haemominutum*' species (Petrie & Watson 2004).

## RESULTS

#### Identified tick species

Eleven tick species were identified from about 3000 ticks which were submitted to the laboratory for analysis. The tick species were specified prior to processing for nucleic acids extraction and included *Amblyomma gemma*, *A. cohaerens*, *A. tholoni*, *A. variegatum*, *Rhipicephalus appendiculatus*, *R. compositus*, *R. (Boophilus) decoloratus*, *R. evertsi*, *R. praetextatus*, *R. pulchellus* and *R. sanguineus*.

#### Infected tick species

Ticks of the species *Rhipicephalus sanguineus* were found to contain DNA of haemotropic *Mycoplasma* species. Four out of six tested *R. sanguineus* pools were positive for *M. haemofelis* and three out of six were positive for 'Candidatus *M. haemominutum*'. All positive pools from *R. sanguineus* were from ticks collected on immobilized lions in the Ngorongoro Crater during disease outbreak in 2001. None of the 440 tick pools tested positive for 'Candidatus *M. turicensis*'. Results are summarized in Table 1.

**Table 1.** Minimum prevalence of haemotropic *Mycoplasma* species in different tick species from Ngorongoro Crater.

Ticks screened	Source of ticks	Pools screened	<i>Mycoplasmas</i> detected	Positive pools	Minimum prevalence
<i>A. gemma</i>	Buffalo, cattle, on ground	7	None	0	–
<i>A. cohaerens</i>	Buffalo	2	None	0	–
<i>A. tholoni</i>	Elephants	10	None	0	–
<i>A. variegatum</i>	Buffalo, cattle, on ground	5	None	0	–
<i>R. appendiculatus</i>	Buffalo, cattle, questing, wildebeest, zebra	372	None	0	–
<i>R. compositus</i>	Buffalo, cattle, questing wildebeest, zebra	52	None	0	–
<i>R. decoloratus</i>	Buffalo	2	None	0	–
<i>R. evertsi</i>	Buffalo, cattle, wildebeest, zebra	16	None	0	–
<i>R. praetextatus</i>	Buffalo, cattle	13	None	0	–
<i>R. pulchellus</i>	Buffalo, questing, zebra	22	None	0	–
<i>R. sanguineus</i>	Lions	6	<i>M. haemofelis</i>	4	19.7%
			' <i>Candidatus</i> <i>M. haemominutum</i> '	3	12.9%
			<i>M. haemominutum</i>		

The difference in prevalence between two *Mycoplasma* species was not significant ( $P > 0.05$ ).

The detection rate in *R. sanguineus* ticks in the Ngorongoro Crater was 19.7% and 12.9% for *M. haemofelis* and '*Candidatus M. haemominutum*', respectively. Two positive pools from *R. sanguineus* (7.8%) tested PCR-positive for both *M. haemofelis* and '*Candidatus M. haemominutum*'. Although more ticks were found to have *M. haemofelis* DNA than '*Candidatus M. haemominutum*', the difference was statistically not significant ( $\chi^2 = 1.4228$ , d.f. = 1,  $P > 0.05$ ).

#### DISCUSSION

PCR analysis revealed the presence of feline haemotropic *Mycoplasma* species in ticks from Ngorongoro Crater. To our knowledge this is the first time that feline haemoplasma species DNA was detected and reported in ticks from free ranging lions in East Africa. We are aware that the agent also could be related to *M. haemocanis* as our assay does not differentiate between *M. haemofelis* and *M. haemocanis*. However, from the observation that all lions in the Serengeti which is contiguous to NCA were positive by PCR for *M. haemofelis* and not *M. haemocanis*, it appears likely that also the ticks in the Ngorongoro Crater are carrying *M. haemofelis* (Willi *et al.* 2007b). However, despite the high proportion of *R. appendiculatus* in the crater of over 70% (Fyumagwa *et al.* 2007), none of the 372 pools tested positive for haemoplasma suggesting that this tick species is not important in the epidemiology of haemoplasmas. The dog tick *R. sanguineus* and the cat flea *Ctenocephalides felis* have been reported as potential reservoirs of haemoplasmas

(Inokuma *et al.* 2004; Shaw *et al.* 2004). However, no information is available on the reservoir status of these ectoparasites in Tanzania and this was part of the justification for conducting this study.

In the PCR analysis for '*Candidatus M. turicensis*', none of the 440 pools tested positive. However, Willi *et al.* (2007b) detected '*Candidatus M. turicensis*' in lion samples collected in 1993/94 during the Canine Distemper Virus (CDV) epidemic in Serengeti National Park, which is contiguous to NCA. Failure to detect '*Candidatus M. turicensis*' DNA in all 440 pools tested is explained by the fact that '*Candidatus M. turicensis*', which was first isolated in Zurich, Switzerland (Willi *et al.* 2005), is probably not as common as the other two species in the crater or is possibly even completely absent.

Retrospective records show that the Ngorongoro Crater has experienced four disease epidemics in its lion population since its establishment in 1959 (Kissui & Packer 2004). In 1962 the crater was infested with swarms of biting flies (*Stomoxys calcitrans*) which caused severe bites to lions with noticeable lion mortality (Kingdon 1977; Kissui & Packer 2004; Lobetti & Tasker 2004). In 1994 and 1997 there were two epidemics shortly after the Serengeti CDV epidemic of 1993/94 (Roelke-Parker *et al.* 1996; Kissui & Packer 2004). The fourth epidemic was in 2001 when about 20 lions died from suspected tick-borne infections due to high tick challenge and swarms of biting flies. Pathogens including *Anaplasma phagocytophilum* which is immunosuppressive and *Babesia* and *Theileria* species were identified in biting flies and in sick lions in the epidemic of 2001 (M.L. Meli *et al.*

unpubl. data; Fyumagwa *et al.* 2007).

High *Rhipicephalus* parasitism is known to cause toxicosis (Norval & Horak 1994; Walker *et al.* 2003; Fyumagwa *et al.* 2007), which compromises the immunity and is likely to predispose haemoplasma-infected animals to clinical disease (Foley & Pedersen 2001; Westfall *et al.* 2001; Sykes *et al.* 2003; Kenny *et al.* 2004; Messick 2004). Co-infection of haemoplasmas with immunosuppressive organisms like Feline Immunodeficiency Virus coupled with stress from high tick infestation and swarms of biting flies (R.D. Fyumagwa & H. Wiik, unpubl. data) is likely to exacerbate the disease problem in the crater. This is also supported by the observation that up to 98% of samples collected in 1993/94 from sick and dead lions in the Serengeti National Park were haemoplasma-positive (Willi *et al.* 2007b). The mean haemoglobin concentration of  $6.62 \pm 1.28$  ( $\pm$ S.D.) (range 5.5–8.4 g/dl) with mean PCV of  $28 \pm 3.44$  ( $\pm$ S.D.) (range 20–32%) observed in 10 immobilized lions in the crater in 2001 indicated anaemia of varying degrees. Since haemotropic *Mycoplasma* species are opportunistic organisms, it is likely to have exacerbated the degree of anaemia in affected lions in the crater in 2001 (R.D. Fyumagwa & H. Wiik, unpubl. data). Apart from lions the ecosystem has small populations of threatened wild carnivores (wild dog, *Lycaon pictus*, and cheetah, *Acinonyx jubatus*) which might be at risk. Therefore, further studies are required to establish the pathological effects of these microorganisms and the host range amongst wild carnivores in the ecosystem.

Detection of these *Mycoplasma* species in ticks has shed some light towards understanding the epidemiology of disease outbreaks in the Ngorongoro Crater. A concerted effort on routine tick control regime is therefore recommended to reduce tick density and decrease the risk of wild animals that succumb to clinical diseases from tick-borne infections.

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#### REFERENCES

- BOBADE, P.A., NASH, A.S. & ROGERSON, D.P. 1988. Feline haemobartonellosis: clinical, haematological and pathological studies in natural infections and the relationship to infection with feline leukaemia virus. *Vet. Rec.* 122: 32–36.
- CRIADO-FORNELIO, A., MARTINEZ-MARCOS, A., BULING-SARANA, A. & BARBA-CARRETERO, J.C. 2003. Presence of *Mycoplasma haemofelis*, *Mycoplasma haemominutum* and piroplasmids in cats from southern Europe: a molecular study. *Vet. Microbiol.* 93: 307–317.
- DEAN, R., HELPS, C.R., GRUFFYDD-JONES, T.J. & TASKER, S. 2005. Use of real-time PCR to detect *Mycoplasma haemofelis* and 'Candidatus *Mycoplasma haemominutum*' in the saliva and salivary glands of haemoplasma-infected cats. In: 48th Annual British Small Animal Veterinary Association Congress. British Small Animal Veterinary Association, Birmingham, U.K.
- EXNER, M.M. & LEWINSKI, M.A. 2003. Isolation and detection of *Borrelia burgdorferi* DNA from cerebral spinal fluid, synovial fluid, blood, urine, and ticks using the Roche MagNA Pure system and real-time PCR. *Diagn. Microbiol. Infect. Dis.* 46: 235–240.
- FOLEY, J.E. & PEDERSEN, N.C. 2001. 'Candidatus *Mycoplasma haemominutum*' a low-virulence erythrocytic parasite of cats. *Int. J. Syst. Evol. Microbiol.* 51: 815–817.
- FYUMAGWA, R.D., RUNYORO, V., HORAK, I.G. & HOARE, R. 2007. Ecology and control of ticks as disease vectors in wildlife of the Ngorongoro Crater, Tanzania. *S. Afr. J. Wildl. Res.* 37(1): 79–90.
- HOELZLE, L.E. 2008. Haemotropic mycoplasmas: recent advances in *Mycoplasma suis*. *Vet. Microbiol.* 130(3-4): 215–226.
- INOKUMA, H., TAROURA, S., OKUDA, M., HISASUE, M., ITAMOTO, K., UNE, S., NAKAICHI, M. & TAURA, Y. 2004. Molecular survey of *Mycoplasma haemofelis* and 'Candidatus *Mycoplasma haemominutum*' infection in cats in Yamaguchi and surrounding areas. *J. Med. Sci.* 66(8): 1017–1020.
- KENNY, M.J., SHAW, S.E., BEUGNET, F. & TASKER, S. 2004. Demonstration of two distinct haemotropic *Mycoplasmas* in French dogs. *J. Clin. Microbiol.* 42(11): 5397–5399.
- KEWISH, K.E., APPELYARD, G.D., MYERS, S.L., KIDNEY, B.A. & JACKSON, M.L. 2004. *Mycoplasma haemofelis* and *Mycoplasma haemominutum* detection by polymerase chain reaction in cats from Saskatchewan and Alberta. *Can. Vet. J.* 45(9): 749–752.
- KINGDON, J. 1977. Lion (*Panthera leo*). In: East African



- mammals. An atlas of evolution in Africa. Vol. III, part A (Carnivores) (pp. 366–395). Academic Press, London.
- KISSUI, B.M. & PACKER, C. 2004. Top-down regulation of a top predator: lions in the Ngorongoro Crater. *Proc. Roy. Soc. Lond. B* 271: 1867–1874.
- LOBETTI, R.G. & TASKER, S. 2004. Diagnosis of feline haemoplasma infection using a real-time PCR assay. *J. S. Afr. Vet. Assoc.* 75(2): 94–99.
- MESSICK, J.B. 2004. Haemotrophic *Mycoplasma* spp (haemoplasmas): a review and new insights into pathogenic potential. *Vet. Clin. Pathol.* 33(1): 2–13.
- NEIMARK, H., HOFF, B. & GANTER, M. 2004. *Mycoplasma ovis comb. Nov.* (formerly *Eperythrozoon ovis*), an eperythrozytic agent of haemolytic anaemia in sheep and goats. *Int. J. Syst. Evol. Microbiol.* 54(2): 365–371.
- NEIMARK, H., JOHANSSON, K-E., RIKIHISA, Y. & TULLY, J.G. 2002. Revision of haemotropic *Mycoplasma* species names. *Int. J. Syst. Evol. Microbiol.* 52: 683.
- NEIMARK, H., JOHANSSON, K-E., RIKIHISA, Y. & TULLY, J.G. 2001. Proposal to transfer some members of the genera *Haemobartonella* and *Eperythrozoon* to the genus *Mycoplasma* with descriptions of '*Candidatus*, *Mycoplasma haemofelis*', '*Candidatus* *Mycoplasma haemomuris*', '*Candidatus* *Mycoplasma haemosuis*', and '*Candidatus* *Mycoplasma wenyoni*'. *Int. J. Syst. Evol. Microbiol.* 51: 891–899.
- NORVAL, R.A.I. & HORAK, I.G. 1994. Vectors: Ticks. In: J.A.W. Coetzer & R.C. Tustin (Eds) *Infectious diseases of livestock*, Vol. 1 (pp. 3–42). Oxford University Press Southern Africa, Cape Town.
- PETRIE, A. & WATSON, P. 2004. The chi-squared test comparing proportions. In: *Statistics for veterinary and animal science* (pp. 101–113, 202). Blackwell Science, Oxford, U.K.
- ROELKE-PARKER, M.E., MUNSON, L., PACKER, C., KOCK, R., CLEAVLAND, S., CARPENTER, M., O'BRIEN, S.J., POPISCHIL, A., HOFMANN-LEHMANN, R., LUTZ, H., MWAMENGELE, G.L.M., MGASA, M.N., MACHANGE, G.A., SUMMERS, B., & APPEL, M.J.G. 1996. A canine distemper virus epidemic in Serengeti lions (*Panthera leo*). *Nature* 379: 441–445.
- RUNYORO, V.A., HOFER, H., CHAUSI, E.B. & MOEHLMAN, P.D. 1995. Long-term trends in the herbivore populations of the Ngorongoro Crater, Tanzania. In: A.R.E. Sinclair & P. Arcese (Eds) *Serengeti II, Dynamics, management and conservation of an ecosystem* (pp. 146–168). University of Chicago Press, Chicago, U.S.A.
- SHAW, S.E., KENNY, M.J., TASKER, S. & BIRTLES, R.J. 2004. Pathogen carriage by the cat flea *Ctenocephalides felis* (Bouche) in the United Kingdom. *Vet. Microbiol.* 102(3–4): 183–188.
- SYKES, J.E., BALL, L.M., BAILIFF, N.L. & FRY, M.M. 2003. Identification of a novel haemotropic *Mycoplasma* in a splenectomized dog with haemic neoplasia. *J. Vet. Int. Med.* 17: 423.
- SYKES, J.E., BALL, L.M., BAILIFF, N.L. & FRY, M.M. 2005. '*Candidatus* *Mycoplasma haematoparvum*' a novel small haemotropic *Mycoplasma* from a dog. *Int. J. Syst. Evol. Microbiol.* 55: 27–30.
- TASKER, S., HELPS, C.R., DAY, M.J., HARBOUR, D.A., SHAW, S.E., HARRUS, S., BANETH, G., LOBETTI, R.G., MALIK, R., BEAUFILS, J.P., BELFORD, C.R. & GRUFFYDD-JONES, T.J. 2003a. Phylogenetic Analysis of haemoplasma species: an international study. *J. Clin. Microbiol.* 41: 3877–3880.
- TASKER, S., HELPS, C.R., DAY, M.J., GRUFFYDD-JONES, T.J. & HARBOUR, D.A. 2003b. Use of real-time PCR to detect and quantify *Mycoplasma haemofelis* and '*Candidatus* *Mycoplasma haemominutum*' DNA. *J. Clin. Microbiol.* 41: 439–441.
- WALKER, A.R., BOUATTOR, A., CAMICAS, J.L., ESTRADA-PENA, A., HORAK, I.G., LATIF, A.A., PEGRAM, R.G. & PRESTON, P.M. 2003. Ticks of domestic animals in Africa: a guide to identification of species (pp. 1–221). Bioscience Reports, Edinburgh, U.K.
- WALTER, S.D., HILDRETH, S.W. & BEATY, B.J. 1980. Estimation of infection rates in populations of organisms using pools of variable size. *Am. J. Epidemiol.* 112: 124–128.
- WESTFALL, D.S., JENSEN, W.A., REAGAN, W.J., RADECKI, S.V. & LAPPIN, M.R. 2001. Inoculation of two genotypes of *Hemobartonella felis* (California and Ohio variants) to induce infection in cats and the response to treatment with azithromycin. *Am. J. Vet. Res.* 62: 687–691.
- WILLI, B., BORETTI, F.S., CATTORI, V., TASKER, S., MELI, M.L., REUSCH, C.E., LUTZ, H. & HOFMANN-LEHMANN, R. 2005. Identification, molecular characterization and experimental transmission of a new haemoplasma isolate from a cat with haemolytic anaemia in Switzerland. *J. Clin. Microbiol.* 43(6): 2581–2585.
- WILLI, B., BORETTI, F.S., BAUMGARTNER, C., TASKER, S., WENGER, B., CATTORI, V., MELI, M.L., REUSCH, C.E., LUTZ, H. & HOFMANN-LEHMANN, R. 2006. Prevalence, risk factor analysis and follow-up of infections caused by three feline haemoplasma species in cats in Switzerland. *J. Clin. Microbiol.* 44: 961–969.
- WILLI, B., BORETTI, F.S., MELI, M.L., BERNASCONI, M.V., CASATI, S., HEGGLIN, D., PUORGER, M., NEIMARK, H., CATTORI, V., WENGLI, N., REUSCH, C.F., LUTZ, H. & HOFMANN-LEHMANN, R. 2007a. Real-time PCR investigation of potential vectors, reservoirs, and shedding patterns of feline haemotropic *Mycoplasmas*. *Appl Environ Microbiol.* 73(12): 3798–802.
- WILLI, B., FILONI, C., CATÃO-DIAS, J.L., CATTORI, V., MELI, M.L., VARGAS, A., MARTÍNEZ, F., ROELKE, M.E., RYSER-DEGIORGIS, M-P, LEUTENEGGER, C.M., LUTZ, H. & HOFMANN-LEHMANN, R. 2007b. Worldwide occurrence of feline hemoplasma infections in wild felid species. *J. Clin. Microbiol.* 45(4): 1159–1166.
- WOODS, J.E., BREWER, M.M., HAWLEY, J.R., WISNEWSKI, N. & LAPPIN, M.R. 2005. Evaluation of experimental transmission of '*Candidatus* *Mycoplasma haemominutum*' and *Mycoplasma haemofelis* by *Ctenocephalides felis* to cats. *Am. J. Vet. Res.* 66: 1008–1012.

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## Paper iv





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Short communication

## Prevalence of *Anaplasma marginale* in different tick species from Ngorongoro Crater, Tanzania

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### ABSTRACT

In 2001, Ngorongoro Crater was infested with high density of ticks on grassland, livestock and wildlife which was also associated with high mortality. Adult ticks were collected, identified, processed for nucleic acids extraction and a molecular analysis was performed to determine the range of tick species harboring *Anaplasma marginale*. The real-time PCR was used in the amplification of rickettsia DNA in tick pools ( $n = 527$ ) from 11 identified tick species. Six tick species were detected with *A. marginale* DNA including *Amblyomma gemma*, *Rhipicephalus appendiculatus*, *R. compositus*, *R. decoloratus*, *R. praetextatus* and *R. pulchellus*. The detection rate in each tick species was 3%, 0.7%, 2%, 13%, 1.8%, and 6.2%, respectively. Five of the positive tick species excluding *R. decoloratus* have previously not been described to transmit *A. marginale*. High diversity of tick species detected with *A. marginale* in Ngorongoro Crater is likely to increase a risk to susceptible animals of contracting the infection.

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### 1. Introduction

*Anaplasma marginale* is an intra-erythrocytic rickettsia which is among *Anaplasma* species that have been taxonomically reorganized from families *Anaplasmataceae* to *Rickettsiaceae* based on phylogenetic sequence similarity of the 16S rRNA gene (Dumler et al., 2001). *A. marginale* is transmitted by ticks as the biological vectors, although mechanical transmission by haematophagous insects or iatrogenically by contaminated fomites has been reported (Kocan et al., 2004). The infection rate of *A. marginale* is determined by the level of rickettsemia in the reservoir host and the ability to infect the midgut of tick vector and

undergo successful biological replication (Eriks et al., 1993; Ueti et al., 2007).

The rickettsia causes anaplasmosis, an infectious disease of economic importance to livestock characterized by debility, fever, constipation, inappetence, severe anaemia, dehydration, abortion and/or death and contributes to great economic loss to livestock productivity (Eriks et al., 1994; de la Fuente et al., 2005). Animals that survive acute infection become carriers serving as the source of contamination for attaching ticks and in reservoir hosts (Kieser et al., 1990; Eriks et al., 1993). Despite its economic importance to livestock in East Africa, little is known on the epidemiology of this rickettsia in wildlife that share the same habitat with livestock (Grootenhuys, 2000). In Tanzania, *A. marginale* is often diagnosed using light microscopy of stained blood smears based on the characteristic feature of the rickettsia in infected erythrocytes (Swai et al., 2005). However, this is not reliable method for proper diagnosis because in sub-clinical cases

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the rickettsemia is usually low and can be overlooked. Little is known regarding the prevalence of *A. marginale* in different tick species in and around Ngorongoro Crater and Tanzania in general due to inadequate resource for surveillance. Epidemiological studies for tick-borne pathogens in free living ticks using molecular technique has been used by many researchers in diagnostic laboratories (Sparagano et al., 1999; Chae et al., 2003).

In 2000/2001, the Ngorongoro Crater experienced a high density and diversity of tick species on grassland, livestock and wildlife which was associated with high mortality (Fyumagwa et al., 2004a; Fyumagwa et al., 2007). Livestock and wildlife mortality from tick-borne diseases prompted the investigation to determine the prevalence of some important tick-borne pathogens in the crater including *A. marginale*. In the present study 11 tick species were identified and used in the molecular analysis for *A. marginale*. Here we report on the detection of *A. marginale* DNA in ticks from Ngorongoro Crater, Tanzania where livestock regularly interacts with wild herbivores.

## 2. Materials and methods

### 2.1. Study area

The Ngorongoro Crater (03°10'S, 35°35'E) is the world's largest intact, inactive caldera occupying approximately 300 km<sup>2</sup> (4%) of the total Ngorongoro Conservation Area (NCA) which has an area of about 8300 km<sup>2</sup> lying at the western edge of the Great Rift Valley in northern Tanzania. The crater is populated by resident wildlife, however, masai livestock frequently go in the crater for grazing. Although a geographically distinct unit, it is part of the greater Serengeti Ecosystem (25,000 km<sup>2</sup>) and regarded 'to some extent' as an ecologically distinct unit (Runyoro et al., 1995).

### 2.2. Sample collection

Ticks were collected from 10 immobilized lions (*Panthera leo*) during a disease outbreak in Ngorongoro Crater lion population in February 2001. Systematic sampling of questing ticks from the crater grassland was performed in 2002 and 2003 by drag and removal sampling (Fyumagwa et al., 2007). Ticks were sampled opportunistically on two dead African elephants (*Loxodonta africana*) in 2002 and 2004 respectively and in 2004 and 2005 three wild herbivore species were immobilized for ticks collection using Etorphine hydrochloride (M99; Norvatis Pty Ltd., South Africa) in combination with a tranquilizer Azaperone tartrate (Kyron Laboratories Pty Ltd., South Africa). The herbivores included 10 African buffalo (*Syncerus caffer*), 15 wildebeest (*Connochaetus taurinus*) and 10 zebra (*Equus burchelli*). Twenty five local zebu cattle (*Bos indicus*) from Maasai bomas near the crater rim with a history of frequent grazing in the crater were sampled during the study. In the study only adult ticks were collected and preserved in liquid nitrogen (−196 °C) and brought to the University of Zurich for laboratory analyses.

### 2.3. Tick identification

All ticks were observed under a dissecting microscope (Wild M38, Heerbrugg, Switzerland) and identified following the scheme as described by Walker et al. (2003). Ticks of the same species which were collected in the same location or same animal species were grouped in pools of five and preserved at −80 °C for subsequent laboratory procedures.

### 2.4. Nucleic acids extraction

Each frozen tick pool was thawed and placed on a sterile parafilm (American National Can™, USA) previously fitted on a sterile glass frame. The ticks were dissected using sterile scalpel blades and were then transferred into 2 ml sterile microcentrifuge tubes. Before preparing the next tick pool the glass frame and scalpel blade were decontaminated with DNA-EX (Inno-Train Diagnostics GmbH, Kronberg, Germany), with 70% ethanol and rinsed with sterile distilled water. To each of the pools 250 µl of phosphate buffered saline (PBS 1×, 14190-136, Invitrogen, Basle, Switzerland), 375 µl of lysis buffer (MagNA Pure LC Total Nucleic Acids Isolation Kit, Roche diagnostics, Rotkreuz, Switzerland) and a 5 mm (diameter) silver metal bead (Schieritz & Hauenstein AG, Arlesheim, Switzerland) was added. The tubes were vortexed (Vortex-Genie 2™, Bender and Hobein, AG, Zurich, Switzerland), fitted into a Mixer mill adapter set (Qiagen, Hombrechtikon, Switzerland) and homogenized in a Mixer Mill MM 300 device (Retsch GmbH, Haan, Germany) at 30,000 Hz for 2 min and then centrifuged at 8000 rpm (Ilettich-EBA12, Switzerland) for 1 min. From each sample 500 µl of the supernatant was used for total nucleic acids (TNA) extraction using the MagNA Pure LC automated system according to manufacturer's instructions (Roche Diagnostics GmbH, Rotkreuz, Switzerland). At the end of the extraction 90 µl of eluted TNA was transferred and preserved at −80 °C for subsequent molecular analysis. The nucleic acids extraction and PCR reactions were performed in separate laboratories. Extraction controls using distilled water were included in each extraction process to monitor for the possibility of cross-contamination.

### 2.5. PCR amplification

A real-time PCR assay was used for the amplification of *A. marginale* from 527 pools using the ABI PRISM™ 7700 Sequence Detector System (Applied Biosystems). The assay amplifies specifically a 65 bp fragment of the MSP4 gene of *A. marginale* (Meli ML, unpublished). Positive, negative and extraction controls were included in each PCR reaction. The PCR reactions contained a final concentration of 0.9 µM of the primers AmarMSP4.520f 5'-TGA CGT GCT GCA CAC AGA TTT-3' and AmarMSP4.586r 5'-AAC AAA GCT TGC GCC TAT CC-3' for forward and reverse primers respectively, and 0.25 µM of the probe MSP4.542p 5'-6FAM-CCT GTG TCC CCG TAT GTA TGT GCC G-3', 12.5 µl of the 2× qPCR Master Mix (Eurogentec) and 5 µl of template TNA in a final volume of 25 µl. The assay was performed using the ABI PRISM 7700 Sequence Detection System (Applied

Biosystems) with an initial step of 50 °C for 2 min and a denaturation at 95 °C for 10 min followed by 45 cycles at 95 °C for 15 s and 60 °C for 1 min.

### 2.6. Data analysis

The rate of infection in tick pools was estimated using the formula: Maximum Likelihood Estimation (MLE) =  $1 - (1 - Y/X)^{1/m}$ , as described by Walter et al. (1980), where  $Y$  = number of positive pools;  $X$  = number of pools and  $m$  = number of organisms per pool. This formula assumes that when a PCR product is positive from a pool of five ticks, only one tick in the pool is considered to be infected (Walter et al., 1980).

## 3. Results

### 3.1. Tick species

By identification of tick samples from more than 2600 ticks picked randomly from Ngorongoro Crater, 11 tick species were found. The tick species which were identified prior to processing for DNA extraction and used for screening of *A. marginale* were: *Amblyomma gemma*, *A. cohaerens*, *A. tholoni*, *A. variegatum*, *Rhipicephalus appendiculatus*, *R. compositus*, *R. (Boophilus) decoloratus*, *R. evertsi*, *R. praetextatus*, *R. pulchellus* and *R. sanguineus* (Table 1). The number of ticks collected from animals and grass and used in the analysis were 30 from lions, 65 from elephants, 250 from cattle, 700 from buffalo, 200 from wildebeest, 490 from zebra and 900 from grass respectively.

### 3.2. PCR amplification

Six out of 11 identified tick species contained *A. marginale* DNA and 29 out of 527 pools tested positive for *A. marginale*. The infected tick pools included *A. gemma*, *R. appendiculatus*, *R. compositus*, *R. decoloratus*, *R. praetextatus* and *R. pulchellus*. Four *R. pulchellus*, seven *R. appendiculatus*, four *R. compositus* and one *R. decoloratus* positive pools were from buffalo; one *R. praetextatus* positive pool was from wildebeest; one *R. appendiculatus* positive pools was from zebra; one *A. gemma* positive pool

from cattle. Five *R. appendiculatus*, two *R. compositus*, one *R. praetextatus* and two *R. pulchellus* positive pools were from questing ticks. The number of tick pools analyzed for *A. marginale* in each of the identified tick species is summarized in Table 1.

## 4. Discussion

This experiment showed that the extraction protocol was efficient and eluates contained DNA that could be used in the PCR amplification for *A. marginale*. To our knowledge this is the first study in Tanzania to establish the molecular prevalence of *A. marginale* involving large number of ticks with high diversity of tick species. The only known epidemiological study in ticks in Tanzania focused on one tick species, *R. appendiculatus* and aimed at establishing the efficacy of PCR in detecting *Theileria parva* in ticks (Ogden et al., 2003). Tick-borne diseases including theileriosis, anaplasmosis, babesiosis and ehrlichiosis account for 72% of all cattle mortality in Tanzania (Fyumagwa et al., 2004b; Leynen et al., 2007). In the current study *A. marginale* was detected in a wide range of tick species. Detection of DNA in ticks does not mean that the ticks are capable of transmitting the infection (Ueti et al., 2007; Leverich et al., 2008). However, in view of the fact that both biological and mechanical transmissions have been described (Kocan et al., 2004), there is high probability for susceptible hosts to contract the infection. *A. gemma*, *R. compositus*, *R. praetextatus* and *R. pulchellus* have previously not been reported to transmit *A. marginale* to animals (Norval, 1994; Walker et al., 2003). These four tick species had relatively high detection rate of the rickettsia suggesting that probably are important in the epidemiology of *A. marginale*. The reason for not recognizing their importance in the transmission of *A. marginale* could be due to insufficient studies and absence of some of these tick species in areas where more research work has been conducted. Despite its abundance in the crater and with more tick pools tested, *R. appendiculatus* showed relatively low detection rate corroborating with the reports of other authors (Norval, 1994; Walker et al., 2003) (Table 1). In the present study none of the 17 *R. evertsi* DNA pools tested positive for *A. marginale*, however, *R. evertsi* has been described to be a biological vector of *A. marginale* (Norval, 1994; Walker et al., 2003) an observation which remain unexplained and requires further investigation.

In a separate study on tick ecology, it was observed that buffalo was host to 13 out of 15 identified tick species and cattle hosted seven tick species consistently (Fyumagwa et al., 2007). Four tick species from buffalo, one from wildebeest, cattle and zebra respectively were detected with *A. marginale* DNA in the present study. As cattle are susceptible to *A. marginale* infection, these results suggest that Maasai cattle around Ngorongoro Crater are at a high risk of contracting the infection. High risk of infection to cattle with clinical anaplasmosis hinders the possibility of introducing improved cattle breeds to the Maasai pastoralists in the area (Eriks et al., 1994; Sparagano et al., 1999).

High numbers of buffalo in the Ngorongoro Crater has been associated with increased tick density in the crater

**Table 1**  
Identified tick species, their estimated abundance, pools tested positive for *A. marginale* DNA and rate of infection in pools from each tick species.

Tick species	Number of pools	Ticks abundance in the crater	Positive pools	Rate of infection in pools (%) <sup>a</sup>
<i>A. gemma</i>	7	1.3%	1	3
<i>A. cohaerens</i>	2	0.6%	0	–
<i>A. tholoni</i>	10	0.8%	0	–
<i>A. variegatum</i>	5	1.1%	0	–
<i>R. appendiculatus</i>	372	70.5%	13	0.7
<i>R. compositus</i>	62	11.7%	6	2
<i>R. decoloratus</i>	2	0.4	1	13
<i>R. evertsi</i>	16	3.2%	0	–
<i>R. praetextatus</i>	23	4.4%	2	1.8
<i>R. pulchellus</i>	22	4.2%	6	6.2
<i>R. sanguineus</i>	6	1.9%	0	–
Pools tested (n)	527			

<sup>a</sup> MLE =  $1 - (1 - Y/X)^{1/m}$ .

grassland because they are the favorite host for ticks (Horak et al., 1983; Fyumagwa et al., 2007). During an epidemic of tick-borne diseases in 2000/2001, anaplasmosis was one of the diseases which affected Maasai cattle in Ngorongoro Crater (Fyumagwa et al., 2004a).

Enhanced livestock productivity around Ngorongoro Crater will help to improve the livelihood of the semi-nomadic Maasai pastoralists. This can be achieved through concerted efforts in the control of tick-borne diseases. Routine livestock dipping is not cost effective because of high cost of acaricides and development of tick resistance to some of acaricides in use (BurrIDGE, 1981; Leynen et al., 2007). Since the vaccine for *A. marginale* is available in developed countries like USA and Australia, it could be appropriate for the government in collaboration with other stakeholders to consider establishing a vaccination programme to control the disease.

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### References

- BurrIDGE, M.J., 1981. Epidemiological approaches to disease control. In: Ristic, M., McIntyre, I. (Eds.), *Diseases of Cattle in the Tropics*. Martinus Nijhoff Publishers, Netherlands, pp. 53–64.
- Chae, J.S., Kim, C.M., Kim, E.H., Hur, E.J., Klein, T.A., Kang, T.K., Lee, H.C., Song, J.W., 2003. Molecular epidemiological study for tick-borne disease (*Ehrlichia* and *Anaplasma* species) surveillance at selected U.S. Military training sites/Installations in Korea. *Ann. N. Y. Acad. Sci.* 990, 118–125.
- de la Fuente, J., Torina, A., Naranjo, V., Caracappa, S., Di Marco, V., Alongi, A., Russo, M., Maggio, A.R., Kocan, K.M., 2005. Infection with *Anaplasma phagocytophilum* in a seronegative patient in Sicily Italy: case report. *Ann. Clin. Microbiol. Antimicrob.* 4, 15–18.
- Dumler, J.S., Barbet, A.F., Bekker, C.P.J., Dasch, G.A., Palmer, G.H., Ray, S.C., Rikihisa, Y., Rurangirwa, F.R., 2001. Reorganization of genera in the families *Rickettsiaceae*: unification of some species of *Ehrlichia* with *Anaplasma*, *Cowdria* with *Ehrlichia* and *Ehrlichia* with *Neorickettsia*, descriptions of six new species combinations and designation of *Ehrlichia equi* and HGE agent as subjective synonyms of *Ehrlichia phagocytophila*. *Int. J. Syst. Evol. Microbiol.* 51, 2145–2165.
- Eriks, I.S., Goff, W.L., Pantone, M., Parish, S.M., McElwain, T.F., Palmer, G.H., 1994. Molecular and biological characterization of a newly isolated *Anaplasma marginale* strain. *J. Vet. Diagn. Invest.* 6 (4), 435–441.
- Eriks, I.S., Stiller, D., Palmer, G.H., 1993. Impact of persistent *Anaplasma marginale* rickettsemia on tick infection and transmission. *J. Clin. Microbiol.* 31 (8), 2091–2096.
- Fyumagwa, R.D., Mkumbo, S.S., Morkel, P.B., 2004a. Remote treatment of black rhinos against babesiosis in Ngorongoro Crater. *Tanzania. Pachy.* 37, 80–83.
- Fyumagwa, R.D., Sudi, F.F., Bahari, M., 2004b. Diseases status in livestock-wildlife interfaces in Tanzania. In: *Proceedings of the WDA-Africa and Middle East Section Annual Conference*, Abu Dhabi, UAE, December 11–13.
- Fyumagwa, R.D., Runyoro, V., Horak, I.G., Hoare, R., 2007. Ecology and control of ticks as disease vectors in wildlife of the Ngorongoro Crater, Tanzania. *S. Afr. J. Wildl. Res.* 37 (1), 79–90.
- Grootenhuys, J.G., 2000. Wildlife, livestock and animal disease reservoirs. In: Heribert, H.T., Prins, Grootenhuys, J.G., Dolan, Thomas T. (Eds.), *Wildlife Research for Sustainable Use*. Kluwer Academic Publishers, Dordrecht, The Netherlands, pp. 81–113.
- Horak, I.G., Potgieter, F.T., Walker, J.B., De Vos, V., Boomker, J., 1983. The ixodid tick burdens of various large ruminant species in South Africa nature reserves. *Onderstepoort J. Vet. Res.* 50, 221–228.
- Kieser, S.T., Eriks, I.S., Palmer, G.H., 1990. Cyclic rickettsemia during persistent *Anaplasma marginale* infection of cattle. *Infect. Immun.* 58, 1117–1119.
- Kocan, K.M., de la Fuente, J., Blouin, E.F., Garcia-Garcia, J.C., 2004. *Anaplasma marginale* (*Rickettsiales: Anaplasmataceae*): recent advances in defining host-pathogen adaptations of a tick-borne rickettsia. *Parasitology* 129, 280–300.
- Leverich, C.K., Palmer, G.H., Knowles, D.P., Brayton, K.A., 2008. Tick-borne transmission of two genetically distinct *Anaplasma marginale* strains following superinfection of the mammalian reservoir host. *Infect. Immun.* 76, 4066–4070.
- Lynen, G., Zeman, P., Bakunam, C., Di Giulio, G., Mtui, P., Sanka, P., Jongejan, F., 2007. Cattle ticks of the genera *Rhipicephalus* and *Amblyomma* of economic importance in Tanzania: distribution assessed with GIS based on an extensive field survey. *Expt. Appl. Acarol.* 43, 303–319.
- Norval, R.A.L., 1994. *Vectors/Ticks*. In: Coetzer, J.A.W., Thomson, G.R., Tustin, R.C. (Eds.), *Infectious Diseases of Livestock with Special Reference to Southern Africa*, vol. 1. Oxford University Press, pp. 1–24.
- Ogden, N.H., Gwakisa, P., Swai, E., French, N.P., Fitzpatrick, J., Kambarage, D., Bryant, M., 2003. Evaluation of PCR to detect *Theileria parva* in field-collected tick and bovine samples in Tanzania. *Vet. Parasitol.* 112 (3), 177–183.
- Runyoro, V.A., Hofer, H., Chausi, E.B., Moehlan, P.D., 1995. Long-term trends in the herbivore populations of the Ngorongoro Crater, Tanzania. In: Sinclair, A.R.E., Peter, Arcese (Eds.), *Serengeti II, Dynamics Management and Conservation of An Ecosystem*. The University of Chicago Press, Chicago, USA, pp. 146–168.
- Sparagano, O.A.E., Allsopp, M.T.E.P., Mank, R.A., Rijpkema, S.G.T., Figueroa, J.V., Jongejan, F., 1999. Molecular detection of pathogen DNA in ticks (Acari: Ixodidae): a review. *Exp. Appl. Acarol.* 23, 929–960.
- Swai, E.S., Karimuribo, E.D., Ogden, N.H., French, N.P., Fitzpatrick, J.L., Bryant, M.J., Kambarage, D.M., 2005. Seroprevalence estimation and risk factors for *A. marginale* on smallholder dairy farms in Tanzania. *Trop. Anim. H. Prod.* 37, 599–610.
- Ueti, M.W., Reagan, J.O., Knowles, D.P., Scoles, G.A., Shkap, V., Palmer, G.H., 2007. Identification of midgut and salivary glands as specific and distinct barriers to efficient tick-borne transmission of *Anaplasma marginale*. *Infect. Immun.* 75 (6), 2959–2964.
- Walker, A.R., Bouattour, A., Camicas, J.L., Estrada-Pena, A., Horak, I.G., Latif, A.A., Pegram, R.G., Preston, P.M., 2003. Ticks of domestic animals in Africa: a guide to identification of species. *Bioscience Reports*. 42 Comiston Drive, Edinburgh EH10 5QR, Scotland, U.K., pp 1–221.
- Walter, S.D., Hildreth, S.W., Beaty, B.J., 1980. Estimation of infection rates in populations of organisms using pools of variable size. *Am. J. Epidemiol.* 112, 124–128.

Paper v





# Molecular detection of *Anaplasma*, *Babesia* and *Theileria* species in a diversity of tick species from Ngorongoro Crater, Tanzania

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Ticks transmit a greater variety of pathogens to mammals than any other blood-sucking arthropod vector, predisposing susceptible individuals to infection with clinical symptoms. A study was conducted to determine the range of haemoparasites in ticks that can pose a health risk to susceptible animals in the Ngorongoro Crater. Questing ticks were collected randomly in crater grassland. *Amblyomma* tick species were collected under moribund grass cover and on hosts; one-host and two-host *Rhipicephalus* tick species were collected on immobilized wild animals. Pools of five ticks were prepared according to species and source and processed for nucleic acid extraction. Haemoparasite DNA was amplified by PCR for *Anaplasma* species ( $n = 118$ ), *Babesia* species ( $n = 102$ ) and *Theileria* species ( $n = 115$ ). Eleven tick species were identified, eight of which were PCR positive for one or more haemoparasites. Sequence analyses for rRNA gene fragments detected *Anaplasma bovis*, *Babesia equi*, *Theileria buffeli* and *Theileria parva*. Therefore, susceptible livestock and endangered wildlife species in crater grassland are at risk of contracting related diseases.

**Key words:** livestock, Ngorongoro Crater, PCR, tick-borne haemoparasites, wildlife.

## INTRODUCTION

Ticks transmit a greater variety of pathogens to mammals than any other blood-sucking arthropod vector, predisposing susceptible individuals to infection with clinical symptoms (Estrada-Pena & Jongejan 1999). *Anaplasma*, *Babesia*, *Ehrlichia* and *Theileria* species are the most important tick-borne haemoparasites to livestock and wildlife worldwide (Kettle 1995; Sparagano *et al.* 1999; Grootenhuys 2000; Steyl *et al.* 2003; Nijhof *et al.* 2003; Penzhorn 2006). The pathogenesis, immunology and pathological effects to susceptible animals have been extensively described (Morzaria 1989; Kettle 1995; Chansiri *et al.* 1999; Grootenhuys 2000; Homer *et al.* 2000; Nijhof *et al.* 2003, 2005; Garcia *et al.* 2004; Kocan *et al.* 2004).

Many of these tick-borne haemoparasites are endemic in Africa and a few occur as epizootic-outbreaks to exotic or naïve hosts (Grootenhuys 2000). Tick-borne diseases, particularly anaplas-

mosis, babesiosis and theileriosis debilitate livestock, precipitating big economic loss as evidenced by high mortality, reduced growth rates, poor carcass yield and reduction in milk production (Stoltsz & Dunsterville 1992; Norval 1994; Sparagano *et al.* 1999; Grootenhuys 2000; Regassa *et al.* 2003; Oliveira-Sequeira *et al.* 2005; Lynen *et al.* 2007).

Co-evolution between wildlife and indigenous pathogens has engendered a *modus vivendi* that assures the survival of both host and parasite populations. Hence the pathogens do not cause devastation to wildlife populations and are regarded as ecological parasites. However, when stressed the innate resistance can be compromised and susceptible individuals succumb to infections with clinical symptoms and noticeable mortality (Horak *et al.* 1983; Uilenberg 1995; Fyumagwa *et al.* 2003, 2004; Pfitzer *et al.* 2004; Nijhof *et al.* 2003, 2005).

In 2000/2001 high mortality of wildlife occurred in Ngorongoro Crater which was associated with tick-borne infections (Nijhof *et al.* 2003; Fyumagwa

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*et al.* 2004, 2007; Estes *et al.* 2006; Munson *et al.* 2008). The death of black rhinos (*Diceros bicornis*) in 2001 from babesiosis was the second incidence in Tanzania (Nijhof *et al.* 2003). The predisposing factor was probably nutritional stress and high tick infestation in the crater grassland (Fyumagwa *et al.* 2004, 2007). Vector-borne diseases are expected to increase in prevalence or new ones may emerge due to the effect of global warming (Alekseev 1998; Osburn *et al.* 2009). The high density and diversity of tick species in Ngorongoro Crater concurrent with wildlife mortality prompted the current study to determine the prevalence of tick-borne haemoparasites that can pose a health risk to susceptible animals. Because of the diversity of tick-borne diseases and the impact they can have on productivity, tick-borne disease research merits, by and large, priority consideration. Here we report on the diversity of tick-borne pathogens detected by polymerase chain reaction (PCR) in different tick species from Ngorongoro Crater.

## METHODS

### Study area

The Ngorongoro Crater (03°10'S, 35°35'E) is the world's largest intact, inactive caldera occupying approximately 300 km<sup>2</sup> (4%) of the total Ngorongoro Conservation Area which has an area of about 8300 km<sup>2</sup> lying at the western edge of the Great Rift Valley in northern Tanzania. The crater is populated by resident wildlife; however, Masai livestock frequently go down the crater for grazing. Although a geographically distinct unit, it is part of the greater Serengeti ecosystem (25 000 km<sup>2</sup>) and regarded to some extent as an ecologically distinct unit (Runyoro *et al.* 1995).

### Collection of ticks

Systematic sampling of questing ticks in the crater grassland was performed in 2002 and 2003 by drag and removal sampling (Fyumagwa *et al.* 2007). *Amblyomma* tick species (three-host tick species) are nocturnal and do not quest on grass. These tick species were collected under moribund grass cover along the roads and animal tracks early in the morning and on hosts. Some ticks were obtained from archived tick samples collected on lions during disease investigation in 2001. In 2004 and 2005 wild herbivore species buffalo (*Syncerus caffer*), blue wildebeest (*Connochaetes taurinus*) and plains zebra (*Equus quagga*) were immobilized to collect ticks that do not quest on grass in adult stage (one-host and two-host *Rhipicephalus*

*decoloratus* and *Rh. evertsi evertsi*, respectively). Immobilizing drugs used were etorphine hydrochloride (M99; Norvartis, South Africa) in combination with a tranquilizer Azaperone tartrate (Kyron Laboratories, South Africa). Collected ticks were preserved in liquid nitrogen (-196°C) and transported in dry shipper to the Clinical laboratory of the Vetsuisse Faculty of the University of Zurich, Switzerland.

### Identification of tick species

Frozen ticks were decanted in a sterile Petri dish placed under a dissecting microscope (Wild M38, Heerbrugg, Switzerland). The identification of tick species was performed using the descriptions of Walker *et al.* (2003). Tick species which are not described by Walker *et al.* (2003), were sent to I.G. Horak at the University of Pretoria, South Africa for identification (Fyumagwa *et al.* 2007). Identified ticks were kept in cold phosphate buffered saline (PBS 1x, Invitrogen, Basle, Switzerland). Ticks of the same species retrieved from the same cryo-vial were pooled in small samples of five ticks, properly labelled and transferred into pre-cooled 1.5-ml Eppendorf tubes (Sarstedt, Numbrecht, Germany) and later on preserved at -80°C for subsequent procedures. Forceps and Petri dishes that were used in the identification were cleaned after every batch of ticks using DNA-EX (DNA contaminate removal solution, Inno-Train Diagnostic GmbH, Kronberg, Germany) followed by 70% ethanol (Kantonsapotheke, Zurich, Switzerland), then rinsed with distilled water.

### Nucleic acids extractions

The frozen tick pools were thawed and placed on biochemically clean parafilm previously fitted on a sterile glass frame (American National Can™, U.S.A). One tick pool at a time was mechanically disrupted using sterile scalpel blades followed by decontamination of the glass frame and scalpel with DNA-EX (Inno-Train Diagnostic GmbH), 70% ethanol and rinsed with distilled water prior to processing the next pool. The mechanically disrupted ticks were transferred into 2 ml micro-centrifuge tubes and to each of the macerated pools 250 µl of phosphate buffered saline (PBS 1x, without MgCl<sub>2</sub> and CaCl<sub>2</sub>, Invitrogen) and 375 µl of lysis buffer containing guanidinium thiocyanate and Triton X-100 (MagNA Pure LC Total Nucleic acid isolation kit, Roche Diagnostics, Rotkreuz, Switzerland) were added together with a 5 mm stainless steel bead (Schieritz and Hauenstein AG,

Arlshheim, Switzerland). The samples were homogenized at 30 000 hz for 2 min in a Mixer Mill MM 300 device (Retsch GmbH, Haan, Germany), cooled in ice for 15 min, homogenized again for 2 min and cooled for 15 min then centrifuged at 8000 rpm for 1 min. From each sample, 500 µl of the lysate was used for nucleic acids extraction using the MagNA Pure LC automated system according to manufacturer's instructions (Roche Diagnostics). Sterile aerosol-barrier tips were used during all procedures. At the end of the extraction procedure 90 µl of the eluate was transferred and preserved at -80°C for subsequent molecular analysis. Extraction controls using distilled water were included in each extraction process to monitor absence of cross-contamination.

#### Determination of 18S rRNA from nucleic acids

The presence of amplifiable nucleic acids in extracted samples was confirmed using a universal TaqMan real-time PCR assay specific for the 18S rRNA gene as described elsewhere (Boretti *et al.* 2009). Twenty eluates from tick DNA were taken randomly and used in the determination of 18S rRNA. Two negative controls were also analysed including extraction control and distilled water.

#### PCR amplification for *Anaplasma* species

A total of 118 tick-DNA pools were amplified for *Anaplasma* species using conventional PCR targeting a gene fragment of 452 bp of the 16S rRNA gene as previously described (Goodman *et al.* 1996). Briefly, the reaction volume was 25 µl, consisting of 2.5 µl of reaction buffer (10x), 2.5 µl of MgCl<sub>2</sub> (25 mM), 0.5 µl of dNTPs (10 mM each), 0.625 µl each of the forward and reverse primers (20 µM of Ehr1:5'-TTT ATC GCT ATT AGA TGA GCC TATG-3' and 20 µM of Ehr2:5'-CTC TAC ACT AGG AGG AAT TCC GCT AT-3', respectively), 0.5 µl of Taq Polymerase (5U/µl, Sigma-Aldrich Chemie GmbH, Buchs, Switzerland), 15.25 µl of distilled water and 2.5 µl of DNA sample. Negative and positive controls were incorporated in the reaction. The PCR reaction was performed using a Tpersonal 48 Thermocycler (Biometra GmbH, Gottingen, Germany). Cycling conditions were: 95°C for 5 min, followed by 35 cycles of 95°C for 30 s, 45°C for 30 s, 72°C for 45 s and finally 72°C for 10 min. The PCR product was visualized in 1.5% agarose gel stained with ethidium bromide after electrophoresis. The DNA pools which yielded PCR products with fragment of interest gel

purified and stored at -20°C for subsequent cloning and sequencing.

#### PCR amplification for *Babesia* species

For *Babesia* species 102 tick-DNA pools were amplified using conventional PCR and the primers used in the analysis were able to detect multi species DNA sequences of 18S rRNA gene fragment of size of about 500 bp. The primers used were broad and are able to amplify *Babesia* species and some *Theileria* species (M.L. Meli unpubl. data). The reaction mixture for each sample was 25 µl consisting of 2.5 µl reaction buffer (10x), 1.5 µl MgCl<sub>2</sub> (25 mM), 0.5 µl dNTPs (10 mM), 0.625 µl each of forward and reverse primers (20 µM of Bbovis.1066f:5'-AAV CTC ACC AGG TCS RGA CA-3' and 20 µM of Bbovis.1528r: 5'-GGA TCA CTC GAT CGG TAG GA-3', respectively), 0.5 µl of Sigma Taq Polymerase (2.5 U/µl, Sigma-Aldrich Chemie GmbH), 2.5 µl of DNA template and 16.25 µl of distilled water. In addition, in each reaction, there were two extraction controls, two negative and two positive controls. The Tpersonal 48 Thermocycler (Biometra) temperature profile was 95°C for 5 min, followed by 35 cycles of 95°C for 30 s, 62°C for 30 s, 72°C for 45 s and finally 72°C for 10 min. PCR products were gel-purified and stored at -20°C for subsequent cloning and sequencing.

#### PCR amplification for *Theileria* species

The amplification of 115 pools for *Theileria* species was conducted targeting a fragment of 250 bp of the 18S rRNA gene. The primers used are also broad and are able to amplify *Theileria* species and some *Babesia* species (M.L. Meli unpubl. data). The total volume of the reaction mixture of 25 µl consisted of 2.5 µl reaction buffer (10x), 2.5 µl MgCl<sub>2</sub> (25 mM), 0.5 µl of dNTPs (10 mM), 0.625 µl of forward and reverse primers (20 µM of Theilspp.f:5'-AAT GAT GGG AAT TTA AAC CYC TTC-3' and 20 µM of Theilspp.r:5'-AAG GCA AAA GCC TGC TTK RAGC-3', respectively), 2.5 µl of DNA template, 0.5 µl Sigma Taq Polymerase (2.5U/µl, Sigma-Aldrich Chemie GmbH), and 15.25 µl of distilled water. Similarly two extraction controls, two negative and two positive controls were incorporated in each of the PCR amplification. The thermal profile for the Tpersonal 48 Thermocycler (Biometra) was 95°C for 5 min, followed by 35 cycles of 95°C for 30 s, 54.4°C for 30 s, 72°C for 45 s and finally 72°C for 10 min. PCR

products were gel-purified and stored at  $-20^{\circ}\text{C}$  for subsequent cloning and sequencing.

#### Cloning and sequencing

The cloning and sequencing was performed from selected PCR positive samples. The cloned and sequenced tick-borne pathogens included *Anaplasma* species, *Babesia* species and *Theileria* species. The purification of DNA fragments was performed using the MinElute Gel Extraction Kit (Qiagen). Purified PCR products were cloned into the vector pCR<sup>®</sup>II-TOPO<sup>®</sup> (TOPO TA Cloning<sup>®</sup> Kit, Invitrogen). Purified plasmid DNA (QIAprep Spin Miniprep Kit, Qiagen) was checked for insert by restriction digestion with *EcoRI* and 10 clones from each positive PCR product were then sequenced from both sides.

Cycle sequencing was performed with approximately 10 ng of DNA and 3.3 pmol plasmid-specific primers (M13 forward, M13 reverse) using the BigDye Terminator Cycle Sequencing Ready Reaction Kit v1.1 (Applied Biosystems, Rotkreuz, Switzerland). Cycling conditions were as follows: 1 min at  $96^{\circ}\text{C}$ , then 25 cycles at  $96^{\circ}\text{C}$  for 10 s and  $50^{\circ}\text{C}$  for 5 s, followed by  $60^{\circ}\text{C}$  for 4 min. Products were purified using the DyeEx Spin column (Qiagen), and analysed on the ABI Prism 310 Genetic Analyzer (Applied Biosystems). The sequences were aligned to one consensus sequence by SeqScape (Version 1.1, Applied Biosystems) and then compared to reference sequences deposited in the Genbank.

#### Data analysis

For statistical purposes, when a PCR product was obtained from a pool of five ticks, only one tick in the pool was assumed to be infected and the estimation of the rate of exposure was determined using the formula: maximum likelihood estimation (MLE) =  $1 - (1 - Y/X)^m$ , as described by Walter *et al.* (1980). Where  $Y$  = number of positive pools;  $X$  = number of pools;  $m$  = number of organisms per pool. Chi-square test ( $\chi^2$ ) was used to compare the infection prevalence in ticks from different tick-borne pathogens (Petrie & Watson 2004).

## RESULTS

#### Tick species

Eleven tick species were identified from about 2000 ticks which were randomly collected in the Ngorongoro Crater. The tick species which were identified prior to processing for total nucleic acids

extraction and used for molecular analyses of selected haemoparasites included *Amblyomma gemma*, *A. cohaerens*, *A. tholoni*, *A. variegatum*, *Rhipicephalus appendiculatus*, *Rh. compositus*, *Rh. decoloratus*, *Rh. evertsi evertsi*, *Rh. praetextatus* and *Rh. pulchellus*, *Rh. sanguineus*.

#### Nucleic acids extraction and quality control

Total nucleic acids were extracted from 335 pools. The cycle threshold values for 18S rRNA from randomly selected tick DNA as determined by real-time PCR ranged from 15.31–35.46. This experiment showed that the extraction protocol was efficient and eluates contained DNA that could be used in the PCR amplification for haemoparasites.

#### *Anaplasma* species

Six out of 11 identified tick species contained *A. marginale* DNA and 29 out of 527 pools tested positive for *A. marginale* (Fyumagwa *et al.* 2009). Sequencing of gene fragments of *Anaplasma* positive pools from *Rh. praetextatus* based on 16S rRNA gene revealed the presence of *Anaplasma bovis* (GU361777–GU361780, Table 1).

#### *Babesia* species

In the PCR amplification of 102 DNA pools from eleven tick species, 37 pools from five tick species tested positive for *Babesia* species including *A. gemma*, *Rh. appendiculatus*, *Rh. compositus*, *Rh. decoloratus* and *Rh. evertsi evertsi*. One pool of *A. gemma*, one pool of *Rh. compositus* from grass cover and questing, respectively; and two *Rh. decoloratus* pools from buffalo which tested positive for *Babesia* species in this study were also positive for *A. marginale* in real time PCR (Fyumagwa *et al.* 2009). Two out of 37 *Babesia* species positive pools from *Rh. evertsi evertsi* had mixed infection with *Anaplasma* species which were not identified as *A. marginale* or *A. phagocytophilum* by real-time PCR (Fyumagwa *et al.* 2009). Sequencing of amplified 18S rRNA fragments of *Babesia* species positive pools from *Rh. appendiculatus* and *Rh. evertsi evertsi* detected *Babesia equi* (GU361784–GU361791). The *Rh. appendiculatus* were from questing ticks and *Rh. evertsi evertsi* positive pools were collected on zebra (Table 1).

#### *Theileria* species

Thirteen of 229 DNA pools comprising of *Rh. appendiculatus* tested positive for *Theileria* species. The sequence analysis of selected ampli-

**Table 1.** Tick-borne haemoparasites detected by conventional PCR and sequencing results from selected pools.

Tick species & pools tested (n)	Source of ticks	Positive pools	Sequencing results	GenBank accession number
<i>A. gemma</i> (5)	Ground	1	No haemoparasites	–
<i>A. variegatum</i> (5)	Ground	3	Not performed	–
<i>Rh. appendiculatus</i> (229)	Questing	32	<i>Babesia equi</i> <i>Theileria parva</i> , <i>Theileria buffeli</i>	GU361785–GU361791 GU361781–GU361794 GU361795–GU361799
<i>Rh. compositus</i> (41)	Questing	17	Not performed	–
<i>Rh. decoloratus</i> (2)	Buffalo		Not performed	–
<i>Rh. evertsi</i> (16)	Buffalo, zebra	7	<i>Babesia equi</i>	GU361784
<i>Rh. praetextatus</i> (16)	Questing	2	<i>Anaplasma bovis</i>	GU361777–GU361780
<i>Rh. pulchellus</i> (13)	Questing	1	Not performed	–
<i>Rh. sanguineus</i> (6)	Lions	2	Not performed	–

fied 18S rRNA fragments from *Rh. appendiculatus* revealed *T. parva* (GU361781–GU361783; GU361792–GU361794) and *T. buffeli* (GU361795–GU361799). The number of positive tick pools from this tick species is summarized in Table 1. The infection prevalence amongst tick species for *Babesia* species, *Anaplasma* species and *Theileria* species were significantly different ( $P < 0.001$ ), with *Babesia* species having higher infection prevalence.

#### DISCUSSION

Ngorongoro Crater has a high diversity of tick species (Fyumagwa *et al.* 2007). Extensive movement of Maasai livestock in northern Tanzania and across the international border with Kenya and high interaction with wildlife in the crater have probably contributed to the high tick diversity (Estes *et al.* 2006; Fyumagwa *et al.* 2007). *Rh. appendiculatus* was by far the most abundant, suggesting its importance in the epidemiology of tick-borne haemoparasites (Norval 1994; Zieger *et al.* 1998; Grootenhuys 2000; Fyumagwa *et al.* 2007). High diversity of infected tick species (eight out of nine) suggests that there is probably a high prevalence of tick-borne pathogens in reservoir hosts in the crater which pose potential risk to susceptible animals when stressed (Dobson & Hudson 1995; Daszak *et al.* 2000).

*Anaplasma* species were detected in six of 11 tick species and had the widest vector range than any haemoparasite detected in this study. *Rh. evertsi evertsi* tested negative for both *A. marginale* and *A. phagocytophilum* in real-time PCR (Fyumagwa *et al.* 2009), and in sequence analysis of the 16S rRNA gene fragments, was

also negative for *Anaplasma bovis*. *Amblyomma variegatum* tested positive for *Anaplasma* species but was negative for *A. marginale* and *A. phagocytophilum* in real-time PCR (Fyumagwa *et al.* 2009). However, *A. variegatum* is described as a biological vector of *Anaplasma bovis* (Goethert & Telford 2003; Walker *et al.* 2003). *Anaplasma bovis* concurrent with high tick infestation can synergistically compromise the immunity of infected susceptible animals (Horak *et al.* 1983; Norval 1994; Walker *et al.* 2003). *Amblyomma gemma* is known to transmit *Ehrlichia ruminantium* (Jongejan & Uilenberg 1994; Wesonga *et al.* 2001) but it was not detected with the rickettsia in this study but was previously detected with *A. marginale* (Fyumagwa *et al.* 2009). On sequence analysis *A. bovis* was detected only in *Rh. Praetextatus* and this tick species was previously not described as an important vector for the rickettsia (Walker *et al.* 2003). The reason for lack of information on its importance in transmission of *Anaplasma* species could be due to insufficient studies and absence of this tick species in areas where more research work has been conducted. As cattle are susceptible to the infection, it is likely that Maasai cattle in Ngorongoro Crater are at high risk of contracting the infection with clinical anaplasmosis leading to high economic loss from retarded growth, treatment cost and mortality (Sparagano *et al.* 1999; Fyumagwa *et al.* 2009).

*Rhipicephalus decoloratus*, *Rh. microplus*, *Rh. evertsi evertsi* and *Rh. sanguineus* are known to transmit *Babesia* species in domestic animals in tropical and subtropical Africa (Regassa *et al.* 2003; Walker *et al.* 2003; Oliveira-Sequeira *et al.* 2005). *Babesia* species were detected in questing



*A. gemma*, *Rh. appendiculatus* and *Rh. compositus* (Table 1) but *A. gemma* has not been reported to harbour *Babesia* species (Regassa *et al.* 2003; Walker *et al.* 2003). On sequence analysis two pools of *Rh. appendiculatus* that were PCR positive for *Babesia* species turned to be *Theileria parva* and two pools were positive for *Babesia equi*, respectively. One pool from *Rh. evertsi* collected on zebra was positive for *Babesia equi* on sequence analysis suggesting that zebras are important reservoir of the haemoparasite. Nineteen positive pools for *Babesia* species were in questing ticks from areas with relatively tall grass (Lerai Forest, Shamba ya Faru, Ngoitokitok, Msalabani, Daraja 3, and Miti 3) (Fyumagwa *et al.* 2007), suggesting that the prevalence of infected ticks was localized. Babesiosis causes high mortality to immuno-compromised animals (Homer *et al.* 2000; Dumler 2005) and *B. bicornis* caused mortality of nutritionally stressed black rhinos in Ngorongoro Crater in 2001 (Nijhof *et al.* 2003; Fyumagwa *et al.* 2004).

Theileriosis due to *Theileria parva* is widespread and causes greatest losses among cattle in eastern, central and southern Africa (Lawrence *et al.* 1994; Uilenberg 1995; Lynen *et al.* 2007). In Tanzania the mortality rate is approximately 12% in local cattle population and it can reach 100% in exotic cattle breeds (Fyumagwa 2004). *Rh. appendiculatus* is the most important tick species in the epidemiology of theileriosis (Zieger *et al.* 1998; Grootenhuis 2000; Walker *et al.* 2003; Lynen *et al.* 2003). Its predominance in abundance amongst tick species can exacerbate the infection prevalence due to frequent exposure to reservoir hosts (Gulland 1995; Dobson & Hudson 1995). About 2.1% of *Rh. compositus* was detected with *Theileria* species but was not sequenced to identify the *Theileria* species. Symptoms of theileriosis were observed in 40% of buffalo calves in Ngorongoro Crater in 2001 (Fyumagwa *et al.* 2003). It has also been described that theileriosis caused 100% mortality in roan antelope calves (*Hippotragus equines*) in South Africa, and other wild ungulate species are also susceptible to theileriosis (Jardine 1992; Stoltz & Dunsterville 1992; Lawrence *et al.* 1994; Steyl *et al.* 2003; Nijhof *et al.* 2005). Therefore, theileriosis is a disease of importance to both livestock and wildlife. *Theileria buffeli* was detected in five clones from questing *Rh. appendiculatus*. In Namibia it has been detected in hartebeest (*Alcelaphus buselaphus*) (Spitalska *et al.* 2005).

The wide range of tick species detected with

haemoparasites suggests that mortality in livestock and wild animals in Ngorongoro Crater in 2000 and 2001 was probably exacerbated by tick-borne diseases. A change in the environment, which favours increase in buffalo numbers, is one of the predisposing factors in the development of high tick density. Detection of *Anaplasma bovis* in *Rh. praetextatus* is a new finding. Many *Rh. appendiculatus* clones had *Theileria* species and *Babesia* species in agreement with previous reports that the tick species is very important in the epidemiology of tick-borne diseases. Failure to detect *Babesia bicornis* which caused mortality to black rhinos in the crater in 2001 and its vectors requires further investigation.

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#### REFERENCES

- ALEKSEEV, A.N. 1998. The possible consequences of the probable global warming of the climate for the spread of blood-sucking ectoparasites and the pathogens they transmit. *Med. Parasitol.* 4: 3–8.
- BORETTI, F.S., PERRETEN, A., MELI, M.L., CATTORI, V., WILLI, B., WENGLI, N., HORNOK, S., HONEGGER, H., HEGGLIN, D., WOELFEL, R., REUSCH, C.E., LUTZ, H. & HOFMANN-LEHMANN, R. 2009. Molecular investigations of *Rickettsia helvetica* infection in dogs, foxes, humans, and *Ixodes* ticks. *Appl. Environ. Microbiol.* 75(10): 3230–3237.
- CHANSIRI, K., KAWAZU, S., KAMIO, T., TERADA, Y., FUJISAKI, K., PHILIPPE, H. & SARATAPHAN, N. 1999. Molecular phylogenetic studies on *Theileria* parasites based on small subunit ribosomal RNA gene sequences. *Vet. Parasitol.* 83(2): 99–105.
- DASZAK, P., CUNNINGHAM, A.A. & HYATT, A.D. 2000. Emerging infectious diseases of wildlife – Threats to biodiversity and human health. *Sci. Compass.* 287: 443–449.
- DOBSON, A.P. & HUDSON, P.J. 1995. Microparasites: Observed patterns in wild animal populations. In: B.T

- Grenfell & A.P. Dobson (Eds), Ecology of infectious diseases in natural populations (pp. 52–89). Publications of the Newton Institute, Cambridge University Press, Cambridge.
- DUMLER, J.S. 2005. *Anaplasma* and *Ehrlichia* infection. *Ann. N.Y. Acad. Sci.* 1063: 361–373.
- ESTES, R.D., ATTWOOD, J.L. & ESTES, A.B. 2006. Downward trends in Ngorongoro Crater ungulate populations: Conservation concerns and the need for ecological research. *Biol. Conserv.* 106: 106–120.
- ESTRADA-PENA, A. & JONGEJAN, F. 1999. Ticks feeding on humans; a review of records on human biting *Ixodidae* with special reference to pathogen transmission. *Expt. Appl. Acarol.* 23: 685–715.
- FYUMAGWA, R.D. 2004. Diseases status in livestock-wildlife interfaces in Tanzania. Proceedings of the WDA-Africa and Middle East Section Annual Conference, Abu Dhabi.
- FYUMAGWA, R.D., MKUMBO, S.S., WIJK, H., AMIYO, T., NYAKI, A. & SOOMBE, C. 2003. Theileriosis in free ranging African buffalo population in Ngorongoro Crater, Tanzania. Proceedings of the TAWIRI Annual Scientific Conference, December 2002, Arusha.
- FYUMAGWA, R.D., MKUMBO, S.S. & MORKEL, P.B. 2004. Remote treatment of black rhinos against babesiosis in Ngorongoro Crater, Tanzania. *Pachy.* 37: 80–83.
- FYUMAGWA, R.D., RUNYORO, V., HORAK, I.G. & HOARE, R. 2007. Ecology and control of ticks as disease vectors in wildlife of the Ngorongoro Crater, Tanzania. *S. Afr. J. Wildl. Res.* 37(1): 79–90.
- FYUMAGWA, R.D., SIMMLER, P., MELI, M.L., HOARE, R., HOFMANN-LEHMANN, R. & LUTZ, H. 2009. Prevalence of *Anaplasma marginale* in different tick species from Ngorongoro Crater, Tanzania. *Vet. Parasitol.* 161: 154–157.
- GARCIA, T.D., FIGUEROA, M.J., ROJAS, M.C., CANTO, A.G., FALCON, N.A. & ALVAREZ, M.J. 2004. Immune response to *Babesia bigemina* infection in pregnant cows. *Ann. N.Y. Acad. Sci.* 1026: 298–301.
- GOETHERT, H.K. & TELFORD, S.R. 2003. Enzootic transmission of *Anaplasma bovis* in Nantucket cotton tail rabbits. *J. Clin. Microbiol.* 41(8): 3744–3747.
- GOODMAN, J.L., NELSON, C., VITALE, B., MADIGAN, J.E., DUMLER, J.S., KURTTI, T.J. & MUNDERLOH, U.G. 1996. Direct cultivation of the causative agent of human granulocytic ehrlichiosis. *N. Engl. J. Med.* 334: 209–215.
- GROOTENHUIS, J.G. 2000. Wildlife, Livestock and Animal Disease Reservoirs. In: H.H.T. Prins, J.G. Grootenhuys & T.T. Dolan (Eds), Wildlife research for sustainable use (pp. 81–113). Kluwer Academic Publishers, Dordrecht.
- GULLAND, F.M.D. 1995. The impact of infectious diseases on wild animal populations – A review. In: B.T. Grenfell & A.P. Dobson (Eds), Ecology of infectious diseases in natural populations (pp. 20–51). Publications of the Newton Institute. Cambridge University Press, Cambridge.
- HOMER, M.J., AGUILAR-DELFIN, I., TELFORD, S.R., KRAUSE, P.J., PERSING, D.H. 2000. Babesiosis. *Clin. Microbiol. Rev.* 13(3): 451–469.
- HORAK, I.G., POTGIETER, F.T., WALKER, J.B., DE VOS, V. & BOOMKER, J. 1983. The ixodid tick burdens in various large ruminant species in South African nature reserves. *Orderstepoort J. Vet. Res.* 50: 221–228.
- JARDINE, J.E. 1992. The pathology of cytauxzoonosis in a tsessebe (*Damaliscus lunatus*). *J. S. Afr. Vet. Assoc.* 63: 49–51.
- JONGEJAN, F. & UILENBERG, G. 1994. Ticks and control methods. *Revue. Sci. Techn. Off. Intern. Epiz.* 13(4): 1201–1226.
- KETTLE, D.S. 1995. Babesiosis and Theileriosis; Typhus and Rickettsial Diseases, In: Medical and veterinary entomology, 2nd edn (pp. 517–536). CABI Publishing, Wallingford.
- KOCAN, K.M., DE LA FUENTE, J., BLOUIN, E.F. & GARCIA-GARCIA, J.C. 2004. *Anaplasma marginale* (Rickettsiales: Anaplasmataceae): recent advances in defining host-pathogen adaptations of a tick-borne rickettsia. *Parasitology* 129: 280–300.
- LAWRENCE, J.A., DE VOS, A.J. & IRVIN, A.D. 1994. East Coast Fever, In: J.A.W. Coetzer, G.R. Thomson & R.C. Tustin (Eds), Infectious diseases of livestock. With special reference to Southern Africa. Vol. 1. (pp. 309–325). University of Cape Town Press, Cape Town.
- LYNEN, G., ZEMAN, P., BAKUNAME, C., DI GIULIO, G., MTUI, P., SANKA, P. & JONGEJAN, F. 2007. Cattle ticks of the genera *Rhipicephalus* and *Amblyomma* of economic importance in Tanzania: distribution assessed with GIS based on an extensive field survey. *Expt. Appl. Acarol.* 43: 303–319.
- MORZARIA, S.P. 1989. Identification of *Theileria* species and characterization of *Theileria parva* stocks. Online at: <http://www.fao.org/wairdocs/ILRI/x5549E/x5549eOt.htm>
- MUNSON, L., TERIO, K.A., KOCK, R., MLENGEYA, T., ROELKE, M.E., DUBOVI, E., SUMMERS, B., SINCLAIR, A.R.E. & PACKER, C. 2008. Climate extremes promote fatal co-infections during canine distemper epidemics in African lions. *PLoS ONE* 3(6): e2545. doi:10.1371/journal.pone.0002545.
- NIJHOF, A.M., PENZHORN, B.L., LYNEN, G., MOLLEL, J.O., MORKEL, P., BEKKER, C.P.J. & JONGEJAN, F. 2003. *Babesia bicornis* sp. nov. and *Theileria bicornis* sp. nov.: tick-borne parasites associated with mortality in the black Rhinoceros (*Diceros bicornis*). *J. Clin. Microbiol.* 41(5): 2249–2254.
- NIJHOF, A.M., PILLAY, V., STEYL, J., PROZESKY, L., STOLTSZ, W.H., LAWRENCE, J.A., PENZHORN, B.L. & JONGEJAN, F. 2005. Molecular characterization of *Theileria* species associated with mortality in four species of African antelopes. *J. Clin. Microbiol.* 43: 5907–5911.
- NORVAL, R.A.I. 1994. Vectors: Ticks, In: J.A.W. Coetzer, G.R. Thomson & R.C. Tustin (Eds), Infectious Diseases of Livestock Vol. 1, with special reference to Southern Africa (pp. 1–24). Oxford University Press, Cape Town.
- OLIVEIRA-SEQUEIRA, T.C.G., OLIVEIRA, M.C.S., ARAUJO, J.P. & AMARANTE, A.F.T. 2005. PCR-based detection of *Babesia bovis* and *B. bigemina* in their natural host *Boophilus microplus* and cattle. *Int. J. Parasitol.* 35(1): 105–111.

- OSBURN, B., SCOTT, C. & GIBBS, P. 2009. One World-one Medicine-one Health: emerging veterinary challenges and opportunities. *Rev. Sci. Tech. Off. Int. Epiz.* 28(2): 481–486.
- PENZHORN, B.L. 2006. Babesiosis of wild carnivores and ungulates. *Vet. Parasitol.* 138: 11–21.
- PETRIE, A. & WATSON, P. 2004. *Statistics for Veterinary and Animal science.* Blackwell Science Ltd, Oxford.
- PFITZER, S., LAST, R. & DE WAAL, D.T. 2004. Death of a buffalo calf (*Syncerus caffer*) due to suspected heart water (*Ehrlichia ruminantium*). *J. S. Afr. Vet. Assoc.* 75(1): 54–57.
- REGASSA, A., PENZHORN, B.L. & BRYSON, N.R. 2003. Attainment of endemic stability to *Babesia bigemina* in cattle on a South African ranch where non-intensive tick control was applied. *Vet. Parasitol.* 116(4): 267–274.
- RUNYORO, V.A., HOFER, H., CHAUSI, E.B. & MOEHLMAN, P.D. 1995. Long-term trends in the herbivore populations of the Ngorongoro Crater, Tanzania. In: A.R.E. Sinclair & P. Arcese (Eds), *Serengeti II, Dynamics, management and conservation of an ecosystem* (pp. 146–168). University of Chicago Press, Chicago.
- SPARAGANO, O.A.E., ALLSOPP, M.T.E.P., MANK, R.A., RIJPKEMA, S.G.T., FIGUEROA, J.V. & JONGEJAN, F. 1999. Molecular detection of pathogen DNA in ticks (Acari; Ixodidae): A review. *Expt. Appl. Acarol.* 23: 929–960.
- SPITALSKA, E., RIDDELL, M., HEYNE, H. & SPARAGANO, O.A.E. 2005. Prevalence of theileriosis in red hartebeest (*Alcelaphus buselaphus caama*) in Namibia. *Parasitol. Res.* 97: 77–97.
- STEYL, J., LAWRENCE, J., PROZESKY, L. & STOLTSZ, H. 2003. Theileriosis in roan antelope. A paper presented at a meeting at Onderstepoort. *Wildlife Group Newsletter* April: 4–9.
- STOLTSZ, W.H. & DUNSTERVILLE, M.T. 1992. In vitro establishment and cultivation of *Cytauxzoon* species (*Theileria* sp) from a sable antelope (*Hippotragus niger*, Harnis 1838). *J. S. Afr. Vet. Assoc.* 63:183.
- UILENBERG, G. 1995. International collaborative research: significance of tick-borne haemoparasitic diseases to world animal health. *Vet. Parasitol.* 57: 19–41.
- WALKER, A.R., BOUATTOUR, A., CAMICAS, J.L., ESTRADA-PENA, A., HORAK, I.G., LATIF, A.A., PEGRAM, R.G. & PRESTON, P.M. 2003. Ticks of domestic animals in Africa: a guide to identification of species. *Bioscience Reports* 42. Edinburgh.
- WALTER, S.D., HILDRETH, S.W. & BEATY, B.J. 1980. Estimation of infection rates in populations of organisms using pools of variable size. *Am. J. Epidemiol.* 112: 124–128.
- WESONGA, F.D., MUKOLWE, S.W. & GROOTENHUIS, J. 2001. Transmission of *Cowdria ruminantium* by *Amblyomma gemma* from infected African buffalo (*Syncerus caffer*) and eland (*Taurotragus oryx*) to sheep. *Trop. Anim. Hlth. Prod.* 33(5): 379–390.
- ZIEGER, U., HORAK, I.G., CAULDWELL, A.E. & UYS, A.C. 1998. Ixodid tick infestations of wild birds and mammals on a game ranch in Central Province, Zambia. *Onderstepoort J. Vet. Res.* 65(2): 113–124.

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Paper vi





Short communication

## Severe *Streptococcus* infection in spotted hyenas in the Ngorongoro Crater, Tanzania

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### Abstract

In a population of spotted hyenas (*Crocuta crocuta*) monitored between 1996 and 2005 in the Ngorongoro Crater, Tanzania, 16 individuals from five of eight social groups displayed clinical signs of an infection, including severe unilateral swelling of the head followed by abscess formation at the mandibular angle, respiratory distress, mild ataxia, and lethargy. Two (12.5%) of these 16 individuals died within days of developing signs. Clinical signs in hyenas were first noted in 2001, and most cases occurred between September 2002 and February 2003, suggesting an outbreak of infection during this period. Histopathological examination of internal organs from one hyena that died with signs revealed morphological changes consistent with severe bacterial infection. Phenotypic examination and phylogenetic analysis of the 16S rRNA gene of the causative agent of infection revealed a Lancefield group C *Streptococcus* with a high level of homology to *S. equi* subsp. *ruminatorum*, a subspecies of *S. equi* recently described in domestic sheep (*Ovis aries*) and goats (*Capra hircus*) with mastitis in Spain. Strains similar to this bacterium were also isolated from two hyenas without obvious clinical signs, suggesting that hyenas may be 'carriers' of this bacterium, and from a sympatric Burchell's zebra (*Equus burchelli*), a herbivore species often consumed by hyenas. To our knowledge this is the first report of a *Streptococcus* infection in these two wildlife species. The high genetic similarity between the hyena and zebra isolates indicates that inter-specific transmission may occur, possibly when hyenas consume infected zebra carcasses.

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**Keywords:** *Crocuta crocuta*; Livestock; Ngorongoro Crater; Spotted hyena; *Streptococcus equi* subsp. *ruminatorum*; Wildlife

### 1. Introduction

The spotted hyena (*Crocuta crocuta*) is the most numerous large carnivore in the Ngorongoro Crater,

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Tanzania. The resident population of hyenas on the 250 km<sup>2</sup> Crater floor consists of eight social groups (clans) that contain between 10 and 60 members (Wachter et al., 2002; Höner et al., 2005). Clan members are highly social and often feed together on carcasses. Although clans defend territories, individuals leave their territories when food availability is low to forage in areas where densities of herbivores are high (Höner et al., 2005). As a result, there is considerable potential for transmission of pathogens between individuals and social groups. An important prey species of hyenas in the Crater is the Burchell's zebra (*Equus burchelli*) (Höner et al., 2002).

Here we report a *Streptococcus* infection of hyenas and a zebra in the Crater, describe the external clinical signs and pathological changes associated with severe infection, and identify the causative agent using standard bacteriological and molecular genetic techniques.

## 2. Methods

### 2.1. Study population and demography

The Ngorongoro Crater is a large caldera in northern Tanzania (3°S, 35°E). All hyenas in the population were individually recognised (Wachter et al., 2002; Höner et al., 2005). Between April 1996 and February 2005, the Crater contained between 171 and 347 spotted hyenas and a mean of 3600 zebras (Höner et al., 2005; Ngorongoro Ecological Monitoring Programme, 2005).

### 2.2. Histopathology and bacteriological analyses

Tissue samples were obtained from one hyena that died with severe clinical signs in 2002, seven hyenas that died without clinical signs between 1997 and 2004, and a swab from a minor skin wound of a zebra that was anaesthetised in 2004 for other purposes. Samples for histopathology were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned at 4 µm and stained with haematoxylin–eosin and Giemsa. Samples for bacteriological culture were stored and transported at –80 °C, and cultivated on Columbia sheep blood agar (5% blood), Chocolate agar, Gassner medium, and McConkey II agar (Oxoid, Wesel, Germany). Agar plates were incubated aerobically (5% CO<sub>2</sub>) and

anaerobically (AnaeroJar 2.51, Oxoid) at 37 °C for 48–72 h. In addition, all samples were incubated in nutrient broth I (Sifin, Berlin, Germany) at 37 °C for 24 h. Isolates were characterised on the basis of Gram staining, cellular morphology, and catalase and oxidase reaction. The Lancefield serological group reaction was determined using the Streptococcal Grouping Kit (Oxoid) according to the manufacturer's instructions.

### 2.3. Sequence analysis and phylogenetic calculations

The phylogenetic relationship of bacterial isolates was investigated by sequencing a 1396 bp segment of their 16S rRNA gene, and comparing the sequences with *Streptococcus* sequences entered in the GenBank (Fig. 2). Cycling conditions were: 3 min 94 °C, 35 × (15 s 94 °C, 30 s 55 °C, 90 s 72 °C), 7 min 72 °C. PCR reaction mixtures (25 µl) contained 0.5 U AmpliTaq DNA polymerase (Applied Biosystems, Darmstadt, Germany), 2.5 µl 10× PCR-buffer, 1.5 mM MgCl<sub>2</sub>, 200 µM dNTPs, 10 pmol of each primer (TPU1 and RTU8; Wyss et al., 1996) and ~50 ng of DNA. PCR products were purified with ExoSAP-IT™ (Amersham Bioscience, Freiburg, Germany), sequenced bidirectionally using the BigDye™ cycle sequencing kit (Applied Biosystems), and visualized on an ABI A3100 automated sequencer. The following sequencing primers were used: Strep2F 5'-TAA CTA ACC AGA AAG GGA CG-3', Strep3R 5'-CAC GAG CTG ACG ACA ACC-3', Strep4F 5'-AGA ACC TTA CCA GGT CTT GAC-3' and Strep5R 5'-TCA GAC TTA TTA AAC CGC CTG-3'. The phylogenetic tree (Fig. 2) was constructed by applying the neighbor-joining algorithm and Kimura-2 parameter distances using MEGA 2.1 (Kumar et al., 2001). We compared these results with those produced by maximum parsimony and maximum likelihood analyses using PAUP\* v.4.0b10 (Swofford, 2002), and found that all analyses revealed trees with similar topologies.

## 3. Results

### 3.1. Clinical signs

Clinical signs were observed in 16 hyenas from five of eight social groups of the Crater. One hyena

expressed and cleared signs on three occasions. Signs were pronounced and included a diffuse, unilateral swelling of the head (Fig. 1a), respiratory distress, serous ocular discharge, mild ataxia, and lethargy. Swellings became localized at the mandibular angle (Fig. 1b), where abscesses developed in 13 (72.2%) of the observed swellings. Eleven abscesses (84.6%) ruptured and drained externally (Fig. 1c), resulting in the clearance of external signs. Clinical signs usually were expressed and cleared within 7–24 days, but in one case the abscess was visible for 9 months. Three hyenas expressed acute signs including severe swelling of head and neck, apathy and marked ataxia (Fig. 1d). While one of these individuals recovered, the two others died within days of developing acute signs.

Although monitoring began in 1996, the first hyena with clinical signs was observed in August 2001. Most hyenas (94%,  $n = 15$ ) with clinical signs were observed between September 2002 and February 2003, suggesting an outbreak of infection during this period. Only one further hyena expressed clinical signs after this period, in December 2004.

### 3.2. Histopathology and bacteriology

Histopathological examination of tissue from one hyena that died with acute signs revealed extensive suppurative inflammation and oedema with numerous bacterial colonies within the soft tissue around the mandibular angle. Additionally, there was severe suppurative necrotizing pneumonia intermixed with



Fig. 1. Spotted hyenas with clinical signs of infection with a *Streptococcus* similar to *S. equi* subsp. *ruminatorum*: (a) hyena with diffuse unilateral swelling of the head that led to temporary occlusion of the right eye, (b) hyena with gross unilateral swelling around and below the right mandibular angle, (c) young hyena after rupture and drainage of an abscess, (d) apathetic and ataxic hyena with neck swelling 2 days before death.

similar bacteria, and marked intraalveolar haemorrhage.

Three phenotypically similar bacterial isolates were cultured from lung, mandibular lymph node, and tissue from the mandibular angle from the hyena that died with clinical signs. Two further phenotypically similar isolates were cultured from the tonsil from one hyena without clinical signs at death and from a minor skin wound of a zebra. Two isolates were obtained from the tonsil from a second hyena without signs at death; one isolate was phenotypically similar to and the other different from the previous cultures. All seven isolates were Gram-positive, catalase-negative, and beta-hemolytic with colony morphology suggestive of *Streptococcus*, and were classified as *Streptococcus* of Lancefield's serogroup C. Samples from an additional five hyenas that died without signs did not reveal streptococci of Lancefield's serogroup C.

### 3.3. Molecular genetic analysis

One of three phenotypically similar isolates from the hyena that died with clinical signs (hyena M149), the two similar isolates from the two hyenas without signs (hyena A084, hyena E118-1), the dissimilar isolate from one of the hyenas without signs (hyena E118-2), and the isolate from the zebra (Zebra EQ003) were sequenced (Fig. 2). All five isolates were genetically highly similar (ingroup mean distance =  $0.001 \pm 0.001$ , overall mean distance =  $0.045 \pm 0.004$ ), and the isolate from one hyena (hyena A084) was identical to the zebra isolate (Fig. 2). The *Streptococcus* isolates from the Crater were identical or highly similar to the *S. equi* subsp. *ruminatorum* type strain CECT 5772<sup>T</sup> (AJ605748; Fig. 2). The five Crater isolates plus the *S. equi* subsp. *ruminatorum* type strain formed a sister clade to the clade containing *S. equi* subsp. *equi* and *S. equi* subsp.

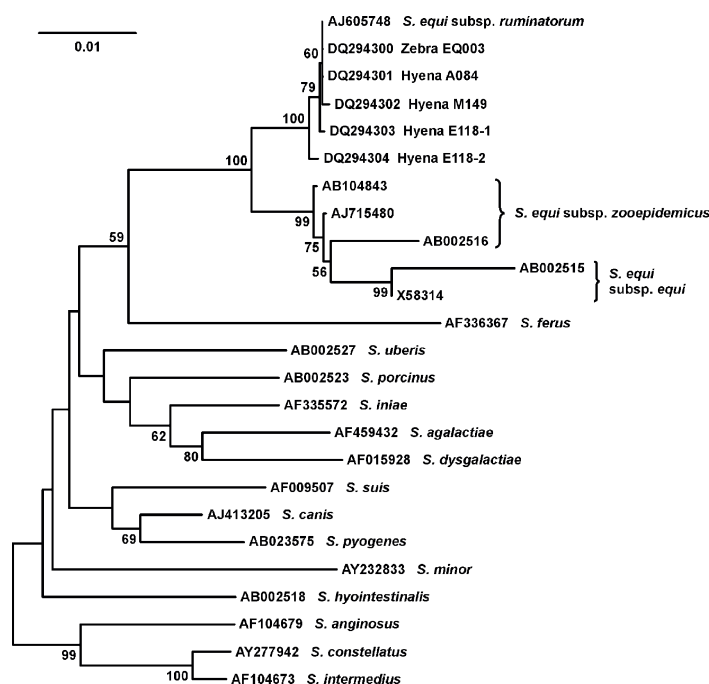


Fig. 2. Phylogenetic relationship between *Streptococcus* isolates based on a 1370 bp segment of the 16S rRNA gene. Included are four 'hyena' isolates from three spotted hyenas in the Ngorongoro Crater (isolates hyena E118-1 and hyena E118-2 were obtained from one hyena), one isolate from a sympatric zebra (zebra EQ003), and isolates from the GenBank (marked by their accession numbers). Numbers located at nodes indicate bootstrap support >50% derived from 1000 pseudoreplications.



*zooepidemicus* as supported by high bootstrap values (Fig. 2).

#### 4. Discussion

We report infection of spotted hyenas in the Ngorongoro Crater, Tanzania, with a beta-hemolytic Lancefield group C *Streptococcus* genetically most closely related to *S. equi* subsp. *ruminatorum*, a bacterium only recently described in domestic goats and sheep with clinical and subclinical mastitis in Spain (Fernández et al., 2004). Infection with this bacterium in some hyenas resulted in clinical signs similar to those of domestic horses (*Equus caballus*) with ‘strangles’, a condition caused by *S. equi* subsp. *equi* (Timoney, 2004). The patho-histological changes found in one hyena that died of infection revealed that internal drainage of the abscess most likely caused the severe fatal infection of the lung. Although a proportion (12.5%) of hyenas that displayed clinical signs of infection died within days of developing signs, most recovered from infection. Similar levels of mortality and recovery have been noted among horses infected with *S. equi* subsp. *equi* (Jorm, 1990; Sweeney et al., 1989). Streptococci with a high level of homology to *S. equi* subsp. *ruminatorum* were also isolated from hyenas without clinical signs, suggesting that hyenas may be ‘carriers’ of infection, as has been demonstrated for horses infected with *S. equi* subsp. *equi* (George et al., 1983; Sweeney et al., 1989).

*S. equi* subsp. *ruminatorum* is closely related to *S. equi* subsp. *equi* and *S. equi* subsp. *zooepidemicus* (Fig. 2). Both *S. equi* subsp. *equi* and *S. equi* subsp. *zooepidemicus* are highly contagious, can spread rapidly through host populations (Sweeney et al., 1989), and cause severe diseases in domestic animals and humans (Balter et al., 2000; Segura and Gottschalk, 2004). They are usually transmitted via oral and nasal routes, but they can also be transmitted indirectly via contaminated water or feed (Jorm, 1992), and between different host species (Soedarmanto et al., 1996). The observation of clinical signs in hyenas from several Crater clans during a relatively short period, and the high genetic similarity between hyena isolates, suggest that this *Streptococcus* may have been transmitted between hyenas, possibly when

saliva was transferred during social interactions between clan members (East et al., 1993, 2001), or when hyenas feed on large carcasses. The isolation of the same *Streptococcus* from a sympatric zebra further suggests that this bacterium may be transmitted between species, possibly when hyenas feed on infected zebra carcasses. To our knowledge this is the first report of *Streptococcus* infection in spotted hyenas and Burchell’s zebra, and the first report of the occurrence of *S. equi* subsp. *ruminatorum* in wildlife species. The epidemiology of *Streptococcus* infections in wildlife in the Crater, possible causes for the outbreak of infection in 2002/2003, and the impact of the outbreak on the hyena population are currently under investigation.

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#### References

- Balter, S., Benin, A., Pinto, S.W.L., Teixeira, L.M., Alvim, G.G., Luna, E., Jackson, D., LaClaire, L., Elliott, J., Facklam, R., Schuchat, A., 2000. Epidemic nephritis in Nova Serrana, Brazil. *Lancet* 355, 1776–1780.
- East, M.L., Hofer, H., Wickler, W., 1993. The erect ‘penis’ is a flag of submission in a female-dominated society: greetings in Serengeti spotted hyenas. *Behav. Ecol. Sociobiol.* 33, 355–370.
- East, M.L., Hofer, H., Cox, J.H., Wulle, U., Wiik, H., Pitra, C., 2001. Regular exposure to rabies virus and lack of symptomatic disease in Serengeti spotted hyenas. *Proc. Natl. Acad. Sci. U.S.A.* 98, 1526–1531.
- Fernández, E., Blume, V., Garrido, P., Collins, M.D., Mateos, A., Domínguez, L., Fernández-Garayzabal, J.F., 2004. *Streptococcus equi* subsp. *ruminatorum* subsp. nov., isolated from mastitis in small ruminants. *Int. J. Syst. Evol. Microbiol.* 54, 2291–2296.

- George, J.L., Reif, J.S., Shiedeler, R.K., Small, C.J., Ellis, R.P., Snyder, S.P., McChesney, A.E., 1983. Identification of carriers of *Streptococcus equi* in a naturally infected herd. *JAVMA* 183, 80–84.
- Höner, O.P., Wachter, B., East, M.L., Hofer, H., 2002. The response of spotted hyenas to long-term changes in prey populations: functional response and interspecific kleptoparasitism. *J. Anim. Ecol.* 71, 236–246.
- Höner, O.P., Wachter, B., East, M.L., Runyoro, V.A., Hofer, H., 2005. The effect of prey abundance and foraging tactics on the population dynamics of a social, territorial carnivore, the spotted hyena. *Oikos* 108, 544–554.
- Jorm, L.R., 1990. Strangles in horse studs: incidence, risk factors and effect of vaccination. *Aust. Vet. J.* 67, 436–439.
- Jorm, L.R., 1992. Laboratory studies on the survival of *Streptococcus equi* subspecies *equi* on surfaces. In: Proceedings of the Sixth International Conference on Equine Infectious Diseases, Cambridge, UK, pp. 39–43.
- Kumar, S., Tamura, K., Jakobsen, I.B., Nei, M., 2001. MEGA 2: Molecular Evolutionary Genetics Analysis Software. Arizona State University, Tempe, USA.
- Ngorongoro Ecological Monitoring Programme, 2005. Ecological Monitoring of the Ngorongoro Conservation Area. Ngorongoro Conservation Area Authority, Ngorongoro, Tanzania. Unpublished annual report.
- Segura, M., Gottschalk, M., 2004. Extracellular virulence factors of streptococci associated with animal diseases. *Front. Biosci.* 9, 1157–1188.
- Soedarmanto, I., Pasaribu, F.H., Wibawan, I.W.T., Lämmler, C., 1996. Identification and molecular characterization of serological group C streptococci isolated from diseased pigs and monkeys in Indonesia. *J. Clin. Microbiol.* 34, 2201–2204.
- Sweeney, C.R., Benson, C.E., Whitlock, R.H., Meirs, D.A., Barningham, S.O., Whitehead, S.C., Cohen, D., 1989. Description of an epizootic and persistence of *Streptococcus equi* infections in horses. *JAVMA* 194, 1281–1286.
- Swofford, D.L., 2002. PAUP\*: Phylogenetic Analysis Using Parsimony (\* and Other Methods). Version 4.0b.10. Sinauer Associates, Sunderland, USA.
- Timoney, J.F., 2004. The pathogenic equine streptococci. *Vet. Res.* 35, 397–409.
- Wachter, B., Höner, O.P., East, M.L., Golla, W., Hofer, H., 2002. Low aggression levels and unbiased sex ratios in a prey-rich environment: no evidence of siblicide in Ngorongoro spotted hyenas (*Crocuta crocuta*). *Behav. Ecol. Sociobiol.* 52, 348–356.
- Wyss, C., Choi, B.K., Schupbach, P., Guggenheim, B., Göbel, U.B., 1996. *Treponema maltophilum* sp. nov., a small oral spirochete isolated from human periodontal lesions. *Int. J. Syst. Bacteriol.* 46, 745–752.



## Paper vii



# Seroprevalence of *Brucella abortus* in buffaloes and wildebeests in the Serengeti ecosystem: A threat to humans and domestic ruminants

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## SUMMARY

A sero-survey was conducted in buffalo and wildebeests in Ngorongoro Crater and Serengeti National Park (SNP) collectively known as Serengeti ecosystem to establish the level of exposure to *Brucella abortus*. Rose Bengal Plate Agglutination test and Competitive ELISA were used serially in the analysis of 205 serum samples. The results indicated that 24% and 17% of buffalo and wildebeest populations respectively are exposed to the bacterium. The difference in the level of exposure of these herbivores to the bacterium in Ngorongoro Crater and SNP was insignificant ( $P>0.05$ ), suggesting that probably the infection is sustainable in the ecosystem. Because wildlife has high interaction with livestock in Ngorongoro Conservation Area, it is likely that livestock are at risk of exposure to the infection. More studies are recommended to understand its epidemiology and isolate the bacterium for characterization. A close monitoring of the wildlife populations in the two protected areas is important to establish the impact of the infection on the reproductive performance. Furthermore public awareness to the communities in the interface should be strengthened to reduce the risk of human exposure to *Brucella* infection.

**Keywords:** *Brucella abortus*, Serengeti ecosystem, odds ratio, relative risk, wildlife

## INTRODUCTION

Brucellosis is a zoonotic disease of great economic importance (Corbel, 1997) and the infection is transmitted through contact with fetal membranes, post-parturient discharges, raw milk and during mating (van der Leek *et al.*, 1993; Godfroid, 2002). The probability of brucellosis becoming established and being maintained in a species depends on a combination of factors including host susceptibility, infection dose, contact with infected animals, management and environmental factors (Godfroid, 2002).

In livestock brucellosis usually leads to abortion, which may result in high economic losses and may also interfere with international trade (Bishop *et al.*, 1994). Late-term abortions, birth of weak calves with poor survival rate, arthritis and bursitis with associated lameness and testicular infection are some of the clinical symptoms which are observed in wildlife and livestock (Davis *et al.*, 1990; Bevins *et al.*, 1996; Bishop *et al.*, 1994; van Tonder *et al.*, 1994; Herr, 1994; Bishop and Bosman, 1994). Basing on pathogenicity and host preference, six bacteria species are recognized, *Brucella abortus*, *B. melitensis*, *B. suis*, *B. ovis*, *B. canis* and *B. neotomae* (Alton *et al.*, 1988). However, the principal

pathogenic species worldwide are *Brucella abortus* (bovine brucellosis), *B. melitensis* (ovine and caprine brucellosis), and *B. suis* (swine brucellosis). In Mediterranean region, *B. melitensis* is the major cause of caprine and ovine brucellosis and is highly pathogenic for humans (van der Leek *et al.*, 1993; Herr, 1994).

In wildlife, brucellosis can be a result of spillover from infected livestock or as a natural sustainable infection within susceptible wildlife population (Bishop *et al.*, 1994; Davis *et al.*, 1990). Studies have shown that there is no difference in the pathogenicity and transmission rate of *B. abortus* from cattle to cattle and from cattle to bison, suggesting the possibility of spillover of the infection between livestock and wildlife (Davis *et al.*, 1990). This is of concern regarding the impact of the infection on wildlife reproductive rates and the possibility of wild animals acting as source of infection for domestic animals and humans (Mathias *et al.*, 1999). Lord and Flores (1983) made 23 isolations of *Brucella* species from a rodent *Capybara* (*Hydrochaeris hydrochaeris*), eight of which were *B. abortus* and 15 isolates were *B. suis*, suggesting that rodents are reservoirs and important in the epidemiology of *Brucella* infection in wildlife and the two *Brucella* species have been isolated worldwide from a great variety of wildlife species (Davis, 1990).

Brucellosis was reported for the first time in livestock in Tanzania in 1920's and *B. abortus* appears to be endemic in pastoral and agro pastoral communities, however, isolated cases have also been reported in smallholder dairy cattle (Fyumagwa *et al.*, 2004). In the northern zone of Tanzania the prevalence against *B. abortus* is about 8% in Maasai cattle and 3.3% in agro pastoral communities (Fyumagwa *et al.*, 2004). The difference in the prevalence is probably attributable to difference in management

practice. In the Annual report of 2003, the Ministry of Water and Livestock Development (MWLD, 2003) by then reported 245 bovine cases which were found to be seropositive for brucellosis but it did not show clearly how many cattle were screened, in which part of the country and therefore the prevalence is not indicated. In this context therefore, most of the information on brucellosis is derived from small sample surveys, which is subject to uncertainty (Bishop *et al.*, 1994). This provides a strong support for the need to conduct a thorough investigation to come up with a reasonable estimate of the prevalence of the infection in Tanzania.

Legal and illegal game hunting is carried out in Tanzania and the two wild herbivore species (buffalo *Syncerus caffer* and wildebeest *Connochaetes taurinus*) are among preferred species. Dressing of carcass in the field without knowledge of the zoonosis from infected carcass has a potential risk of spreading the infection to livestock and humans (Drew *et al.*, 1992). The wildlife populations in the Serengeti and Ngorongoro share the grazing ground and water sources with livestock. However, the effect of brucellosis on the reproductive performance to livestock and risk of zoonosis to human has not been considered seriously (Mathias *et al.*, 1999). In southern Africa, brucellosis with abortion symptoms has been reported in the lowveld of Mpumalanga and Limpopo (Bester, 2002). The observation suggests that the infection causes infertility in susceptible population in wildlife and can have a significant impact on the population growth. While this may represent a potential reservoir for infection to livestock, the actual level of risk to livestock remains unknown (Bishop *et al.*, 1994). Currently it is not known what level of infection exists in wild herbivores in Tanzania and its adverse effect on wildlife

population growth has not been established.

The aim of this study was to establish the prevalence of exposure to *B. abortus* in these wildlife species in the Serengeti Ecosystem and its possible impact to sustainable wildlife conservation and livestock production in the interface given the wild animal migration/grazing habits.

## **MATERIALS AND METHODS**

### **Study area and sample collection**

The study was conducted in Serengeti ecosystem which constitutes the Ngorongoro Conservation Area (NCA) which is a multiple land use area and the Serengeti National Park (SNP). The two protected areas are contiguous and located in the northern zone of Tanzania. The wild herbivores serum samples, which were used in the study include those of buffaloes and wildebeests. The serum samples were retrieved from the serum and tissue bank at the Serengeti Wildlife Research Center (SWRC) Veterinary laboratory. Sera were collected during rinderpest sero-surveillance in 2001 to 2004, wildebeest migration and reproductive physiology studies conducted in 2001 to 2003 and from tick ecology and tick borne pathogens study in Ngorongoro Crater in 2004.

### **Serum samples**

A total of 205 serum samples including 103 from buffaloes and 102 wildebeests were used in the serological analysis to determine the exposure of the two wild herbivores to brucellosis. For the buffaloes 27 samples were from NCA and 76 samples were from SNP and for wildebeest 31 samples were from NCA and 71 samples were from SNP.

### **Serological analysis**

To evaluate for exposure to *Brucella* organisms among selected herbivore species two serological tests were used. The Rose Bengal Plate Test (RBPT) using the standardized *B. abortus* Rose Bengal Plate Test Antigen, PA0060 Batch 266, Veterinary Laboratories Agency, New Haw, Addlestone, Surrey KT15 3NB, UK). This is a qualitative procedure involving visual assessment of agglutination of serum on glass slides. The RBPT was conducted at SWRC veterinary laboratory and tests were interpreted as positive or negative (Chernysheva *et al.*, 1980). The competitive ELISA (BRUCELLA 400 Veterinary Laboratories Agency, Weybridge UK) was conducted at Central Veterinary Laboratory (CVL) in Dar es Salaam by a procedure described by Harlow and Lane (1996).

### **Data analysis**

The sero-survey results for brucellosis in buffaloes and wildebeests were presented as an overall prevalence for the Serengeti ecosystem as well as location specific prevalence by stratifying the serum samples on their origin because the two protected areas (NCA and SNP) have different levels of interaction between wildlife and livestock and different management systems with the former having high interaction with Maasai livestock. The odds ratio (OR) and relative risk (RR) were established to be able to understand whether there is an association between location and prevalence of the infection. Chi-square test ( $\chi^2$ ) was used to compare the statistical significance in prevalence between the two protected areas (Petrie and Watson, 2004).

## **RESULTS**

### **Prevalence**

Both RBPT and competitive ELISA produced the same test results as shown in table 1 below. In buffaloes the overall prevalence was 24% that is 25 out of 103 sera tested positive for brucellosis in the Serengeti ecosystem. However location specific prevalence showed that prevalence of brucellosis in buffaloes was 30% in

NCA where as in SNP prevalence of brucellosis in buffaloes was 22%. In wildebeest the overall prevalence of brucellosis was 17% that is 17 out of 102 sera tested positive for brucellosis in the Serengeti ecosystem. The location specific prevalence was 19% in NCA and 15% in SNP.

**Table 1.** Positive reactors and prevalence rate of *Brucella abortus* using competitive ELISA and RBPT on blood sera from buffaloes and Wildebeests

Location/ Herbivore spp	NCA				SNP			
	Tests +ve	Tests -ve	Prevalence (%)	N	Tests +ve	Tests -ve	Prevalence (%)	N
Buffaloes	8	19	29.6	27	17	59	22.4	76
Wildebeests	6	25	19	31	11	60	15.5	71
Total	14	44	24	58	28	119	19	147

Note: NCA- Ngorongoro Conservation Area, SNP-Serengeti National Park

**Effect of interaction with livestock**

The NCA is a multiple land use area and is considered to have the highest livestock-wildlife interaction than any other protected area in Tanzania. The measure of biological association between locations as a contributing factor to the difference in the rate of exposure to the *Brucella* organisms in the two protected areas was determined using odds ratio (OR) and relative risk (RR). The OR for buffalo and wildebeest in NCA and SNP populations were 1.46, and 1.3 respectively and the RR for both species was 1.3.

The 95% confidence interval (CI) for buffalo and wildebeest in the two protected areas were 3.065 to 6.634 and 11.176 to 18.887 respectively. Because the OR for both buffalo and wildebeest are not contained within the CI and the RR is the same for both animal species, it is likely that herbivores in NCA and SNP are at equal risk of exposure to the microorganisms. Statistically the difference in the rate of exposure to *Brucella*

organism between the two protected areas was insignificant for both buffalo and wildebeest ( $\chi^2=0.245$ ;  $df=1$ ;  $P>0.05$  and  $\chi^2=0.037$ ;  $df=1$ ;  $P>0.05$  respectively).

**DISCUSSION**

The status of brucellosis in wildlife in different protected areas in Tanzania is not clearly known. High prevalence of exposure to *Brucella abortus* in the Serengeti ecosystem (24% and 17%) for buffalo and wildebeest populations respectively was an unexpected observation. In both buffalo and wildebeest populations the prevalence of exposure was higher in NCA than in SNP, however, the difference was statistically insignificant ( $P>0.05$ ). This suggests that the source of exposure for wildlife is not from livestock but rather from the contaminated environment or unknown wild reservoir. Although the difference in exposure is not statistically significant, it can be speculated that the disparity is due to high contamination of the environment in NCA because of the sedentary nature of wild

herbivores in the crater. Anthropogenic activities outside the crater in NCA and sustainable availability of forage and water in the Ngorongoro Crater restrict wild herbivores within the crater while in SNP wildlife migrates more extensively.

In the analysis Rose Bengal Plate Agglutination Test (RBPT) was highly sensitive and all serum samples which tested positive by competitive ELISA were also detected as positive by RBPT. This suggests that the qualitative test (RBPT) can be used for routine diagnosis under field conditions and the quantitative test can be used only for confirmation purpose corroborating with the report of Chernysheva *et al.* (1980).

Several workers have described that brucellosis can cause infertility in wild herbivores (Davis *et al.*, 1990; Bishop *et al.*, 1994; Bester, 2002). In Serengeti ecosystem, abortions have been observed in wildebeest during late December (Cosmas Soombe, 2003; 2004, Sayalel Kuya, 2007, pers comm). However, no attempts have been carried out to isolate the bacterium from aborted fetuses. Buffaloes prefer habitat with wooded tall grassland, making it difficult to observe any abortion symptoms, however, it is possible that abortions do occur similar to what has been reported elsewhere (Davis *et al.* 1990; Bishop *et al.*, 1994; Bester, 2002). In 2001 and 2007 two cases of abortions in buffalo were observed in Ngorongoro Crater grassland. From wildlife conservation point of view such high prevalence of exposure probably has an adverse effect on the reproductive performance. The low buffalo population growth in Serengeti is reported to be attributed to high poaching pressure (Dublin *et al.*, 1990). However, demographic studies have not been conducted on this local population to understand the age structure and establish

the recruitment rate. Such information could in understanding the effect of infertility from *Brucella* infections (Mathias *et al.*, 1999).

The determination of OR and RR was based on the assumption that in NCA wild herbivores are at high risk of exposure due to high interaction with livestock than in SNP. However, the analysis has shown that wildlife populations in the two protected areas under different management systems are at the same risk of exposure to the bacterium (RR=1.3). Since the prevalence of exposure to the bacterium is very high in buffalo and wildebeest (OR & RR > 1 the null value) compared to livestock (Fyumagwa *et al.*, 2004) it is likely that livestock are at risk of contracting the infection from wildlife and the infection is probably sustainable in wildlife population. Seroprevalence is very subjective because it cannot distinguish between current and previous exposure to the organisms, therefore, there is a need to isolate and characterize the microorganisms. This will help to understand its epidemiology and devise proper control measures. Because the bacterium has a zoonotic potential it is important to create public awareness to communities adjacent to the protected areas especially the Maasai pastoralists in NCA and agro pastoral communities in western Serengeti on the risk of infection from infected milk and beef and infected game meat, respectively.

More studies are recommended to isolate the bacterium for characterization and compare with the strains circulating in livestock. Demographic studies should be carried out to establish its effect to the study wildlife populations on reproductive performance.

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## REFERENCES

- Alton GG, Jones LM, Angus RD, Verger JM. Techniques for the brucellosis laboratory, 1<sup>st</sup> Ed. Institut National de la Recherche Agronomique, Paris, 190 pp. 1988
- Bester P. Disease prevention and management on game ranches. In: B. L. Penzhorn (Ed.). Game ranch planning and Management. Proceedings of a Symposium on Game ranch Planning and Management, Onderstepoort, South Africa. November 1-2, Pp 54-59, 2002
- Bevins JS, Blake JE, Adams LG, Templeton JW, Morton JK, Davis DS. The pathogenicity of *Brucella suis* biovar 4 for bison. *J Wildlife Dis* 32: 581-585, 1996
- Bishop GC, Bosman PP, Herr S. Bovine brucellosis; In: Infectious diseases of livestock with special reference to Southern Africa. Volume 2 (Coetzer, J.A.W., Thomson, G.R., and Tustin, R.C. Eds). Oxford university Press, Cape Town. Pp 1053-1066, 1994
- Bishop GC, Bosman PP. *Brucella suis* infection; In: Infectious diseases of livestock with special reference to Southern Africa. Volume 2 (Coetzer, J.A.W., Thomson, G.R., and Tustin, R.C. Eds). Oxford University Press, Cape Town. Pp 1076-1077, 1994
- Chernysheva MI, Gubina FA, Zheludkov MM, Perekopskaia TI. Use of acidic rose Bengal antigen in the plate agglutination test for brucellosis in humans. *J Microb Epid Immun* 6: 84-88, 1980.
- Corbel MJ. Brucellosis: an overview. *Emerg Infec dis* 3: 213-221, 1997
- Davis DS. Brucellosis in wildlife. In: Animal brucellosis (K. Nielsen and R.J. Duncan, eds). CRC Press, Florida, 321-334, 1990.
- Davis DS, Templeton JW, Ficht TA, Williams JD, Kopec JD, Adams LG. *Brucella abortus* in captive bison. I. Serology, bacteriology, pathogenesis and transmission to cattle. *J Wildlife Dis* 26: 360-371, 1990
- Drew ML, Jessup DA, Burr AA, Franti CE. Serologic survey for brucellosis in feral swine, wild ruminants and black bear of California, 1977 to 1989. *J Wildlife Dis* 28: 355-363, 1992.
- Dublin HT, Sinclair ARE, Boutin S, Anderson E, Jago M, Arcese P. Does competition regulate ungulate populations? Further evidence from Serengeti, Tanzania. *Oecologia* 82: 283-288, 1990.
- Fyumagwa RD, Sudi FF, Bahari M. Diseases Status in livestock-wildlife interfaces in Tanzania. In: Proceedings of the WDA- Africa and Middle East Section annual conference, in Abu Dhabi, UAE, 11<sup>th</sup>-13<sup>th</sup> December 2004.
- Godfroid J. Brucellosis in wildlife. *Off Int Epiz* 21: 277-286, 2002
- Harlow E, Lane D. Antibodies- A Laboratory Manual. Cold Spring Harbor Laboratory. 1996.
- Herr S. *Brucella mellitensis* infection; In: Infectious diseases of livestock with special reference to Southern Africa. Volume 2 (Coetzer, J.A.W., Thomson, G.R., and Tustin, R.C. Eds). Oxford. Pp 1073-1075, 1994
- Lord VR, Flores RC. *Brucella* spp. From the capybara (*Hydrochaeris hydrochaeris*) in Venezuela: Serologic studies and metabolic characterization of isolates. *J Wildlife Dis* 19: 308-314, 1983
- Mathias LA, Girio RJS, Duarte JMB. Sero-survey for antibodies against *Brucella abortus* and *Leptospira interrogans* in Pampas Deer from Brazil. *J Wildlife Dis* 35: 112- 114, 1999
- MWLD. Annual report. FAO/ OIE/ WHO Questionnaire-2003, Tanzania. Unpublished. 2003
- Petrie A, Watson P. The Chi-squared test comparing proportions, In: Statistics for Veterinary and Animal science. Pp 101- 113, 202. Blackwell Science Ltd, UK 2004
- van der Leek ML, Becker HN, Humphrey P, Adams CL, Belden RC, Frankenberger WB, Nicoletti PL. Prevalence of *Brucella* sp. Antibodies in feral swine in Florida. *J Wildlife Dis* 29: 410-415, 1993.
- van Tonder EM, Herr S, Bishop GC, Bosman PP. *Brucella ovis* infection; In: Infectious diseases of livestock with special reference to Southern Africa. Volume 2 (Coetzer, J.A.W., Thomson, G.R., and Tustin, R.C. Eds). Oxford. Pp 1067-1072, 1994.



## Paper viii



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## Fatal canine distemper infection in a pack of African wild dogs in the Serengeti ecosystem, Tanzania

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### ABSTRACT

In 2007, disease related mortality occurred in one African wild dog (*Lycaon pictus*) pack close to the north-eastern boundary of the Serengeti National Park, Tanzania. Histopathological examination of tissues from six animals revealed that the main pathologic changes comprised interstitial pneumonia and suppurative to necrotizing bronchopneumonia. Respiratory epithelial cells contained numerous eosinophilic intracytoplasmic inclusion bodies and multiple syncytial cells were found throughout the parenchymal tissue, both reacting clearly positive with antibodies against canine distemper virus (CDV) antigen. Phylogenetic analysis based on a 388 nucleotide (nt) fragment of the CDV phosphoprotein (P) gene revealed that the pack was infected with a CDV variant most closely related to Tanzanian variants, including those obtained in 1994 during a CDV epidemic in the Serengeti National Park and from captive African wild dogs in the Mkomazi Game Reserve in 2000. Phylogenetic analysis of a 335-nt fragment of the fusion (F) gene confirmed that the pack in 2007 was infected with a variant most closely related to one variant from 1994 during the epidemic in the Serengeti National Park from which a comparable fragment is available. Screening of tissue samples for concurrent infections revealed evidence of canine parvovirus, *Streptococcus equi* subsp. *ruminantium* and *Hepatozoon* sp. No evidence of infection with *Babesia* sp. or rabies virus was found. Possible implications of concurrent infections are discussed. This is the first molecular characterisation of CDV in free-ranging African wild dogs and only the third confirmed case of fatal CDV infection in a free-ranging pack.

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### 1. Introduction

Although diseases are listed as one of the top five causes of extinction, currently there is insufficient information to

determine the level of threat diseases pose to the viability of many wildlife populations (Smith et al., 2006). The African wild dog (*Lycaon pictus*) is an endangered species that has declined in numbers throughout much of its former range, mostly due to habitat loss and human persecution (Fanshawe et al., 1991). The African wild dog is a cooperative breeder that lives in tightly bonded social packs (Moehlman and Hofer, 1997). African wild dogs have been studied in several African countries but despite this large research effort the impact of diseases on the

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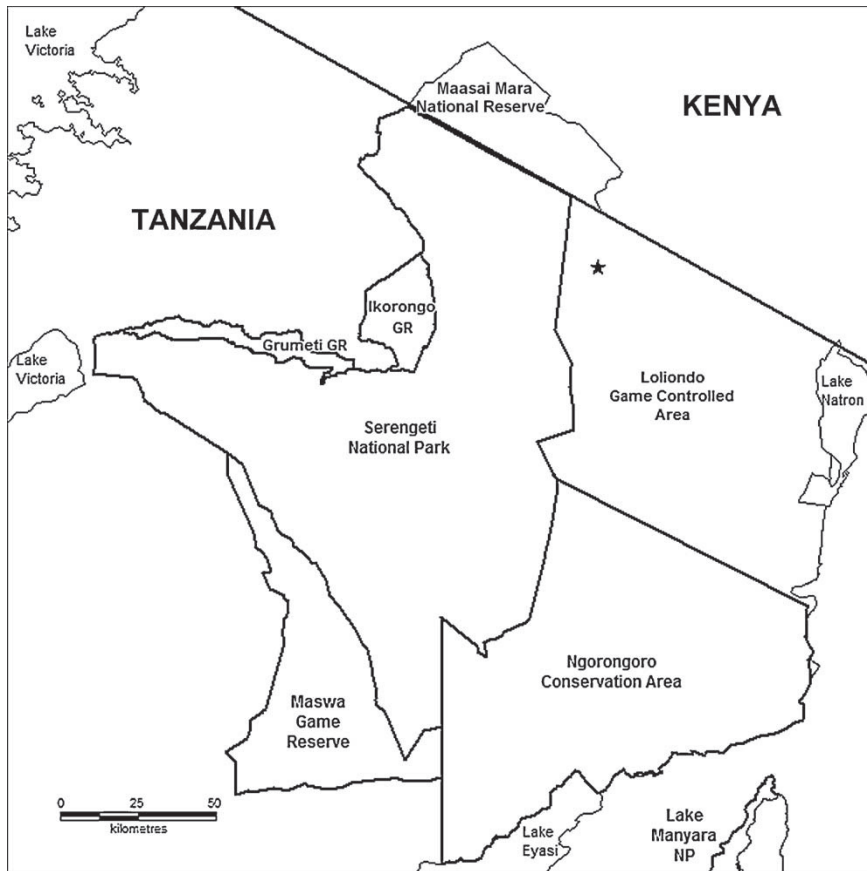


Fig. 1. Map of the Serengeti National Park, Ngorongoro Conservation Area and Loliondo Game Controlled Area in Tanzania and the Maasai Mara National Reserve, Kenya. The location of the African wild dog pack infected with canine distemper virus is marked with a black star (GR, Game Reserve; NP, National Park).

population dynamics of this species is unclear (Fanshawe et al., 1991; Burrows et al., 1994; Creel et al., 1997; Alexander et al., 2010).

Here we report an outbreak of disease in a pack of free-ranging African wild dogs in the Loliondo Game Controlled Area, 12 km from the north-eastern boundary of the Serengeti National Park, Tanzania (Fig. 1). Described are clinical signs, internal pathologic changes, and the identification of CDV as the primary causative agent of mortality in the pack members examined. We provide genetic sequence data for a fragment of the relatively conserved phosphoprotein (P) gene and the more variable fusion (F) gene from the CDV variant that infected the pack.

CDV infection causes reduced immune function in hosts (von Messling et al., 2004) which is likely to increase the susceptibility of CDV infected hosts to further infection by other pathogens (Beldomenico et al., 2008). Pre-existing infections are also thought to influence the clinical course of morbillivirus infections (Akinaden et al., 2005; Munson et al., 2008). We therefore screened the samples for the presence of the following pathogens (i) *Babesia* sp. because severe infection with this tick-borne blood parasite is

suggested to increase expression of clinical disease in CDV infected lions (Munson et al., 2008); (ii) *Hepatozoon* sp. because infection with this tick-borne blood parasite may impair immune function, especially in young hosts (East et al., 2008), and because prevalence of *Hepatozoon* sp. infection can be high in African wild dog populations (van Heerden et al., 1995); (iii) *Streptococcus equi* subsp. because this bacterium was identified as a concurrent infection during a mass epidemic of phocine distemper virus (PDV) in European harbour seals (*Phoca vitulina*) (Akinaden et al., 2005) and was the likely causative agent of a recent disease outbreak in spotted hyenas (*Crocuta crocuta*) (Höner et al., 2006; Speck et al., 2008) in the Ngorongoro Conservation Area (Fig. 1); (iv) rabies virus because all tested African wild dog carcasses from the Serengeti ecosystem between 1989 and 1992 were rabies positive (Burrows et al., 1994; East and Burrows, 2001; Woodroffe, 2001); and (v) canine parvovirus (CPV) which is an important and comparatively recent disease of canids (Parrish and Kawaoka, 2005) that is known to have infected African wild dogs in the Serengeti ecosystem (Burrows et al., 1994).

**Table 1**  
Primers and target genes used for pathogen screening, plus amplicon size from target genes.

Pathogen	Primer	Sequence (5' → 3')	Target gene	Amplicon size [nt]	Reference
CDV	P1	ATGTTTATGATCACAGCGGT	P	429	Barrett et al. (1993)
	P2	ATTGGTTGCACCACTTGTC			
CDV	FC1	GGACTGATAATGTCATTA	F	372	Liermann et al. (1998)
	FC2	ATAGCTTTGTTAGACTGTT			
CPV	555for	CAGGAAGATATCCAGAAGGA	VP2	583	Buonavoglia et al. (2001)
	555rev	GGTGCTAGTTGATGTAATAACA			
Rabies virus	JW12 F	ATGTAACACCYCTACAATG	N	110	Wakeley et al. (2005)
	N165–146R	GCAGGGTAYTTRTACTATA			
<i>Streptococcus equi</i> subsp. <i>ruminatorum</i>	LysGT1 P	ACAAGATTGATTCAAAGCAATAATCAG	16S rRNA	1369	Wyss et al. (1996)
	TPU1	AGAGTTTGATCMTGGCTCAG			
	RTU8	AAGGAGGTGATCCAACCCCA	Sequencing primers		Höner et al. (2006)
	Strep2F	TAACCTAACAGAAAGGGACG			
	Strep3R	CACGAGCTGACGACAACC			
	Strep4F	AGAACCTTACCAGGTCTTGAC	18S rRNA	462	East et al. (2008)
	Strep5R	TCAGACTTATTAAACCGCTG			
	Hemo3	TGTGTACAAAGGGCAGGACG			
<i>Babesia</i> sp.	Hemo4	GCGGCTTAATTTGACTCAACAC	18S rRNA	687	This study
	Babspec for	CCGTGTAATTSTAGGGCTAATAC			
	Babspec rev	AACCAAAGCTCTACTTATTCCATG			

CDV, canine distemper virus; CPV, canine parvovirus.

## 2. Materials and methods

The Serengeti ecosystem is defined as the area covered by the migratory movements of wild herbivores (Schaller, 1972).

### 2.1. Histopathology

Tissue samples from six relatively fresh carcasses (four pups, two adults) including liver, spleen, lung, heart, brain and kidney were stored in 10% neutral buffered formalin embedded in paraffin, sectioned at 4 µm and stained with hematoxylin eosin. For immunohistochemistry tissue sections were deparaffinized and after antigen retrieval (in a pressure cooker for 2 min in 0.01 M citrate buffer, pH 6.0) slides were processed in an automated immunohistochemistry slide stainer (DAKO, Hamburg, Germany) through the following steps: 30 min incubation with a 1:3000 dilution of mouse anti-canine-distemper-virus antibodies (AbD Serotec MCA2538H, Serotec, Düsseldorf, Germany); washing with Tris–HCl buffer; 20 min incubation with biotinylated secondary antibody (Zyto-Chem-Plus-AP-Kit, Zytomed, Berlin, Germany); washing with Tris–HCl buffer; 20 min incubation with Streptavidin-AP-Conjugate (Zyto-Chem-Plus-AP-Kit, Zytomed, Berlin, Germany); washing with Tris–HCl buffer; staining with Fast Red (F4648, Sigma, Bad Homburg, Germany) twice for 5 min; washing with Tris–HCl buffer. Hematoxylin (S 3301, DAKO, Hamburg, Germany) was used as counterstaining.

### 2.2. RT-PCR and CDV sequence analysis

Tissue samples from two pup carcasses were preserved with RNAlater<sup>®</sup> (Sigma–Aldrich Co, St. Louis, MO, USA) following the manufacturer's instructions, stored and transported at –10 °C and then stored at –80 °C. Viral

RNA and DNA were isolated simultaneously using the MinElute virus spin Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. Reverse transcriptase-polymerase chain reaction (RT-PCR) was performed using *morbillivirus*-specific primers that amplify a 388-nt fragment of the P gene and a 335-nt fragment of the F gene (Table 1). PCR products were purified using Qiagen PCR purification Kit (Qiagen, Hilden, Germany). Sequencing was bidirectional and conducted using the fluorescent Big Dye Terminator Cycle sequencing Kit 3.1 (ABI, Darmstadt, Germany) following the manufacturer's instructions. Sequences were analysed on an ABI model 3130xl Genetic Analyzer (ABI). Editing and alignment of the sequences was carried out with BIOEDIT v.7.0.9.0 (Hall, 1999). Neighbour-joining trees of the P and F gene fragments were generated using the Tamura Nei parameter with 1000 bootstrap pseudo-replications as implemented in Mega 4 (Tamura et al., 2007). Geographical origin, host species and GenBank accession numbers of variants included in the phylogenetic analyses are indicated in the phylogenetic trees (Fig. 3). Nt sequence data for variant SNP\_hyena\_94-177 see Carpenter et al. (1998).

### 2.3. Detection of concurrent infections

Screening for CDV, CPV, *Hepatozoon* sp. and *Babesia* sp. was conducted at the Leibniz Institute for Zoo and Wildlife Research, Germany. DNA was isolated from various tissues using DNeasy Blood and Tissue Kit according to the manufacturer's instructions (Qiagen, Hilden, Germany). The primer pairs used and publications detailing the methods applied for pathogen screening are described in Table 1. PCRs were replicated at least twice and products confirmed by sequencing. The presence of *Streptococcus equi* was determined by cultivation of bacteria and molecular

**Table 2**  
Summary of pathological changes found in different organs from six African wild dogs.

Organs and pathological changes	1	2	3	4	5	6
Lung: bronchopneumonia	+++ <sup>a</sup>	+++ <sup>a</sup>	+++ <sup>a</sup>	++ <sup>a</sup>	+++ <sup>a</sup>	+++ <sup>a</sup>
Lung: syncytial cells	–	–	–	++ <sup>a</sup>	++ <sup>a</sup>	–
Lung: interstitial oedema	+++	++	++	–	++	+++
Lung: intra-alveolar haemorrhage	–	+	+	–	++	++
Respiratory epithelium: intracytoplasmic inclusion bodies	+++ <sup>a</sup>	+++ <sup>a</sup>	++ <sup>a</sup>	++ <sup>a</sup>	+++ <sup>a</sup>	+++ <sup>a</sup>
Small intestine: coccidial infection	–	++	nd	+	+	+++
Spleen: depletion of lymph follicles	++	++	nd	++	nd	nd
Heart: focal myocarditis associated with protozoal cyst	nd	+	nd	nd	nd	nd
Lymph nodes: follicular hyperplasia	nd	++	nd	nd	nd	nd
Liver	NAD	NAD	NAD	NAD	NAD	NAD
Kidney	NAD	NAD	NAD	NAD	NAD	NAD
Brain	NAD	nd	nd	nd	nd	nd
Degree of autolysis	–	–	++	+++	+	+

Individuals 1–4 were pups (<12 months of age) and individuals 5–6 were adults.

–, none; +, mild; ++, moderate; +++, severe histological changes; NAD, nothing abnormal detected; nd, not determined.

<sup>a</sup> Containing cells positive for canine distemper virus antigen.

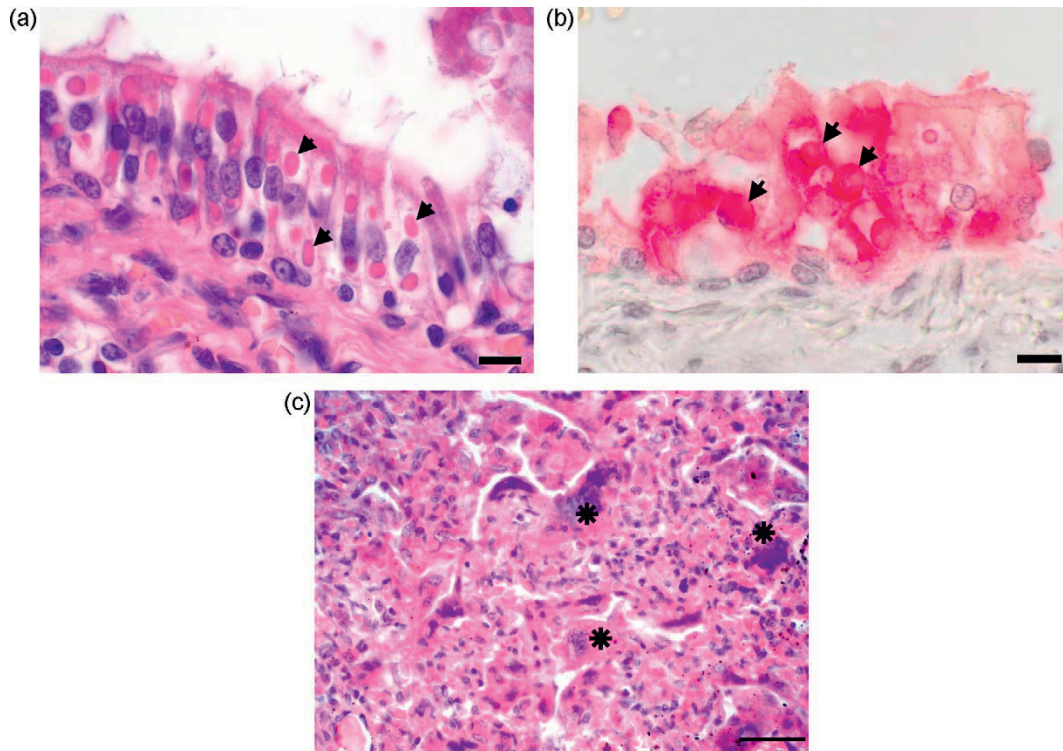
methods as described by Höner et al. (2006). Tissues were screened for the presence of *Babesia* sp. using specific primers (Table 1). Rabies screening was carried out at the Federal Research Centre for Animal Health, Germany. Brain samples from six animals were preserved in phosphate buffered 50% glycerol solution and screened for rabies virus. Rabies virus antigen in brain smears was detected using the standard fluorescent antibody test (FAT) (Dean et al., 1996) and confirmed by a quantitative real-time PCR (qRT-PCR)

targeting the nucleoprotein (N) gene of rabies virus essentially as described (Wakeley et al., 2005).

### 3. Results

#### 3.1. Disease outbreak

On 3rd October 2007, 23 (nine adults, six yearlings and eight pups) of approximately 38 members of one pack of African wild dogs (the Ololosokwan pack) were found dead



**Fig. 2.** (a) Bronchiolar epithelium with multiple eosinophilic viral intracytoplasmic inclusion bodies (arrows) (bar = 10 μm). (b) Positive immunohistochemistry reaction of bronchiolar epithelial cells and inclusion bodies (arrows) against canine distemper virus antigen (bar = 10 μm). (c) Lung tissue, multiple syncytial cells (asterisks) (bar = 50 μm).



or dying during a period of 8 days in the Loliondo Game Controlled Area (Fig. 1). Affected animals showed clinical signs of ataxia, weakness, soiling of the perineum and dehydration. Some carcasses were markedly decomposed.

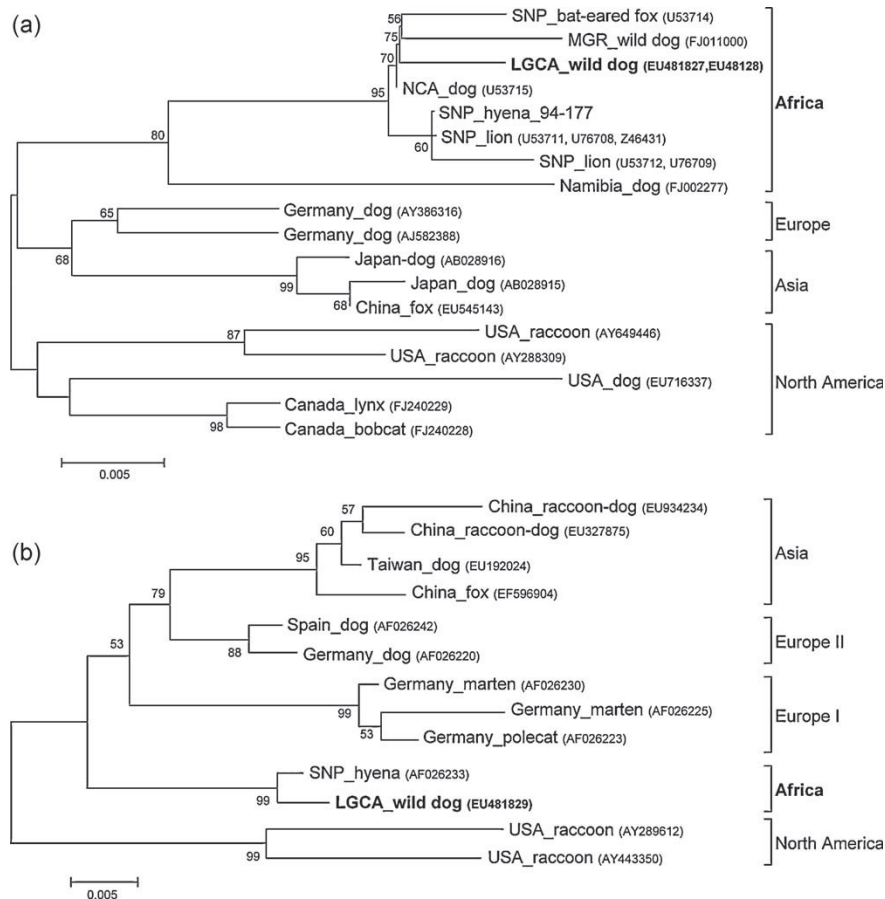
### 3.2. Histopathology

Histopathological examination of the organs from six animals (four pups and two adults) identified that the main pathological changes were located in the lungs (Table 2). Moderate to severe multilobular suppurative to necrotizing bronchopneumonia was found with extensive intra-alveolar and interstitial infiltration with mononuclear inflammatory cells and some neutrophilic granulocytes, as well as marked (secondary) bacterial colonisation. Epithelial lining cells of bronchi and bronchioli contained clearly visible eosinophilic intracytoplasmic inclusion bodies (Fig. 2a). Immunohistochemistry revealed that respiratory

epithelial cells and their inclusion bodies reacted clearly positive with antibodies against canine distemper virus antigen (Fig. 2b). Additionally, a few animals had formation of multiple syncytial cells within the parenchymal tissue (Fig. 2c), some also reacting positive with antibodies against canine distemper virus antigen. The severity of the findings is consistent with fatal canine distemper virus infection. Further pathological changes of other organs were considered incidental findings and were mostly confined to generalised congestion.

### 3.3. RT-PCR and CDV P and F gene fragment sequences

CDV sequences generated in this study were submitted to GenBank. Two sequences were obtained for a 388-nt fragment of the P gene (EU481827, EU481828) and phylogenetic comparison of sequence data from these fragments with published information (Fig. 3a) revealed



**Fig. 3.** Phylogenetic relationship of the canine distemper viral variant from an African wild dog pack in the Loliondo Game Controlled Area, northern Tanzania to published variants from Tanzania and elsewhere. (a) A neighbour-joining tree based on a 388-nt fragment of the phosphoprotein (P) gene generated using the Tamura Nei parameter with statistical support for nodes obtained by bootstrapping (1000 replicates); values  $\geq 50\%$  are shown. When identical nt sequences for this fragment were available from a particular host species, only one sequence was used in the analysis. (b) A neighbour-joining tree based on a 355-nt fragment of the fusion (F) gene generated using the method described for the P gene fragment. Geographical origin, host species and GenBank accession numbers of variants included in the phylogenetic analyses are indicated in the tree (LGCA, Loliondo Game Controlled Area; SNP, Serengeti National Park; NCA, Ngorongoro Conservation Area; MGR, Mkomazi Game Reserve).



**Table 3**  
Concurrent pathogens found infecting six African wild dogs from one pack.

Method	Organ	1	2	3	4	5	6
CDV RT-PCR	Brain, lung	+	+	nd	nd	nd	nd
CPV PCR	Lung, intestine	+	+	nd	nd	nd	nd
Rabies virus qRT-PCR	Brain	–	–	–	–	–	–
<i>Streptococcus equi</i> subsp. culture PCR	Lung	+	–	nd	nd	nd	nd
<i>Hepatozoon</i> sp. PCR	Liver, kidney	+	+	+	+	+	+
<i>Babesia</i> sp. PCR	Lung	–	–	–	–	–	–

The organs listed were screened using various methods for viruses (canine distemper virus (CDV), canine parvovirus (CPV) and rabies) two tick-borne blood parasites and one bacterium. Individuals 1–4 were pups (<12 months of age); individuals 5–6 were adults. –, negative; +, positive; nd, not determined.

that the African wild dog pack in 2007 was infected with a CDV variant most closely related to a CDV variant described from a domestic dog (100% identity) in the Ngorongoro Conservation Area in 1994 and closely related to a variant described in a captive African wild dog (99% homology) in north-eastern Tanzania in 2000, and to CDV variants previously described from lions (*Panthera leo*), a spotted hyena, and a bat-eared fox (*Otocyon megalotis*) in the Serengeti National Park in northern Tanzania in 1993 and 1994. Only one other P gene fragment from Africa is available which is from a domestic dog in Namibia. All P gene fragments from African CDV variants clustered together with the Tanzania variants forming a distinct and tight sub-cluster.

Similarly, the 335-nt fragment of the F gene from one pup (EU481829) was most closely related (99% homology) to the variant that infected a spotted hyena during the CDV outbreak among wild carnivores in the Serengeti National Park in 1993 and 1994 (AF026233), the only available F gene fragment from Tanzania (Fig. 3b).

#### 3.4. Concurrent infections

The results of molecular screening for concurrent infections in each of the six animals examined are summarized in Table 3. Of two pups tested for CPV infection, both yielded positive results. One of these two pups was also positive for *Streptococcus equi* subsp. *ruminatorum* infection. All six pack members screened for *Hepatozoon* sp. infection were positive. None of six pack members were positive for rabies or *Babesia* sp.

#### 4. Discussion

Our histopathological results (Fig. 2) from six members of one African wild dog pack in the Serengeti ecosystem in 2007 indicate fatal CDV infection. We describe for the first time a CDV variant from a free-ranging pack using fragments of the P gene and F gene (Fig. 3). Phylogenetic analysis of the P gene fragment revealed high homology between the CDV variant that infected the pack in 2007 and other CDV variants previously described from Tanzania, including variants from fatal CDV infection of captive African wild dogs in the Mkomazi Game Reserve in 2000 (van de Bildt et al., 2002) (now upgraded to a National Park) and from a CDV epidemic in wild carnivore hosts (lions, spotted hyenas and a bat-eared fox) in the Serengeti National Park, and a domestic dog in the Ngorongoro

Conservation Area (Fig. 1) in 1993 and 1994 (Haas et al., 1996; Roelke-Parker et al., 1996; Carpenter et al., 1998). We also found high homology between one CDV F gene fragment from the pack in 2007 and one variant from a spotted hyena host in the Serengeti National Park in 1994 (Liermann et al., 1998). Evidence for one or more concurrent infections with CPV, *Hepatozoon* sp. and *Streptococcus equi* subsp. *ruminatorum* was also found in the pack members examined. These concurrent infections may have occurred as a consequence of the immunological suppression caused by CDV infection (von Messling et al., 2004). It is also possible that some pack members were already infected with one or more pathogens when first exposed to CDV, and these infections, particularly CPV (Parrish and Kawaoka, 2005), may have increased susceptibility to and the pathological consequences of CDV infection, irrespective of the sequence in which concurrent infections were acquired (Beldomenico et al., 2008). Thus our results indicate that the primary cause of morbidity and mortality in the pack was CDV disease and that co-infections may have contributed to the fatal outcome of CDV infection in some animals. Although all previous clinical diagnoses of African wild dog carcasses in both the Tanzanian and Kenyan sector of the Serengeti ecosystem have been positive for rabies infection (Burrows et al., 1994; East and Burrows, 2001; Woodroffe, 2001) none of the animals screened in this study were positive for rabies. Sera from lions in the Serengeti National Park provide evidence of increased CDV exposure in 2006 without infection causing disease in this host species (Munson et al., 2008) and we are unaware of any evidence of clinical signs of CDV infection in any wild carnivore host species inside the park in either 2006 or 2007, even though several host species were closely monitored by researchers.

Although CDV and related viruses in the genus *Morbillivirus* are associated with disease epidemics in wildlife (Taubenberger et al., 1996; Harder and Osterhaus, 1997; Akineden et al., 2005), current knowledge of the impact of CDV on the population dynamics of African wild dogs is largely unknown. Serological surveys indicate relatively high exposure to CDV in some African wild dog populations without apparent evidence of fatal disease, for example, in the Okavango Delta, Botswana (Alexander et al., 2010), and the Selous Game Reserve, Tanzania (Creel et al., 1997). Both Creel et al. (1997) and Alexander et al. (2010) suggest that African wild dog populations can remain demographically healthy despite high exposure to CDV. There is also evidence that African wild dogs on the

Maasai steppe, Tanzania (Visee, 2001), and in Namibia (Laurenson et al., 1997) are exposed to CDV. Although no evidence of exposure to CDV was found in packs in the Kruger National Park, South Africa in the 1990s (van Heerden et al., 1995), a more recent study has revealed evidence of exposure (Snoeren, 2009).

We know of only two previous clinically confirmed outbreaks of CDV infections in free-ranging African wild dog packs. CDV infection was established in one pack in Chobe National Park, Botswana in 1994 using immunohistochemistry on lung sections from one juvenile, however no genetic sequence data were obtained from the CDV variant and the pack of 12 animals was reduced to two surviving females following the death of six juveniles and four males (Alexander et al., 1996). The second possible CDV infected pack occurred in 1968 in the Serengeti ecosystem when 5 adults and 11 pups disappeared from a pack during a period of weeks leaving five adults and five pups, and one pup postmortem revealed haemorrhagic gastritis and enteritis, and heavy blood loss (Schaller, 1972). Although these clinical signs are typical of the gastro-intestinal form of distemper they are not exclusive to CDV infection. The most extensive mortality reported in African wild dogs associated with CDV infection occurred in 2000 in fenced breeding enclosures in the Mkomazi Game Reserve, Tanzania, when 49 of 52 captive animals, most of which had been vaccinated against CDV (Visee, 2001), died (van de Bildt et al., 2002). Interestingly, nine of 12 wild caught pups obtained from the Maasai steppe to start this captive breeding facility had positive CDV titres before they were vaccinated (Visee, 2001).

There is evidence suggesting that CDV infection has occurred in wild carnivore hosts in the Serengeti ecosystem for the past few decades and not always with fatal consequences. A distemper-like disease reduced silver-backed jackal numbers (*Canis mesomelas*) between 1978 and 1979 (Moehlman, 1983) without affecting sympatric golden jackals (*Canis aureus*) or African wild dog packs (Burrows et al., 1994). Serological evidence indicates CDV exposure among wild carnivores for many years before the start of the CDV epidemic in 1993, and high seroprevalence in 2006 occurred without evidence of clinical disease in infected species (Harrison et al., 2004; Munson et al., 2008).

Why one African wild dog pack close to the Serengeti National Park boundary (Fig. 1) in 2007 suffered fatal CDV infection when other wild carnivore host species in the park, and other African wild dog packs in the Loliondo Game Controlled Area (Fig. 1) displayed no clinical signs of CDV infection or increased mortality in that year, is unclear. It is possible that the immune status of the pack may have been compromised by intrinsic and extrinsic factors (Hofer and East, 1998; Lee, 2006; Beldomenico et al., 2008; Munson et al., 2008) that resulted in CDV infection causing fatalities among pack members. High contact rates between pack members would ensure rapid transmission of infectious pathogens to all susceptible pack members, and infections that reduced pack hunting success would be expected to accelerate immunological decline (Beldomenico et al., 2008).

## 5. Conclusion

Despite the fact that this study reports on confirmed fatal CDV infection in free-ranging African wild dogs, it is currently unclear what the demographic consequences of CDV are for African wild dog populations and why some packs survive exposure to CDV while others succumb to fatal infection. The level of natural herd immunity to CDV within a population is likely to be one key factor determining the impact of CDV (Guiserix et al., 2007), another may be the effect of concurrent infections on immune status. Although CDV infection can be fatal to African wild dogs, there is currently little evidence that this virus threatens large demographically healthy populations. Even so, high CDV mediated mortality within one or a few packs could result in a significant population reduction in small, isolated populations of this endangered canid.

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## References

- Akineden, Ö., Hassan, A.A., Alber, J., El-Sayed, A., Estoepangestie, A.T.S., Lämmler, C., Weiss, R., Siebert, U., 2005. Phenotypic and genotypic properties of *Streptococcus equi* subsp. *zooepidemicus* isolated from harbor seals (*Phoca vitulina*) from the German North Sea during the phocine distemper outbreak in 2002. *Vet. Microbiol.* 110, 147–152.
- Alexander, K.A., Kat, P.W., Munson, L.A., Kalake, A., Appel, M.J.G., 1996. Canine distemper-related mortality among wild dogs (*Lycan pictus*) in Chobe National Park, Botswana. *J. Zoo Wildl. Med.* 27, 426–427.
- Alexander, K.A., McNutt, J.W., Briggs, M.B., Stander, P.E., Funston, P., Hemson, G., Keet, D., van Vuuren, M., 2010. Multi-host pathogens and carnivore management in southern Africa. *Comp. Immunol. Microbiol. Infect. Dis.* 33, 249–265.
- Barrett, T., Visser, I.K.G., Mamaev, L., Goatley, L., van Bresse, M.F., Osterhaus, A.D.M.E., 1993. Dolphin and porpoise morbilliviruses are genetically distinct from Phocine distemper virus. *Virology* 193, 1010–1012.
- Beldomenico, P.M., Telfer, S., Gebert, S., Lukomski, L., Bennett, M., Begon, M., 2008. Poor condition and infection: a vicious cycle in natural populations. *Proc. R. Soc. Lond. B* 275, 1753–1759.
- Buonavoglia, C., Martella, V., Pratelli, A., Tempesta, M., Cavalli, A., Buonavoglia, D., Bozzo, G., Decaro, N., Carmichael, L.E., 2001. Evidence for evolution of canine parvovirus type-2 in Italy. *J. Gen. Virol.* 82, 1555–1560.
- Burrows, R., Hofer, H., East, M.L., 1994. Demography, extinction and intervention in a small population: the case of the Serengeti wild dogs. *Proc. R. Soc. Lond. Series B* 256, 281–292.
- Carpenter, M.A., Appel, M.J.G., Roelke-Parker, M.E., Munson, L., Hofer, H., East, M.L., O'Brien, S.J., 1998. Genetic characterization of canine distemper virus in Serengeti carnivores. *Vet. Immunol. Immunopathol.* 65, 259–266.

- Creel, S., Creel, N.M., Munson, L., Sanderlin, D., Appel, M.J.G., 1997. Serosurvey for selected viral diseases and demography of African wild dogs in Tanzania. *J. Wildl. Dis.* 33, 823–832.
- Dean, D.J., Abelseth, M.K., Athanasiu, P., 1996. The fluorescence antibody test. In: Meslin, F.-X., Kaplan, M.M., Koprowski, H. (Eds.), *Laboratory Techniques in Rabies*. 4th edition. World Health Organization, Geneva, pp. 88–93.
- East, M.L., Burrows, R., 2001. The efficacy of inactivated rabies vaccine for the protection of African wild dogs (*Lycaon pictus*) against viral challenge: a review. *Verh. ber. Erkr. Zootiere* 40, 233–242.
- East, M.L., Wibbelt, G., Lieckfeldt, D., Ludwig, A., Goller, K., Wilhem, K., Schares, G., Thierer, D., Hofer, H., 2008. A *Hepatozoon* species genetically distinct from *H. canis* infecting spotted hyenas in the Serengeti ecosystem, Tanzania. *J. Wildl. Dis.* 44, 45–52.
- Fanshawe, J.H., Frame, L.H., Ginsberg, J.R., 1991. The wild dog—Africa's vanishing carnivore. *Oryx* 25, 137–146.
- Guiserix, M., Bahi-Jaber, N., Fouchet, D., Sauvage, F., Pontier, D., 2007. The canine distemper epidemic in the Serengeti: are lions victims of a new highly virulent canine distemper virus strain, or is pathogen circulation stochasticity to blame? *J. R. Soc. Interface* 17, 1127–1134.
- Haas, L., Hofer, H., East, M., Wohlsein, P., Liess, B., Barrett, T., 1996. Canine distemper virus infection in Serengeti spotted hyenas. *Vet. Microbiol.* 49, 147–152.
- Hall, T.A., 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucl. Acids Symp. Ser.* 41, 95–98.
- Harder, T.C., Osterhaus, A.D.M.E., 1997. Canine distemper virus—a morbillivirus in search of a new host? *Trends Microbiol.* 5, 120–124.
- Harrison, T.M., Mazet, J.K., Holecamp, K.E., Dubovi, E., Engh, A.L., Nelson, K., van Horn, R.C., Munson, L., 2004. Antibodies to canine and feline viruses in spotted hyenas (*Crocuta crocuta*) in the Masai Mara National Reserve. *J. Wildl. Dis.* 40, 1–10.
- Hofer, H., East, M.L., 1998. Biological conservation and stress. *Adv. Study Behav.* 27, 405–525.
- Höner, O.P., Wachter, B., Speck, S., Wibbelt, G., Ludwig, A., Fyumagwa, R.D., Wohlsein, P., Lieckfeldt, D., Hofer, H., East, M.L., 2006. Severe *Streptococcus* infection in spotted hyenas in the Ngorongoro Crater, Tanzania. *Vet. Microbiol.* 115, 223–228.
- Laurenson, K., van Heerden, J., Stander, P., van Vuuren, M.J., 1997. Seropidemiological survey of sympatric domestic and wild dogs (*Lycaon pictus*) in Tsumkwe District, north-eastern Namibia. *Onderstepoort J. Vet. Res.* 64, 313–316.
- Lee, K., 2006. Linking immune defenses and life history at the levels of the individual and the species. *Int. Comp. Biol.* 46, 1000–1015.
- Liermann, H., Harder, T.C., Löchelt, M., von Messling, V., Baumgärtner, W., Moennig, V., Haas, L., 1998. Genetic analysis of the central untranslated genome region and the proximal coding part of the F gene of wildtype and vaccine distemper morbilliviruses. *Virus Genes* 17, 259–270.
- Moehlman, P.D., 1983. Socioecology of silver-backed and golden jackals, *Canis mesomelas* and *C. aureus*. In: Eisenberg, J.F., Kleiman, D.G. (Eds.), *Recent Advances in the Study of Mammalian Behavior*. American Society of Mammalogists, Lawrence, Kansas, pp. 423–438.
- Moehlman, P.D., Hofer, H., 1997. Cooperative breeding, reproductive suppression, and body mass in canids. In: Solomon, N., French, J.A. (Eds.), *Cooperative Breeding in Mammals*. Cambridge University Press, Cambridge, pp. 76–128.
- Munson, L., Terio, K.A., Kock, R., Mlengeya, T., Roelke, M.E., Dubovi, E., Summers, B., Sinclair, A.R.E., Packer, C., 2008. Climate extremes promote fatal co-infections in African lions. *Plos One* 6, e2545.
- Parrish, C.R., Kawaoka, Y., 2005. The origins of new pandemic viruses: the acquisition of new host ranges by canine parvovirus and influenza A virus. *Annu. Rev. Microbiol.* 59, 553–586.
- Roelke-Parker, M.E., Munson, L., Packer, C., Kock, R., Cleaveland, S., Carpenter, M., O'Brien, S.J., Pospischil, A., Hofmann-Lehmann, R., Lutz, H., Mwamengele, G.L.M., Mgasa, M.N., Machange, G.A., Sumner, B.A., Appel, M.J., 1996. A canine distemper virus epidemic in Serengeti lions (*Panthera leo*). *Nature* 379, 441–445.
- Schaller, G.B., 1972. *The Serengeti Lion*. Chicago University Press, Chicago.
- Smith, K.F., Sax, D.F., Lafferty, K.D., 2006. Evidence for the role of infectious disease in species extinction and endangerment. *Cons. Biol.* 20, 1349–1357.
- Snoeren, A., 2009. The prevalence of canine distemper virus antibodies in wild carnivores in the Kruger National Park. MSc Thesis, University of Pretoria, South Africa.
- Speck, S., Höner, O.P., Wachter, B., Fickel, J., 2008. Characterization of *Streptococcus equi* subsp. *ruminatorum* isolated from spotted hyenas (*Crocuta crocuta*) and plains zebras (*Equus burchelli*), and identification of a M-like protein (SrM) encoding gene. *Vet. Microbiol.* 128, 148–159.
- Tamura, K., Dudley, J., Nei, M., Kumar, S., 2007. MEGA4: Molecular evolutionary genetics analysis (MEGA) software version 4.0. *Mol. Biol. Evol.* 24, 1596–1599.
- Taubenberger, J.K., Tsai, M., Krafft, A.E., Lichy, J.H., Reid, A.H., Schulman, F.Y., Lipscomb, T.P., 1996. Two morbilliviruses implicated in bottleneose dolphin epizootics. *Emerg. Infect. Dis.* 2, 213–216.
- van de Bildt, M.W.G., Kuiken, T., Visee, A.M., Lema, S., Fitzjohn, T.R., Osterhaus, A.D.M.E., 2002. Distemper outbreak and its effect on African wild dog conservation. *Emerg. Infect. Dis.* 8, 211–213.
- van Heerden, J., Mills, M.G.L., Van Vuuren, M.J., Kelly, P.J., Dreyer, M.J., 1995. An investigation into health status and disease of wild dogs (*Lycaon pictus*) in the Kruger National Park. *J. S. Afr. Vet. Assoc.* 66, 18–27.
- Visee, A.M., 2001. Distemper, rabies and parvovirus vaccination in a captive breeding programme for the African wild dog (*Lycaon pictus*) in Northern Tanzania. *Verh. ber. Erkr. Zootiere* 40, 243–250.
- von Messling, V., Milksevic, D., Cattaneo, R., 2004. Tropism illuminated: Lymphocyte-based pathways blazed by lethal morbillivirus through the host immune system. *Proc. Natl. Acad. Sci. U.S.A.* 101, 14216–14221.
- Wakeley, P.R., Johnson, N., McElhinney, L.M., Marston, D., Sawyer, J., Fooks, A.R., 2005. Development of a real-time, TaqMan reverse transcription-PCR assay for detection and differentiation of lyssavirus genotypes 1, 5 and 6. *J. Clin. Microbiol.* 43, 2786–2792.
- Woodroffe, R., 2001. Assessing the risk of intervention: immobilization, radio-collaring and vaccination of African wild dogs. *Oryx* 35, 234–244.
- Wyss, C., Choi, B.K., Schupbach, P., Guggenheim, B., Göbel, U.B., 1996. *Treponema maltophilum* sp. nov., a small oral spirochete isolated from human periodontal lesions. *Int. J. Syst. Bacteriol.* 46, 745–752.



Paper ix



## Chapter 22

# Diseases of Economic and Conservation Significance in the Livestock-Wildlife Interface in Tanzania

Robert D. Fyumagwa

### 22.1. Summary

*Tanzania is endowed with a diversity of wild animals and they are more abundant than anywhere else in the world, but veterinary services for wildlife are in their infancy. As a result, little information is available regarding the prevalence of many wildlife diseases. At the moment, Tanzania is free from rinderpest, but serosurveillance is still ongoing. Foot-and-mouth disease virus (FMD) strains A and O are exotic, but SAT-1, 2 and 3 are indigenous and endemic in African buffalo, although only O and SAT-1 and SAT-2 have been reported in livestock in Tanzania. Canine distemper virus (CDV) is endemic in domestic dogs, but occurs sporadically in wild carnivores, and epidemics occurred in December 2001 and October 2007 in captive, breeding wild dogs in the Mkomazi Game Reserve, now the Mkomazi National Park, and the Loliondo Game Controlled Area, respectively. Malignant Catarrhal Fever (MCF) is a fatal disease in cattle and is associated with wildebeest. Isolated cases of TB (*Mycobacterium bovis*) have been observed in cattle and various wild species, but its prevalence in wildlife is not known. Brucellosis is endemic with a prevalence of 8 % in pastoral areas and an average prevalence of 3.3 % in agropastoral areas. A serosurvey is taking place in agropastoral communities in western Serengeti, where livestock-wildlife interaction is very high. Some seropositive results from*

*brucellosis have been observed in buffalo and wildebeest, and the prevalence of their exposure to the Brucella organism is 24 % and 17 %, respectively. Newcastle disease decimates about 45 % of chickens in Tanzania each year and killed hundreds of thousands of laughing doves in northern Tanzania in 2006. Tick-borne diseases claim more than 12 % of cattle in Tanzania, and mortality in wildlife was observed in 2000 and 2001 in herbivore and lion populations in the Ngorongoro Crater. Rabies is endemic in domestic dogs and the domestic canid strain has been detected sporadically in jackals, bat-eared foxes and white-tailed mongooses. Recently, a unique strain of rabies was isolated in 13 % of spotted hyenas in the Serengeti National Park. Some emerging diseases encountered in wildlife include infectious otitis in giraffes, genital infection in baboons, streptococcus pneumonia in spotted hyenas, pneumonia in chimpanzees and algal toxins in lesser flamingos. Rift Valley Fever epidemics have been associated with heavy rains and occurred during El Niño in 1998. Many livestock and more than 100 people died in central Tanzania in the most recent one in 2006-07.*

## 22.2. Introduction

Tanzania is the largest nation in East Africa, with an area of approximately 947 087 km<sup>2</sup>, and the third country in Africa in terms of its cattle population, after Ethiopia and Sudan. Because of its large size, Tanzania has an extensive international boundary (3861 km) bordering eight countries: Kenya, Uganda, Rwanda, Burundi, the Democratic Republic of Congo (DRC), Zambia, Malawi and Mozambique.

Tanzania is endowed with a diversity of wild animals and has the largest wildlife population in the world, with over 4 million herbivores (Mduma & Fyumagwa 2003, unpublished report). Due to her commitment to wildlife conservation, Tanzania has placed almost 30 % of her land area in protected areas. Tanzania has 15 national parks (> 4 %), 30 game reserves (15 %), 38 game controlled areas (8 %), the Selous Niassa Wildlife Corridor (1 %) and the Ngorongoro Conservation Area (1 %). Wildlife plays an important role in obtaining local and foreign currency from tourism (game viewing and sport hunting) and the sale of live animals, game products and by-products. It is also an important source of animal protein to local people. The wildlife sector contributes 13 % of the national gross domestic product (Meghji 2002).

By virtue of having an extensive international boundary, Tanzania is faced with a problem of transboundary animal disease transmission due



to livestock and wildlife movement among bordering countries and in the interface within the country. A good example is the largest population of migratory wildebeest and zebra from the Serengeti National Park in Tanzania to the Maasai Mara National Reserve in Kenya and back. African buffalo are known to migrate long distances across international boundaries from northern Serengeti and the Mkomazi National Park to Kenya. These migrants know nothing of international boundaries, neither do their diseases. The movement of Maasai pastoralists between Kenya and Tanzania, and refugees from Burundi, DRC and Rwanda is another possible way of transboundary transmission of animal diseases.

Both livestock and wildlife are susceptible to a variety of diseases, most of which are endemic in Tanzania and a few occur as epizootic outbreaks. Most of the endemic diseases debilitate livestock, thus precipitating big economic losses, as evidenced by reduced growth rates, poor carcass yield and lower milk production. Outbreaks of epizootic diseases such as rinderpest and contagious bovine pleuropneumonia (CBPP) cause devastation to a large number of animals when they strike a highly susceptible population. The major diseases range from those caused by ectoparasites, protozoa and bacteria to viruses. Because of the diversity of such diseases and the impact they can have on productivity, animal disease research in Tanzania merits, by and large, priority consideration. It is a wide and complex subject involving numerous domestic and wild animals and a variety of specialised disciplines and skills which cut across all animal species.

With a total human population of about 34 million (United Republic of Tanzania URT 2002) and the third largest cattle population in Africa after Ethiopia and Sudan, the wildlife and livestock sectors are faced with a problem of disease spillover at their interface. Because they fall under two different ministries, the Ministry of Livestock Development and Fisheries (MoLDF) and the Ministry of Natural Resources and Tourism (MNRT), collaborative disease monitoring and surveillance in wildlife is only limited to transboundary animal diseases and zoonotic diseases. Since wildlife veterinary practice is in its infancy in Tanzania, little information is available regarding the prevalence of many diseases in wildlife. Because of the vast land area reserved for wildlife (30 %), Tanzania has an extensive livestock-wildlife interface. Due to constant movement of animals in the interface, there is high interaction between livestock and wildlife competing for food, water and space. A large human population adjacent to some of the protected areas is an ideal setting for disease spillover from livestock to wildlife and *vice versa*, for instance the Ser-

engeti ecosystem has some 2 million people, which is equal to the total population of Botswana.

### 22.3. Livestock Population in Tanzania

The estimated population of domestic animals in Tanzania is shown in Table 22.1. Many of these animals are found in varying numbers throughout the country, but there are insignificant numbers of cattle or none at all in coastal areas and the southern part of the country. However, in 2006, the government started shifting pastoralists from the Usangu plains to the Lindi and Mtwara regions of southern Tanzania. During the past 10 years, the trend has been for an increase in the number of cattle (local and exotic breeds) and an increase in local chicken, pig and commercial poultry farming, the latter two being in sub-urban and urban areas. Livestock farming in Tanzania is predominantly pastoral and agropastoral on communal land, with few private commercial farms, and many government farms and ranches have been privatised.

At the national level, livestock contribute significantly to the national income through taxes accrued during animal auctions and by the sale of meat and other animal products like milk, skins and hides; they account for 23 % of the production (Winrock-International 1992). In agropastoral communities, livestock are used as draught animals and to provide organic manure and fuel.

<i>S/n</i>	<i>Animal species</i>	<i>Total number</i>
1	Cattle	18 500 000
2	Goats	12 500 000
3	Sheep	4 700 000
4	Pigs	850 000
5	Donkeys	200 000
6	Horses	2000
7	Domestic dogs	5 000 000
8	Poultry	47 000 000

**Table 22.1:** Livestock population for selected species in Tanzania (sources: (MWLD 2003; Packer 2004; Winrock-International 1992))

## 22.4 Wildlife Population in Tanzania

Wildlife censuses in Tanzania have mostly been performed for large herbivores (see Table 22.2). It would be very difficult and expensive to establish the populations of small herbivores, carnivores and other small mammals. The populations of large carnivores in Tanzania that are known are of lions, hyenas, cheetahs and wild dogs. The Serengeti ecosystem alone has about 3000 lions, 300 leopards, 250 cheetahs and 7000 spotted hyenas. Data from other protected areas are mere estimates due to difficult terrain and insufficient human resources and equipment to conduct regular censuses in all the protected areas.

<i>S/n</i>	<i>Wild animal</i>	<i>Estimated number</i>
1	Herbivores	>4 000 000
2	Lions	15 000
3	Hyenas	30 000
4	Wild dogs	1000
5	Cheetahs	800

**Table 22.2:** Wildlife population in protected areas of Tanzania (source: (Packer 2004))

## 22.5. Major Livestock and Wildlife Diseases

Every animal harbours at least a few parasites, seemingly without adverse consequences. In nature, parasites, bacteria, fungi and viruses are component parts of the ecosystems in which wildlife is found, but they do not necessarily cause disease. Millions of years of co-evolution have engendered a *modus vivendi* that assures the survival of both host and parasite populations; hence, parasites do not cause devastation to wild animal populations and are regarded as ecological parasites.

The important wildlife diseases have been introduced from livestock or are newly emerging due to habitat change that influences some of the indigenous parasites to invade new, naïve hosts. The wildlife diseases that are of economic importance are those which cause clinical symptoms in wild animals. However, disease surveillance in wildlife is conducted to understand the ecology of the disease in protected areas and because it may potentially infect livestock.

## 22.5.1 Viral Infections

### 22.5.1.1. Rinderpest

Rinderpest (RP) can decimate large populations of domesticated and wild ungulates. It is caused by a *Morbilivirus* that is closely related to the causative agents of measles, canine distemper and peste des petits ruminants. Being a disease of public interest, it is the responsibility of the government to control and eradicate it with a total countrywide annual mass vaccination campaign which was stopped in 1997. This was a prerequisite for pursuing the International Organisation for Epizootic Diseases (OIE) pathway for eradication of the disease. To accomplish this pathway, certain conditions must be fulfilled to verify the absence of both clinical rinderpest and infection in livestock and wild animals. Active surveillance in wildlife and livestock is one means of achieving this objective. At present, wildlife and livestock in Tanzania are free from RP, but because of outbreaks in the Meru National Park in 2001 (R. Kock 2002, pers. comm.) and in livestock in Garisa in 2003 (Adela Mrosso 2003, pers. comm.), both in Kenya, Tanzania is continuing to conduct serosurveillance in wildlife and livestock. The last outbreak of RP in wildlife in Tanzania was in 1992 in the Mkomazi National Park, then a game reserve, which borders onto the Tsavo National Park in Kenya, and the last outbreak in livestock occurred in 1997 in the Ngorongoro, Monduli, Karatu and Hai districts of the northern zone (J.O. Mollel 2002, pers. comm.). In 2004, Tanzania accomplished the OIE pathway for RP eradication and was therefore declared freed from the disease in 2005.

Before 1960, when mass ring vaccinations of livestock against rinderpest were not well established, the population of wild ungulates was very low. For instance, wildebeest in the Serengeti ecosystem ranged from 200 000 to 300 000 (Sinclair 1973). However, following effective annual ring vaccinations in cattle, the wildebeest population rose steadily to 1.3-1.4 million in the 1970s.

### 22.5.1.2. Foot-and-Mouth Disease

Foot-and-mouth disease (FMD) occurs in cloven-hoofed animals and causes great economic losses in terms of animal productivity and death to exotic, naïve animals (cattle and pigs) (Figure 22.1). Most wild animals are resistant, but they act as reservoirs of infections for domestic animals. However, some wild animals like elands, kudu and impala show clinical symptoms in the same way that cattle and pigs do (C. Foggini 2001,



**Figure 22.1:** Foot-and-mouth disease in cattle

pers. comm.). Although the disease is endemic in Tanzania, it appears to have epidemic episodes. FMD was first reported in Tanzania in the 1920s. Four serotypes, O, A, South African Territory 1, 2 (SAT-1, 2) were reported to be prevalent in the country, but recent serosurveillance in wildlife and livestock has revealed that SAT-1, 2 and 3 in buffalo (Wiik & Fyumagwa 2002) and O, SAT-1 and 2 circulate in livestock, although SAT-3 has not been reported in livestock (MWLD 2003). In 2003, there were 160 outbreaks with 19 915 cases in cattle, out of which 347 died (MWLD 2003).

FMD is a disease of public interest and the government has responsibility for controlling it, but this is very expensive. A control and eradication policy is currently being formulated. The strategy is mass vaccination twice a year for each prevalent strain. At the moment, however, smallholder dairy cattle owners, dairy farms and beef ranches are responsible for controlling the disease on their respective farms through vaccination.

### **22.5.1.3. African Swine Fever Virus**

African swine fever (ASF) is a disease of domestic pigs, initially transmitted by soft ticks (*Ornithodoros* sp.) and later by contact when there is an overt clinical symptom. Highly infectious, fatal epidemics occurred in the Mbeya region in 1987 and the Kilimanjaro and Arusha regions in 1988 (MWLD 2000). Further epidemics occurred in Mbeya and Dar es Salaam in 2001 and the Arusha region in 2003. The 2004 outbreak in

the Kigoma region on Lake Tanganyika has been associated with movements of refugees from Burundi, who are blamed for poaching game in the adjacent protected areas.

ASF control rests on rigorously preventing contact between domestic pigs and wild reservoirs (warthogs and bush pigs), strict procedures for eradication and disinfection when the disease does occur, strict quarantine measures, restriction of animal movements and restriction on feeding insufficiently sterilised food scraps and air-dried bonemeal and leftovers from game meat (warthogs and bush pigs). Surveillance in the wild reservoirs (bush pigs and warthogs) has been conducted in the northern zone of Tanzania to characterise the circulating virus and try to establish the epidemiology of the epidemic disease in domestic pigs in the respective areas. All warthog serum samples were seropositive for the ASF virus, suggesting that exposure to the virus is very high and 50 % of blood samples were detected with the genetic material of the virus using reverse transcriptase-polymerase chain reaction (RT-PCR).

#### **22.5.1.4 Canine Distemper Virus**

Canine Distemper Virus (CDV) infection is a highly contagious and fatal disease which affects both domestic dogs and wild carnivores. However, in Tanzania, the livestock sector pays little attention to controlling the disease in domestic dogs because it is not zoonotic, even though it causes considerable mortality in dogs. The occurrence of a clinical Canine Distemper Virus (CDV) infection in susceptible wild carnivores such as lions and wild dogs (Figure 22.2) is unpredictable and occurs sporadically. In the 1980s, 22 of 77 screened lions (29 %) in the Serengeti ecosystem were seropositive for CDV (Kennedy-Stoskopf 1998), but the population was free from clinical symptoms of the disease. During this time, the rinderpest virus was sporadically circulating in wildlife and livestock. Whether the 29 % reactors were due to feeding on carcasses infected with the rinderpest virus (*Mobilivirus* – similar to CDV) remains unexplained and is mere speculation. However, the CDV epidemic which is most remembered occurred in 1993-94, when 30 % (1000) of the lion population in the Serengeti National Park died from the disease (Roelke-Parker et al. 1996). In 2001, about 94 % (49 out of 52) of captive, breeding wild dogs in the Mkomazi National Park died from CDV. They were receiving annual vaccinations of CDV vaccine using the phocine distemper recombinant vaccine. The outbreak in vaccinated wild dogs suggests that the vaccine was probably not conferring cross-protection against





**Figure 22.2:** Canine distemper (CDV) in African wild dogs

the particular CDV strain which was circulating in an unknown reservoir host in that area. In October 2007, 66 % (25 out of 38) of wild dogs in the Oloolosokwan pack in the Loliondo Game Controlled Area died from a CDV infection and the strain has been established to be similar to that which killed lions in the Serengeti National Park in the 1993-94 epidemic.

#### **22.5.1.5. Rabies Virus Infection**

This encephalitic disease can affect literally all warm-blooded animals, including humans. Rabies was reported for the first time in Tanzania in 1932 (Rweyemamu et al. 1973). Epidemiological observations by 1983 showed the disease to be enzootic in the country, and all the regions except Mtwara and Lindi in southern Tanzania and the Zanzibar Islands were infected (Loretu 1988; Magembe 1985). Over 90 % of cases were in domestic dogs and 3-4 % in wildlife, including jackals, mongooses, bat-eared foxes and spotted hyenas (Swanepoel 1994).

At the moment, the policy in Tanzania is for the individual owner of an animal to buy veterinary services, and vaccinations are to be annual. Due to the high cost of vaccination and the withdrawal of the government from conducting an annual mass vaccination campaign, only about 10 % of domestic dogs are vaccinated annually (Fyumagwa et al. 2002). This low percentage has precipitated the disease problem in Tanzania. However, as rabies is a disease of great public health importance, the government conducts some mass vaccinations in certain localities where rabies cases reach epidemic proportions. From 2003 to 2004, there was a rabies epidemic in domestic dogs in western Serengeti which caused human fatalities, especially among children who were bitten by rabid

dogs and did not receive post-exposure vaccines. In contrast to the epizootic situation outside the park, no case of symptomatic rabies was observed in wild carnivores inside the park between 2003 and 2005. This suggests that rabies that is epizootic in a rural domestic dog population close to protected areas does not lead to an increase in the incidence of rabies in wild carnivores (East et al. 2005).

The disease occurs sporadically in wild carnivores, and the domestic dog type is the most common strain. It caused the extinction of wild dogs in the Serengeti National Park around 1991. In 2003, there were 45 bovine cases and 523 dog cases, which caused 38 and 510 deaths, respectively (MWLD 2003). Recent surveillance has shown that 13 % of spotted hyenas in the Serengeti National Park have a unique rabies virus with 8.5 % sequence divergence from the dog strain. This has not been reported elsewhere in Africa, but resembles the strain found in the Middle East and it is non-virulent (East & Hofer 2001). Surveillance has also revealed the rabies virus in jackals, mongooses and bat-eared foxes, with a strain similar to the domestic canid strain.

#### **22.5.1.6. Malignant Catarrhal Fever**

In East Africa, Malignant Catarrhal Fever (MCF) is associated with wildebeest and the virus, *Alcelaphine herpes virus-1* (AHV-1), is shed by nasal discharges from newly born wildebeest calves and uterine membranes (Grootenhuis 2000). The disease outbreaks therefore occur when cattle come in contact with calving wildebeest, which happens when the migration is on the Serengeti short-grass plains, the Maasai steppe in Simanjiro and other adjacent dispersal areas. Maasai pastoralists have learned to live with the disease and move their cattle to the highlands during the wildebeest calving, thus abandoning the short-grass plains despite their high nutritive value. However, MCF is a particularly major problem in years without adequate rainfall, when the short-grass plains have a shortage of forage forcing the wildebeest to extend their grazing range into the highlands that would usually only be utilised by Maasai cattle. In 2000, for instance, 3000 heads of cattle are estimated to have died from MCF (J.O. Mollel, pers. comm.). The disease is also of significance in the differential diagnosis for rinderpest. It is also important to understand the seroprevalence in wildebeest to get more insight into the epidemiology of the disease to livestock. In the past few years, efforts have been made to establish a vaccine for cattle and plans are under way to conduct an experimental study on vaccine trials against MCF. The



impact of vaccine development for the sustainable conservation of the Serengeti ecosystem requires some consideration.

#### **22.5.1.7. Newcastle Disease**

Poultry is the major source of animal protein for the majority of rural people in Tanzania. However, local chickens are faced with a highly fatal viral disease, which decimates 45 % of the population every year (MWLD 2000). Clinical and pathological data of confirmed Newcastle disease (NCD) in Tanzania indicate a high frequency of the viscerotropic form of the disease and further laboratory investigations indicate the occurrence of velogenic-viscerotropic NCD infection.

Newcastle disease is controlled by restricting the movement and banning the import, export and slaughter of affected birds. Birds may be vaccinated with a thermo-stable vaccine produced at the Central Veterinary Laboratory in Dar es Salaam. Small doses are available for small flocks of indigenous chickens at a very low price (1 US \$ per 400 doses).

Previously, the disease was thought to pose little threat to wild birds. However, in 2006, doves in northern Tanzania, particularly laughing doves and a few mourning doves, were affected by a virus closely related to the Newcastle disease virus (*Paramyxovirus-1*). Reports of dove mortality were received from TANAPA staff, birdwatchers, tour operators and communities adjacent to affected areas. The sick birds were seen resting on trees or near water sources and when disturbed they flew and fell to the ground and could not fly anymore. The clinical symptoms include general weakness (lack of endurance and stamina to fly), whitish and/or greenish watery diarrhoea, salivation, twisting of the head and neck, unable to hold the head and neck upwards, unable to fly when approached, subnormal temperatures (38.2 to 38.4 °C) and infestation with biting flies. The significant post-mortem findings were dehydration, emaciation, haemorrhages in the intestines, proventriculus and brain and under the skin, and liver congestion. The epidemic in doves did not coincide with Newcastle disease in poultry in adjacent communities and no other wild bird species died from the disease, suggesting that the viral strain was species-specific to doves only. The disease disappeared after the onset of the short rains in November 2006, suggesting that its transmission to many susceptible individuals was exacerbated by severe drought which forced many birds to concentrate at a few water sources.

Retrospective information from local communities indicates that mass deaths of doves in Tanzania were observed in 1991 in Loliondo (Ndelani

Ndiko 2006, pers. comm.). However, poor communication facilities at the time meant that the relevant authorities were perhaps not informed and no investigation was conducted to establish the cause of the mortality. The 2006 outbreak began in March with a few doves reported to have died in Babati (Kaaya, pers. comm.) and widespread high mortality was observed from the end of September 2006 (Swai and Morris Kilewo, pers. comm.). Dove deaths from the epidemic were reported in the Babati, Karatu, Monduli, Simanjiro, Longido and Rombo districts. Because Tanzania is the largest exporter of live wild birds in the world, its presence in poultry and wild birds threatens the sustainability of the trade. It also costs a great deal to screen all the birds and hold them for more than 21 days prior to shipment to their designated destination.

#### **22.5.1.8. Rift Valley Fever Virus**

Rift Valley Fever Virus (RVFV) is an arbovirus associated with periodic outbreaks, mostly on the African continent. The infection causes a febrile disease accompanied by abortion in livestock and a febrile illness that can be associated with a severe, fatal haemorrhagic syndrome in humans. RVFV outbreaks occur when there are unusually heavy rains, but little is known of how the virus is maintained during the inter-epidemic period when there is no disease in livestock or humans. At least seven wildlife species, African buffalo, black rhinoceros, kudu, impala, African elephants, hartebeest and waterbucks, have been detected with RVFV neutralising antibodies (Evans et al. 2007). The highest neutralising antibody titres have been observed in the buffalo, suggesting that it is probably an important wild animal species in the epidemiology of the disease (Evans et al. 2007). An RVFV epidemic occurred in Tanzania in 1998 during the heavy *El Niño* rains, at which time some small Maasai stocks in northern Tanzania were affected. The most recent RVFV epidemic occurred in 2006-2007 in northern pastoral communities and central Tanzania, especially the Dodoma region. Many livestock died from the disease and hundreds of people were affected with more than 100 people reported to have died from the epidemic. The disease caused widespread panic in Tanzania and many people were scared of eating beef. The epidemic started in Kenya in early 2006 and spread southwards to northern and central Tanzania. The exposure status among wildlife in Tanzania is still under investigation, but preliminary results show that 3 % of the wildlife in northern Tanzania is exposed to the infection. In the Serengeti ecosystem, the wild animals found positive for RVFV include

wildebeest, buffalo, spotted hyenas, zebras and topi. Competitive ELISA (c-ELISA) for detection of RFV Immunoglobulin G was used in the analysis and the cut-off point was for bovid which is not species-specific.

#### **22.5.1.9. Equine Herpes Viruses**

Equine herpes viruses (EHV) are a group of viruses infecting equids. The viral diseases caused by EHV are important because there is no cure and infections of susceptible naïve animals are fatal. About 11 equine herpes viruses (EHV) are known to infect equids, but the most important ones are EHV-1, 2, 3, 4, 5 and 9. EHV-1, 3, 4 and 9 cause neurological symptoms and respiratory disease (*rhinopneumonitis*). Other symptoms include abortion, paresis or paralysis in domestic horses, and they show latency in sensory ganglia. EHV-2 and EHV-5 are non-pathogenic and show latency in the lympho-reticular system. Equine arteritis virus (EAV) is also pathogenic to domestic horses. EHV-9 has been reported to be highly pathogenic and caused fatal infection in Thomson's gazelles in a zoo in Japan.

Viral research on Burchell's zebras in the Serengeti ecosystem, which is one of the long-term projects under the TAWIRI veterinary programme, has found that:

1. 60 % of the population is exposed to EHV-9 (the virus DNA has been detected and the report submitted for publication)
2. 11 % is exposed to EHV-1
3. 2 % is exposed to EHV-4
4. 0 % is exposed to EHV-2, but it has been observed in zebras in Namibia
5. 24 % of the Burchell's zebra population is exposed to EAV

#### **22.5.1.10. African Horse Sickness**

African Horse Sickness (AHS) is a fatal disease in exotic horses. It is endemic in Tanzania, but its distribution is restricted to the distribution of the arthropod vector. Many horses imported in 1978 without prior vaccination died more or less immediately on arrival and mortality rates approached 100 % in such places as Morogoro and Mabuki, in Mwanza (Semuguruka 2003, pers. comm.). It is recommended that African horse sickness vaccines used in Tanzania should contain virus types

1-9 (MWLD 2000). Although clinical symptoms have not been seen in wild equids, serosurveys in Burchell's zebras and donkeys have shown that they are exposed to the virus, suggesting that they may be reservoirs of the virus. However, more research is required to understand the epidemiology of the diseases in horses and the reservoir status of zebras and donkeys to such viruses.

## **22.5.2. Bacterial Infections**

### **22.5.2.1. Tuberculosis (TB)**

Isolated cases of *Mycobacterium bovis* have been reported in dairy cattle and local cattle, but the prevalence of the disease is not well established. Most TB cases are diagnosed on slaughter slabs during meat inspection. Routine screening using a tuberculin test is done in dairy farms and national ranches, and rarely in pastoral cattle.

Isolated cases of *M. bovis* have been reported in buffalo, giraffes, wildebeest, elands and lions. However, the prevalence of the disease is not yet established. The presence of *M. bovis* is an indication of spillover from livestock. Systematic screening of 20 buffalo in the Serengeti ecosystem using gamma interferon assay and ELISA gave negative results.

### **22.5.2.2. Contagious Bovine Pleuropneumonia**

Contagious Bovine Pleuropneumonia (CBPP) is a bacterial disease (lung sickness) in cattle caused by mycoplasma (*Mycoplasma mycoides mycoides*). It is widespread in sub-Saharan Africa. In Tanzania, the disease mainly affects pastoral cattle because systematic annual vaccination is hindered by unrestricted movement. The disease has decimated quite a large proportion of cattle. A vaccination campaign is currently taking place throughout the country, starting in the southern highlands where there is an epidemic and moving towards the northern zone where the disease is endemic. However, it is very difficult to restrict the movement of pastoral cattle which reside in remote areas and trek cattle at night.

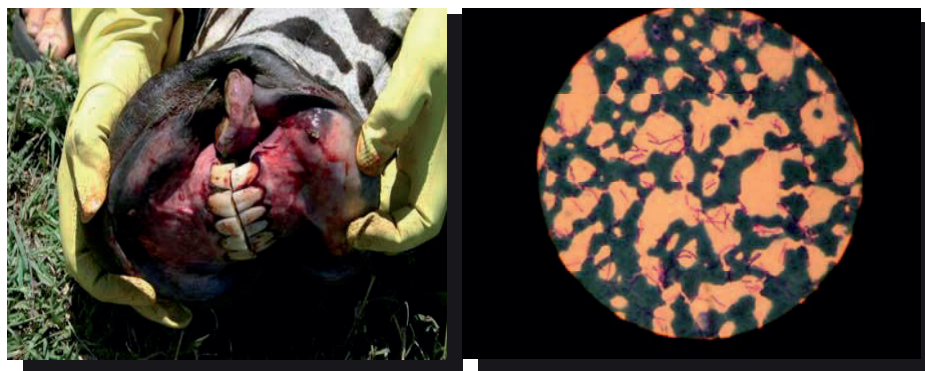
The disease is of great economic importance and it is the responsibility of the government to import the vaccine and distribute it to district veterinary officers for vaccination at a subsidised cost. Although the disease does not affect wild ruminants like buffalo it is important to conduct serosurveys in areas where cattle are most affected to get scientific confirmation and understand more of the role of wildlife in the epidemiology of the disease.

### 22.5.2.3. Anthrax

The disease is caused by *Bacillus anthracis* (A and B) and occurs sporadically, but is basically enzootic in Tanzania and is controlled by routine vaccination of valuable stock in and around areas of outbreaks (MWLD 2000). Individual livestock keepers are responsible for seeking advice on how to conduct vaccinations. However, when there is an outbreak, the government is responsible for securing a vaccine and supervising the campaign.

The bacterium affects domestic and wild animals, but zebras (Figure 22.3) are more susceptible than buffalo and wildebeest (unpublished data). The disease is endemic in some protected areas, but occurs sporadically. For instance, in 1984, an outbreak reported in the Lake Manyara National Park killed a range of wild animal species, although impala were most affected. There were also concurrent outbreaks in domestic stock (cattle, sheep and goats) in neighbouring areas, indicating a close association between livestock and wildlife.

In 1989, several hippopotami died from the disease in the Ruaha National Park (Kapela & Maiseli 1990) and in 1998 an outbreak occurred in the northern part of the Serengeti National Park, killing more than 1000 impala and other herbivores. In 2000 and 2001, again hippopotami died in the Ugalla Game Reserve and at the Mtera Dam, respectively. In 2003, an outbreak occurred in the Ngorongoro Conservation Area and the Serengeti National Park; in the latter, impala were most affected. In 2003, hippopotami were again affected in the Ruaha National Park and 10 buffalo died in the Arusha National Park. In January 2004, 385 herbi-



**Figure 22.3:** Anthrax in zebras and Anthrax bacteria (*Bacillus anthracis*) in a blood smear (Giemsa stain)

vores died in the Lake Manyara National Park, 360 of which were impala, 4 elephants, 2 giraffes and 4 buffalo. Besides these major outbreaks, some isolated cases occur from time to time in different areas. Unfortunately, efforts have not been made to find out the type of *B. anthracis* which is responsible for the outbreak in livestock and wildlife.

#### **22.5.2.4. Brucellosis**

Brucellosis was first reported in Tanzania in the 1920s. *Brucella abortus* appears to be endemic in local cattle in Tanzania with an average prevalence of 11 % (MWLD 2000), but isolated cases have also been reported in smallholder dairy cattle. In the Southern Highlands, 20 % of screened goats were shown to be infected with *B. melitensis*. In the northern zone, the prevalence is about 8 % in Maasai cattle and 3.3 % in agropastoral communities (MWLD 2000). However, information on prevalence derives from small-sample surveys and is therefore subject to uncertainty (Berman 1981). In 2003, the Ministry of Water and Livestock Development reported 245 bovine cases, but it is not clear how many cattle were screened and in which part of the country (MWLD 2003). As a serosurvey of livestock in western Serengeti is in progress, the exposure status of agropastoral livestock in the area is not available for inclusion here.

Serosurveys have been conducted to detect antibodies against *B. abortus* in wildebeest, buffalo, zebras and other wild herbivore species. Seropositive results have been observed only in buffalo and wildebeest, with a general exposure status of 24 % and 17 %, respectively (Fyumagwa et al. 2007c). The serosurveys involved 102 and 106 serum samples, respectively, from the Ngorongoro Crater and the Serengeti National Park. The findings show that the exposure status is higher in wildlife than in Maasai cattle, suggesting that the infection has probably become established in wildlife and could be a potential source of spillover infection to livestock. Plans are underway to try to isolate the bacterium in wildlife to achieve a better understanding of the epidemiology of the disease in livestock.

### **22.5.3. Vector-borne Diseases**

#### **22.5.3.1. Tick-borne Diseases**

Tick-borne diseases account for about 72 % of the total livestock mortality in Tanzania (Lynen et al. 2007). Theileriosis, in particular East Coast Fever (ECF), is widespread and causes the greatest losses among cattle,



with a mortality rate of approximately 12 % of the local cattle population, but it can reach 100 % for exotic cattle breeds if not treated (MWLD 2000). Other tick-borne diseases of importance to livestock are Anaplasmosis, Babesiosis and Ehrlichiosis. Under national disease control policy, it is the responsibility of the individual farmer to buy veterinary services for treatment and disease control measures. The main disease control strategy is to dip livestock in acaricides to reduce the tick burden on the animals. However, constraints are the cost of the acaricides and development of tick resistance to some of the acaricides in use (Lynen et al. 2007).

*Theileria*, *Babesia*, hemotropic *Mycoplasma*, *Hepatozoon* and *Ehrlichia* species were the major blood parasites that affected wild herbivores and lions in the Ngorongoro Crater in 2000 and 2001, respectively, killing more than 1000 buffalo (Figure 22.4), three black rhinoceros, hundreds of other herbivores and about 20 lions (Fyumagwa et al. 2008). The predisposing factors were probably stress from starvation due to drought the preceding year and high tick infestation with means of  $42 \pm 7.4$  and  $819 \pm 152.95$  ( $\pm$  SE) for adult and immature ticks per square metre, respectively, concurrent with swarms of biting flies (*Stomoxys calcitrans*) (Fyumagwa et al. 2007b).

The black rhinoceros died from *Babesia bicornis*, a newly reported haemoparasite in Tanzania (Fyumagwa et al. 2004b; Nijhof et al. 2003), but its vector is still unknown. The study on tick ecology and vegetation structure in the Ngorongoro Crater identified about 15 tick species. In a subsample of ticks collected in the crater grassland and on wildlife, 11 tick species were identified and by molecular analysis for haemoparasi-



**Figure 22.4:** High tick infestation on buffalo in the Ngorongoro Crater

tes, nine species were infected by one or several of the tick-borne parasites (Fyumagwa et al. 2009). One tick species, *Rhipicephalus sanguineus*, from lions was found to harbour haemotropic *Mycoplasma haemofelis*, *Candidatus* 'Mycoplasma haemominutum' (Fyumagwa et al. 2008) and *Hepatozoon felis* (unpublished).

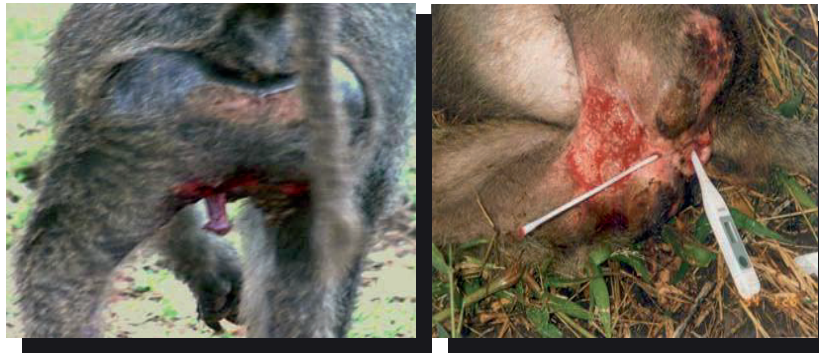
### 22.5.3.2. Trypanosomosis

In Tanzania, all trypanosomes are transmitted by tsetse flies. Over 60 % of the land is tsetse infested and over 75 % of the cattle population is exposed to tsetse flies, hence trypanosomosis is virtually enzootic in the greater part of Tanzania (MWLD 2000). Four *Trypanosoma* species, *T. congolense*, *T. vivax*, *T. simiae* and *T. brucei*, are important for livestock in Tanzania. The first two are important for cattle and the third causes acute and fatal trypanosomosis in domestic pigs (Fyumagwa 2000). *T. brucei* is less important for cattle but can cause acute disease in domestic dogs and the subspecies, *T. brucei rhodesiense*, causes sleeping sickness in humans, which is fatal and difficult to treat. Almost all the protected areas are tsetse infested, but the disease is not a problem to wildlife due to millennia of co-evolution with the protozoa. Wildlife is therefore a major reservoir of the disease to livestock at the interface and trypanosomosis is the second killer of livestock after ECF in Tanzania. An increase in the tsetse population following the *El Niño* event of 1997-98 caused an outbreak of sleeping sickness in the Serengeti National Park and adjacent areas concurrent with a trypanosomosis epidemic in livestock in the interface. Trypanosomosis in horses has been a big problem in a tourist investment (Grumeti Reserves Ltd) adjacent to the Ikorongo-Grumeti Game Reserve bordering the Serengeti National Park, and at least four deaths have been reported (Auty et al. 2008).

### 22.5.4. Emerging Diseases

These are diseases whose pathogens appear for the first time and infect naïve populations with devastating effects. They can result from a previously non-virulent pathogen which can turn into a virulent form in the original host as a result of mutation or genetic re-assortment with other pathogens or when it jumps to a new naïve host in the same ecological zone and causes clinical symptoms in the new host with some devastating effects. The change in virulence can also be a result of climate change like the current effects of global warming. A number of diseases have





**Figure 22.5:** Olive baboon with genital disease (*Treponema pallidum pallidum*)

emerged in recent years in Tanzania, including cerebral theileriosis in cattle, babesiosis in rhinoceros, ear disease in giraffes, genital infection in baboons (Figure 22.5) and algal toxins in lesser flamingos.

#### **22.5.4.1. Cerebral Theileriosis**

Theileriosis is a group of diseases in livestock and wildlife caused by many *Theileria* species which are tick-borne Apicomplexan protozoa (Grootenhuis 2000). Cerebral theileriosis or ormilo (Maasai vernacular) is a new disease caused by *Theileria taurotragi*, which previously was described as non-pathogenic to cattle. The disease was reported for the first time in the Ngorongoro Conservation Area in 1987. The protozoan used to be considered a benign parasite of elands (J.O. Mollel 2002, pers. comm.). Cerebral theileriosis occurs as a result of accumulation of infected lymphoblasts in the central nervous system (Kettle 1995). The protozoan is transmitted by *Rhipicephalus purchellus* and the *R. appendiculatus* group of ticks, including *R. zambeziensis* and *R. duttoni*, which is restricted to eastern, central and southern Africa (Walker et al. 2003).

#### **22.5.4.2. Babesiosis in Black Rhinoceros**

A fatal *Babesia* infection in black rhinoceros occurred for the first time in the Ngorongoro Crater in 2000-2001. The new haemoparasite, later identified as *Babesia bicornis*, caused an acute infection with the mortality of at least two black rhinoceros in 2001 (Fyumagwa et al. 2004a; Nijhof et al. 2003). The epidemic was associated with high tick infestation on crater grassland and wild herbivores subsequent to severe drought in the preceding year of 2000 (Fyumagwa et al. 2007b). It is postulated that

long-term weather changes with cycles of severe drought followed by wet years and lack of prescribed burning in the crater exacerbated tick infestation in the crater. Severe drought caused herbivores nutritional stress which is immunosuppressive and, hence, wildlife succumbed to clinical infections from multiple infectious agents (Fyumagwa et al. 2007b). The disease outbreak among the rhinoceros population in the Ngorongoro Conservation Area is a newly reported phenomenon. The death of the rhinoceros was most probably a result of infection with *Babesia bicornis* (Nijhof et al. 2003), which was opportunistic as a result of immunosuppression brought on by stress from severe drought in the preceding months and concurrent with a high level of tick infestation in the crater grassland (Fyumagwa et al. 2004a). Thus, the evidence suggests that latent infection with a stress trigger is more likely than sudden exposure to a naïve infection due to poor immune response.

#### **22.5.4.3. *Streptococcus* Infection in Spotted Hyenas**

In 2002 and 2003, spotted hyenas were affected by *Streptococcus equi* subsp. *ruminatorum*, a bacterium which caused severe pneumonia. In general, 12.5 % of the affected hyenas in the crater population from five out of eight affected clans died, reducing the population by 4.3 % (Honer et al. 2006). It was the first time a *Streptococcus* infection caused clinical disease with noticeable mortality in hyenas in Tanzania. The bacterium resembles *S. pneumoniae* var. *zooepidermicus*, which recently caused mortality in sheep in Spain (Honer et al. 2006).

#### **22.5.4.4. *Genital Infection in Olive Baboons***

A previously unreported genital infection (Figure 22.5) was observed in several olive baboon troops in the Gombe National Park in western Tanzania in 1988. In 1994, a similar disease was reported in the Lake Manyara National Park in the Eastern Rift Valley (Fyumagwa et al. 2003). The infections caused such severe structural damage that articles in the popular media described animals dying with «rotting genitals». Laboratory analysis using PCR of genital swabs from affected baboons revealed the presence of the bacterium *Treponema pallidum*. Specifically, it was shown that: 1) *T. pallidum*, the agent responsible for the human diseases, yaws, bejel and syphilis, could be found in the lesions of infected animals, 2) infection with the bacterium was statistically associated with the

clinical signs of disease, 3) these clinical signs differ from those previously described in non-venereal treponemal infections of wild primates, but are similar to those seen in venereal syphilis in humans, 4) the strain identified is genetically distinct from other characterised strains of the bacterium, and 5) the infection appears to be relatively new and spreading between adjacent baboon populations. Although outward signs of infection can be severe, ending in sterility and occasionally death, most infected baboons do not show visible signs of disease. Finally, the genetic comparison of this baboon strain to human strains indicates that the human *T. pallidum* infection was passed down from our anthropoid ancestors. If, as the evidence suggests, *T. pallidum* plays a causative role in the disease, a new transmission mode may have evolved in Tanzanian baboons and the affected populations could provide a model system for understanding the emergence of syphilis, and perhaps other sexually transmitted diseases (STDs), in humans.

#### **22.5.4.5. Ear Infection in Giraffe**

A fatal ear disease has been spreading in giraffes in the Mikumi National Park and the Selous Game Reserve (Mikumi-Selous ecosystem) since 1999. The disease is characterised by drooping ears, swarms of flies around the ears, and affected animals being reluctant to browse and spending much of the time hiding their heads in tall grass in an effort to avoid flies. The animals finally lose condition, terminating in death. In 2002, it was estimated that 90 % of the giraffes in the Mikumi-Selous ecosystem were affected, and 13 % were in advanced stages of infection (Mlengeya et al. 2002).

Preliminary investigations indicated that the disease could be associated with ticks, biting flies and bacterial infection. Environmental factors, such as decreased browsing material, animals being concentrated in small areas and a high incidence of ticks, were considered to be contributing to the spread of the disease. However, studies are ongoing to clearly identify the ecological factors and link them to the suspected causative agents of the giraffe ear problem.

The Mikumi-Selous ecosystem has one wet and one dry season, each lasting roughly six months from late-November to early-May and from mid-May to early-November, respectively, and a definite dry spell in January-February. The normal annual rainfall varies from 650 to 950 mm, although rainfall is very variable from year to year.

The park harbours a good number of wildlife species including giraffes, lions, buffalo, Burchell's zebras, white-bearded wildebeest, African elephants, hippopotami, elands, warthogs, common waterbucks, Lichtenstein's hartebeest, sable antelopes, greater kudus, Bohor reedbucks, black-backed jackals, African hunting dogs, African civets, leopards, black and white colobus monkeys, yellow baboons and a large variety of birds and insects.

Whereas the 2002 survey revealed that 90 % of giraffes in the Mikumi National Park were infected, with 13 % in advanced stages (26 out of 202 observed), a total of 274 giraffes were observed in surveys carried out between October 2003 and September 2005, and only 14 (5.11 %) showed signs of infection. The tick density in the park in the 2002 survey was estimated to be  $500 \pm 255.28$  ( $\pm$  SD) and the prevalence of the advanced stage of ear disease was 13 %. In 2003 and 2004, the period prevalence (5.1 %) of the advanced stage of ear disease was significantly lower ( $P < 0.01$ ) than the point prevalence (13 %) observed in August 2002. The linear regression analysis with an equation  $Y = 0.017X + 4.388$  had a coefficient of determination of 0.774, suggesting that 77.4 % of the cases could not occur if there were no ticks in the ecosystem.

Could this ear disease be indigenous to giraffes, getting worse during wet seasons and spontaneously recovering after the rains? Was the disease always present, but was only noticed in 1999? Although the number of giraffes observed during the reported surveys was limited, the results show that more giraffes suffered from the disease during the wet than the dry season. Pier (1981) reported that the disease is transmitted by arthropod vectors, including ticks and flies, moisture enhances the transmission of the infection, major outbreaks in a local population or a geographical area are usually related to rainfall, and the rate of new cases recedes when the rainy season wanes and flies and ticks abate. The positive gradient in the regression line is also indicative of a probable link between the number of ticks in the habitat and the severity of ear infection in giraffes. The standard deviation of the mean tick density was very high, indicating that the tick infestation in the Mikumi National Park was localised to certain areas. It is probably influenced by the type of vegetation, which indirectly determines the distribution of herbivores. Different herbivore species have variable natural resistance against ticks. The mean tick density has declined concomitantly with the improvement of the ear infection from August 2002 to September 2005 and was positively correlated with the decrease in tick density in the park. Studies have shown that the bont tick, *Amblyomma variegatum*, is closely associated

with dermatophilosis and is considered to be responsible for aggravating the condition (Lloyd & Walker 1993). In addition to *A. variegatum*, a closely related tick species, *A. gemma*, was always present and abundant on giraffes and buffalo.

*Dermatophilus congolensis* is a gram-positive, non-acid fast, facultative anaerobic bacterium which causes dermatophilosis (streptothricosis), an exudative, pustular dermatitis that mainly affects cattle, sheep and horses, but has also been reported in goats, many wild mammals, reptiles and occasionally humans (Jones et al. 1995). Dermatophilosis has been reported worldwide and, in East Africa, cases have been observed in sheep, goats, cattle and camels (Gitao et al. 1990; Msami et al. 2001). The infection occurs when the integrity of the skin is impaired, as in long exposure to rains or traumatic injuries resulting from arthropod bites, for example, ticks and flies (Zaria 1993). The arthropods also serve as mechanical transmitters of *D. congolensis* (Msami et al. 2001). Other bacteria found in lesions include *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Streptococcus* spp. and *Klebsiella* spp. The multiple secondary bacterial infections are likely to have exacerbated the problem.

#### **22.5.4.6. Mass Die-offs of Lesser Flamingos**

The lesser flamingo is endemic in Africa south of the Sahara and is categorised on the IUCN Red List as 'near threatened' or 'vulnerable' across its entire range (McCulloch et al. 2003). Lack of enough breeding sites and vulnerability to both natural and anthropogenic disturbance during breeding have a significant impact on the population growth (Anderson 2000; Simons 2000). The species is a phytoplankton feeder mainly consuming the cyanobacterium (*Arthrospira* sp.) that prevails in soda lakes. A mass die-off of lesser flamingos was observed for the first time in Tanzania at the Empakaai Crater in August 2000 (Figure 22.6) when more than 1000 birds were estimated to have died. In 2002, high mortality of lesser flamingos was noted in Lake Natron, which is the most important breeding site for flamingos in eastern and southern Africa (Simons 2000; Tuite 2000). Efforts were made to find the cause of the problem, and water and tissue samples from the affected areas were collected and submitted for analysis of pesticide residues at the Government Chief Chemists' Laboratory in Dar es Salaam, Tanzania. Since the analyses did not provide conclusive answers that could easily be linked to mass die-off, and because some flamingos that were dying were presenting central nervous signs that could result from cyanotoxins and pathologi-



**Figure 22.6:** Flamingo mass mortality from cyanotoxins

cal features indicating bacterial infection, attempts were made to assess possible toxins in the lake that might have originated from cyanobacterial toxins and histopathological examination for bacterial involvement. During the mass die-off of lesser flamingos in soda lakes in Tanzania in 2000, 2002 and 2004, clinicopathological and toxicological investigations were made to elucidate the likely cause of mortality. Water and tissue samples were collected from the lakes and from dead flamingos, respectively. Water samples and tissues were analysed for pesticide residues and tissues were also analysed for cyanotoxins. The significant pathological lesions observed in fresh carcasses included oedema in lungs, enlarged liver, haemorrhages in liver with multiple necrotic foci, haemorrhages in kidneys and haemorrhages in intestines with erosion of the inner lining (mucosa). Analysis of cyanotoxins revealed the presence of neurotoxin (anatoxin-a) and hepatotoxins (microcystins LR, RR). Concentrations of microcystins LR were significantly higher ( $P = 0.0003$ ) in liver than in other tissues. Based on clinicopathological findings and concentrations of the detected cyanotoxins, it is suspected that cyanobacterial toxins concurrent with secondary bacterial infection were the likely cause of the observed mortalities in flamingos (Fyumagwa et al. 2007a; Lugomela et al. 2006).

#### **22.5.4.7. Respiratory Disease in Chimpanzees**

Recent outbreaks of pneumonia in chimpanzees in the Gombe and Mahale national parks caused by *Streptococcus pneumoniae* and measles are a threat which is influencing the demography of the species. The investigation to establish the epidemiology of the infection is still ongoing, but because chimpanzees are closely related to humans,



researchers suspect that the infections are probably a spillover from humans.

## 22.6. National Wildlife Disease Control Strategies

The recent outbreaks of diseases in wildlife made the wildlife sector realise the importance of having wildlife veterinary officers in Tanzania. In 1992, there was only one wildlife veterinary officer. However, the number had increased to 11 by 2008 and they are fully engaged in disease surveillance in wildlife. At the Faculty of Veterinary Medicine at Sokoine University of Agriculture (SUA), some newly recruited academic staff have started to specialise in wildlife medicine. At the inter-ministerial level between the Ministry of Livestock Development and Fisheries (MoLDF) and the Ministry of Natural Resources and Tourism (MNRT), the Director of Veterinary Services (DVS) and the Director of Wildlife (DW) have signed a memorandum of understanding (MoU) to put more emphasis on monitoring and surveillance of diseases in wildlife. Under this new MoU, the DVS is the spokesperson for all disease problems in wildlife, as is also the case for livestock on the Tanzanian mainland.

## 22.7. Major Constraints and the Way Forward

Because major diseases occur in the interface between wildlife and livestock in remote areas, the accessibility of these areas is a big challenge. Lack of enough trained manpower to maintain vigilance in disease monitoring and surveillance is another constraint. Availability of reliable sources of funding and equipment to conduct disease monitoring and surveillance is another obstacle. As Tanzania is endowed with the highest population of wildlife in the world and has the third highest population of cattle among African countries, it encounters some difficulty in effectively controlling disease spill-over from wild animals to livestock and *vice versa*. Since some of the disease agents, like *Bacillus anthracis*, are free-living in the environment, with such vast protected areas it is impossible to eradicate them and they will remain a potential source of infection.

The communal land tenure system also has an impact when it comes to movement of livestock. The task ahead is for the livestock and wildlife

sectors and other stakeholders and regional development institutions to come together and formulate strategies for sustainable disease control with the limited resources available. The overall purpose should be to reduce the disease problems in the interface at the regional level and put in place sustainable disease surveillance.



A snared zebra in Serengeti National Park (Photo : E. Røskaft)







**Doctoral theses in Biology**  
**Norwegian University of Science and Technology**  
**Department of Biology**

<b>Year</b>	<b>Name</b>	<b>Degree</b>	<b>Title</b>
1974	Tor-Henning Iversen	Dr. philos Botany	The roles of statholiths, auxin transport, and auxin metabolism in root gravitropism
1978	Tore Slagsvold	Dr. philos Zoology	Breeding events of birds in relation to spring temperature and environmental phenology
1978	Egil Sakshaug	Dr. philos Botany	"The influence of environmental factors on the chemical composition of cultivated and natural populations of marine phytoplankton"
1980	Arnfinn Langeland	Dr. philos Zoology	Interaction between fish and zooplankton populations and their effects on the material utilization in a freshwater lake
1980	Helge Reinertsen	Dr. philos Botany	The effect of lake fertilization on the dynamics and stability of a limnetic ecosystem with special reference to the phytoplankton
1982	Gunn Mari Olsen	Dr. scient Botany	Gravitropism in roots of <i>Pisum sativum</i> and <i>Arabidopsis thaliana</i>
1982	Dag Dolmen	Dr. philos Zoology	Life aspects of two sympatric species of newts ( <i>Triturus</i> , <i>Amphibia</i> ) in Norway, with special emphasis on their ecological niche segregation
1984	Eivin Røskaft	Dr. philos Zoology	Sociobiological studies of the rook <i>Corvus frugilegus</i>
1984	Anne Margrethe Cameron	Dr. scient Botany	Effects of alcohol inhalation on levels of circulating testosterone, follicle stimulating hormone and luteinizing hormone in male mature rats
1984	Asbjørn Magne Nilsen	Dr. scient Botany	Alveolar macrophages from expectorates – Biological monitoring of workers exposed to occupational air pollution. An evaluation of the AM-test
1985	Jarle Mork	Dr. philos Zoology	Biochemical genetic studies in fish
1985	John Solem	Dr. philos Zoology	Taxonomy, distribution and ecology of caddisflies ( <i>Trichoptera</i> ) in the Dovrefjell mountains
1985	Randi E. Reinertsen	Dr. philos Zoology	Energy strategies in the cold: Metabolic and thermoregulatory adaptations in small northern birds
1986	Bernt-Erik Sæther	Dr. philos Zoology	Ecological and evolutionary basis for variation in reproductive traits of some vertebrates: A comparative approach
1986	Torleif Holthe	Dr. philos Zoology	Evolution, systematics, nomenclature, and zoogeography in the polychaete orders <i>Oweniimorpha</i> and <i>Terebellomorpha</i> , with special reference to the Arctic and Scandinavian fauna
1987	Helene Lampe	Dr. scient Zoology	The function of bird song in mate attraction and territorial defence, and the importance of song repertoires
1987	Olav Hogstad	Dr. philos Zoology	Winter survival strategies of the Willow tit <i>Parus montanus</i>
1987	Jarle Inge Holten	Dr. philos Botany	Autecological investigations along a coast-inland transect at Nord-Møre, Central Norway

1987 Rita Kumar	Dr. scient Botany	Somaclonal variation in plants regenerated from cell cultures of <i>Nicotiana glauca</i> and <i>Chrysanthemum morifolium</i>
1987 Bjørn Åge Tømmerås	Dr. scient. Zoology	Olfaction in bark beetle communities: Interspecific interactions in regulation of colonization density, predator - prey relationship and host attraction
1988 Hans Christian Pedersen	Dr. philos Zoology	Reproductive behaviour in willow ptarmigan with special emphasis on territoriality and parental care
1988 Tor G. Heggberget	Dr. philos Zoology	Reproduction in Atlantic Salmon ( <i>Salmo salar</i> ): Aspects of spawning, incubation, early life history and population structure
1988 Marianne V. Nielsen	Dr. scient Zoology	The effects of selected environmental factors on carbon allocation/growth of larval and juvenile mussels ( <i>Mytilus edulis</i> )
1988 Ole Kristian Berg	Dr. scient Zoology	The formation of landlocked Atlantic salmon ( <i>Salmo salar</i> L.)
1989 John W. Jensen	Dr. philos Zoology	Crustacean plankton and fish during the first decade of the manmade Nesjø reservoir, with special emphasis on the effects of gill nets and salmonid growth
1989 Helga J. Vivås	Dr. scient Zoology	Theoretical models of activity pattern and optimal foraging: Predictions for the Moose <i>Alces alces</i>
1989 Reidar Andersen	Dr. scient Zoology	Interactions between a generalist herbivore, the moose <i>Alces alces</i> , and its winter food resources: a study of behavioural variation
1989 Kurt Ingar Draget	Dr. scient Botany	Alginate gel media for plant tissue culture
1990 Bengt Finstad	Dr. scient Zoology	Osmotic and ionic regulation in Atlantic salmon, rainbow trout and Arctic charr: Effect of temperature, salinity and season
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1990 Åse Krøkje	Dr. scient Botany	The mutagenic load from air pollution at two workplaces with PAH-exposure measured with Ames Salmonella/microsome test
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1990 Magne Husby	Dr. scient Zoology	Breeding strategies in birds: Experiments with the Magpie <i>Pica pica</i>
1991 Tor Kvam	Dr. scient Zoology	Population biology of the European lynx ( <i>Lynx lynx</i> ) in Norway
1991 Jan Henning L'Abée Lund	Dr. philos Zoology	Reproductive biology in freshwater fish, brown trout <i>Salmo trutta</i> and roach <i>Rutilus rutilus</i> in particular
1991 Asbjørn Moen	Dr. philos Botany	The plant cover of the boreal uplands of Central Norway. I. Vegetation ecology of Sølendet nature reserve; haymaking fens and birch woodlands
1991 Else Marie Løbersli	Dr. scient Botany	Soil acidification and metal uptake in plants
1991 Trond Nordtug	Dr. scient Zoology	Reflectometric studies of photomechanical adaptation in superposition eyes of arthropods
1991 Thyra Solem	Dr. scient Botany	Age, origin and development of blanket mires in Central Norway

1991 Odd Terje Sandlund	Dr. philos Zoology	The dynamics of habitat use in the salmonid genera <i>Coregonus</i> and <i>Salvelinus</i> : Ontogenic niche shifts and polymorphism
1991 Nina Jonsson	Dr. philos	Aspects of migration and spawning in salmonids
1991 Atle Bones	Dr. scient Botany	Compartmentation and molecular properties of thioglucoside glucohydrolase (myrosinase)
1992 Torgrim Breiehagen	Dr. scient Zoology	Mating behaviour and evolutionary aspects of the breeding system of two bird species: the Temminck's stint and the Pied flycatcher
1992 Anne Kjersti Bakken	Dr. scient Botany	The influence of photoperiod on nitrate assimilation and nitrogen status in timothy ( <i>Phleum pratense</i> L.)
1992 Tycho Anker-Nilssen	Dr. scient Zoology	Food supply as a determinant of reproduction and population development in Norwegian Puffins <i>Fratercula arctica</i>
1992 Bjørn Munro Jenssen	Dr. philos Zoology	Thermoregulation in aquatic birds in air and water: With special emphasis on the effects of crude oil, chemically treated oil and cleaning on the thermal balance of ducks
1992 Arne Vollan Aarset	Dr. philos Zoology	The ecophysiology of under-ice fauna: Osmotic regulation, low temperature tolerance and metabolism in polar crustaceans.
1993 Geir Slupphaug	Dr. scient Botany	Regulation and expression of uracil-DNA glycosylase and O <sup>6</sup> -methylguanine-DNA methyltransferase in mammalian cells
1993 Tor Fredrik Næsje	Dr. scient Zoology	Habitat shifts in coregonids.
1993 Yngvar Asbjørn Olsen	Dr. scient Zoology	Cortisol dynamics in Atlantic salmon, <i>Salmo salar</i> L.: Basal and stressor-induced variations in plasma levels and some secondary effects.
1993 Bård Pedersen	Dr. scient Botany	Theoretical studies of life history evolution in modular and clonal organisms
1993 Ole Petter Thangstad	Dr. scient Botany	Molecular studies of myrosinase in Brassicaceae
1993 Thrine L. M. Heggberget	Dr. scient Zoology	Reproductive strategy and feeding ecology of the Eurasian otter <i>Lutra lutra</i> .
1993 Kjetil Bevanger	Dr. scient Zoology	Avian interactions with utility structures, a biological approach.
1993 Kåre Haugan	Dr. scient Bothany	Mutations in the replication control gene trfA of the broad host-range plasmid RK2
1994 Peder Fiske	Dr. scient. Zoology	Sexual selection in the lekking great snipe ( <i>Gallinago media</i> ): Male mating success and female behaviour at the lek
1994 Kjell Inge Reitan	Dr. scient Botany	Nutritional effects of algae in first-feeding of marine fish larvae
1994 Nils Røv	Dr. scient Zoology	Breeding distribution, population status and regulation of breeding numbers in the northeast-Atlantic Great Cormorant <i>Phalacrocorax carbo carbo</i>
1994 Annette-Susanne Hoepfner	Dr. scient Botany	Tissue culture techniques in propagation and breeding of Red Raspberry ( <i>Rubus idaeus</i> L.)
1994 Inga Elise Bruteig	Dr. scient Bothany	Distribution, ecology and biomonitoring studies of epiphytic lichens on conifers
1994 Geir Johnsen	Dr. scient Botany	Light harvesting and utilization in marine phytoplankton: Species-specific and photoadaptive responses
1994 Morten Bakken	Dr. scient Zoology	Infanticidal behaviour and reproductive performance in relation to competition capacity among farmed silver fox vixens, <i>Vulpes vulpes</i>

1994 Arne Moksnes	Dr. philos Zoology	Host adaptations towards brood parasitism by the Cockoo
1994 Solveig Bakken	Dr. scient Bothany	Growth and nitrogen status in the moss <i>Dicranum majus</i> Sm. as influenced by nitrogen supply
1994 Torbjørn Forseth	Dr. scient Zoology	Bioenergetics in ecological and life history studies of fishes.
1995 Olav Vadstein	Dr. philos Botany	The role of heterotrophic planktonic bacteria in the cycling of phosphorus in lakes: Phosphorus requirement, competitive ability and food web interactions
1995 Hanne Christensen	Dr. scient Zoology	Determinants of Otter <i>Lutra lutra</i> distribution in Norway: Effects of harvest, polychlorinated biphenyls (PCBs), human population density and competition with mink <i>Mustela vison</i>
1995 Svein Håkon Lorentsen	Dr. scient Zoology	Reproductive effort in the Antarctic Petrel <i>Thalassoica</i> <i>antarctica</i> ; the effect of parental body size and condition
1995 Chris Jørgen Jensen	Dr. scient Zoology	The surface electromyographic (EMG) amplitude as an estimate of upper trapezius muscle activity
1995 Martha Kold Bakkevig	Dr. scient Zoology	The impact of clothing textiles and construction in a clothing system on thermoregulatory responses, sweat accumulation and heat transport
1995 Vidar Moen	Dr. scient Zoology	Distribution patterns and adaptations to light in newly introduced populations of <i>Mysis relicta</i> and constraints on Cladoceran and Char populations
1995 Hans Haavardsholm Blom	Dr. philos Bothany	A revision of the <i>Schistidium apocarpum</i> complex in Norway and Sweden
1996 Jorun Skjærmo	Dr. scient Botany	Microbial ecology of early stages of cultivated marine fish; impact fish-bacterial interactions on growth and survival of larvae
1996 Ola Ugedal	Dr. scient Zoology	Radiocesium turnover in freshwater fishes
1996 Ingridbjørg Einarsdottir	Dr. scient Zoology	Production of Atlantic salmon ( <i>Salmo salar</i> ) and Arctic charr ( <i>Salvelinus alpinus</i> ): A study of some physiological and immunological responses to rearing routines
1996 Christina M. S. Pereira	Dr. scient Zoology	Glucose metabolism in salmonids: Dietary effects and hormonal regulation
1996 Jan Fredrik Børseth	Dr. scient Zoology	The sodium energy gradients in muscle cells of <i>Mytilus</i> <i>edulis</i> and the effects of organic xenobiotics
1996 Gunnar Henriksen	Dr. scient Zoology	Status of Grey seal <i>Halichoerus grypus</i> and Harbour seal <i>Phoca vitulina</i> in the Barents sea region
1997 Gunvor Øie	Dr. scient Bothany	Eevaluation of rotifer <i>Brachionus plicatilis</i> quality in early first feeding of turbot <i>Scophthalmus maximus</i> L. larvae
1997 Håkon Holien	Dr. scient Botany	Studies of lichens in spurce forest of Central Norway. Diversity, old growth species and the relationship to site and stand parameters
1997 Ole Reitan	Dr. scient. Zoology	Responses of birds to habitat disturbance due to damming
1997 Jon Arne Grøttum	Dr. scient. Zoology	Physiological effects of reduced water quality on fish in aquaculture
1997 Per Gustav Thingstad	Dr. scient. Zoology	Birds as indicators for studying natural and human- induced variations in the environment, with special emphasis on the suitability of the Pied Flycatcher
1997 Torgeir Nygård	Dr. scient Zoology	Temporal and spatial trends of pollutants in birds in Norway: Birds of prey and Willow Grouse used as Biomonitors

1997 Signe Nybø	Dr. scient. Zoology	Impacts of long-range transported air pollution on birds with particular reference to the dipper <i>Cinclus cinclus</i> in southern Norway
1997 Atle Wibe	Dr. scient. Zoology	Identification of conifer volatiles detected by receptor neurons in the pine weevil ( <i>Hylobius abietis</i> ), analysed by gas chromatography linked to electrophysiology and to mass spectrometry
1997 Rolv Lundheim	Dr. scient Zoology	Adaptive and incidental biological ice nucleators
1997 Arild Magne Landa	Dr. scient Zoology	Wolverines in Scandinavia: ecology, sheep depredation and conservation
1997 Kåre Magne Nielsen	Dr. scient Botany	An evolution of possible horizontal gene transfer from plants to soil bacteria by studies of natural transformation in <i>Acinetobacter calcoaceticus</i>
1997 Jarle Tufto	Dr. scient Zoology	Gene flow and genetic drift in geographically structured populations: Ecological, population genetic, and statistical models
1997 Trygve Hesthagen	Dr. philos Zoology	Population responses of Arctic charr ( <i>Salvelinus alpinus</i> (L.)) and brown trout ( <i>Salmo trutta</i> L.) to acidification in Norwegian inland waters
1997 Trygve Sigholt	Dr. philos Zoology	Control of Parr-smolt transformation and seawater tolerance in farmed Atlantic Salmon ( <i>Salmo salar</i> ) Effects of photoperiod, temperature, gradual seawater acclimation, NaCl and betaine in the diet
1997 Jan Østnes	Dr. scient Zoology	Cold sensation in adult and neonate birds
1998 Seethaledsumy Visvalingam	Dr. scient Botany	Influence of environmental factors on myrosinases and myrosinase-binding proteins
1998 Thor Harald Ringsby	Dr. scient Zoology	Variation in space and time: The biology of a House sparrow metapopulation
1998 Erling Johan Solberg	Dr. scient. Zoology	Variation in population dynamics and life history in a Norwegian moose ( <i>Alces alces</i> ) population: consequences of harvesting in a variable environment
1998 Sigurd Mjøen Saastad	Dr. scient Botany	Species delimitation and phylogenetic relationships between the Sphagnum recurvum complex (Bryophyta): genetic variation and phenotypic plasticity
1998 Bjarte Mortensen	Dr. scient Botany	Metabolism of volatile organic chemicals (VOCs) in a head liver S9 vial equilibration system in vitro
1998 Gunnar Austrheim	Dr. scient Botany	Plant biodiversity and land use in subalpine grasslands. – A conservation biological approach
1998 Bente Gunnveig Berg	Dr. scient Zoology	Encoding of pheromone information in two related moth species
1999 Kristian Overskaug	Dr. scient Zoology	Behavioural and morphological characteristics in Northern Tawny Owls <i>Strix aluco</i> : An intra- and interspecific comparative approach
1999 Hans Kristen Stenøien	Dr. scient Botany	Genetic studies of evolutionary processes in various populations of nonvascular plants (mosses, liverworts and hornworts)
1999 Trond Arnesen	Dr. scient Botany	Vegetation dynamics following trampling and burning in the outlying haylands at Sølendet, Central Norway
1999 Ingvar Stenberg	Dr. scient Zoology	Habitat selection, reproduction and survival in the White-backed Woodpecker <i>Dendrocopos leucotos</i>
1999 Stein Olle Johansen	Dr. scient Botany	A study of driftwood dispersal to the Nordic Seas by dendrochronology and wood anatomical analysis

1999 Trina Falck Galloway	Dr. scient Zoology	Muscle development and growth in early life stages of the Atlantic cod ( <i>Gadus morhua</i> L.) and Halibut ( <i>Hippoglossus hippoglossus</i> L.)
1999 Marianne Giæver	Dr. scient Zoology	Population genetic studies in three gadoid species: blue whiting ( <i>Micromisistius poutassou</i> ), haddock ( <i>Melanogrammus aeglefinus</i> ) and cod ( <i>Gradus morhua</i> ) in the North-East Atlantic
1999 Hans Martin Hanslin	Dr. scient Botany	The impact of environmental conditions of density dependent performance in the boreal forest bryophytes <i>Dicranum majus</i> , <i>Hylocomium splendens</i> , <i>Plagiochila asplenigides</i> , <i>Ptilium crista-castrensis</i> and <i>Rhytidiadelphus lokeus</i>
1999 Ingrid Bysveen Mjølnerød	Dr. scient Zoology	Aspects of population genetics, behaviour and performance of wild and farmed Atlantic salmon ( <i>Salmo salar</i> ) revealed by molecular genetic techniques
1999 Else Berit Skagen	Dr. scient Botany	The early regeneration process in protoplasts from <i>Brassica napus</i> hypocotyls cultivated under various g-forces
1999 Stein-Are Sæther	Dr. philos Zoology	Mate choice, competition for mates, and conflicts of interest in the Lekking Great Snipe
1999 Katrine Wangen Rustad	Dr. scient Zoology	Modulation of glutamatergic neurotransmission related to cognitive dysfunctions and Alzheimer's disease
1999 Per Terje Smiseth	Dr. scient Zoology	Social evolution in monogamous families: mate choice and conflicts over parental care in the Bluethroat ( <i>Luscinia s. svecica</i> )
1999 Gunnbjørn Bremset	Dr. scient Zoology	Young Atlantic salmon ( <i>Salmo salar</i> L.) and Brown trout ( <i>Salmo trutta</i> L.) inhabiting the deep pool habitat, with special reference to their habitat use, habitat preferences and competitive interactions
1999 Frode Ødegaard	Dr. scient Zoology	Host spesificity as parameter in estimates of arthropod species richness
1999 Sonja Andersen	Dr. scient Bothany	Expressional and functional analyses of human, secretory phospholipase A2
2000 Ingrid Salvesen	Dr. scient Botany	Microbial ecology in early stages of marine fish: Development and evaluation of methods for microbial management in intensive larviculture
2000 Ingar Jostein Øien	Dr. scient Zoology	The Cuckoo ( <i>Cuculus canorus</i> ) and its host: adaptions and counteradaptions in a coevolutionary arms race
2000 Pavlos Makridis	Dr. scient Botany	Methods for the microbial econtrol of live food used for the rearing of marine fish larvae
2000 Sigbjørn Stokke	Dr. scient Zoology	Sexual segregation in the African elephant ( <i>Loxodonta africana</i> )
2000 Odd A. Gulseth	Dr. philos Zoology	Seawater tolerance, migratory behaviour and growth of Charr, ( <i>Salvelinus alpinus</i> ), with emphasis on the high Arctic Dieset charr on Spitsbergen, Svalbard
2000 Pål A. Olsvik	Dr. scient Zoology	Biochemical impacts of Cd, Cu and Zn on brown trout ( <i>Salmo trutta</i> ) in two mining-contaminated rivers in Central Norway
2000 Sigurd Einum	Dr. scient Zoology	Maternal effects in fish: Implications for the evolution of breeding time and egg size
2001 Jan Ove Evjemo	Dr. scient Zoology	Production and nutritional adaptation of the brine shrimp <i>Artemia</i> sp. as live food organism for larvae of marine cold water fish species
2001 Olga Hilmo	Dr. scient Botany	Lichen response to environmental changes in the managed boreal forest systems



2001 Ingebrigt Uglem	Dr. scient Zoology	Male dimorphism and reproductive biology in corkwing wrasse ( <i>Symphodus melops</i> L.)
2001 Bård Gunnar Stokke	Dr. scient Zoology	Coevolutionary adaptations in avian brood parasites and their hosts
2002 Ronny Aanes	Dr. scient	Spatio-temporal dynamics in Svalbard reindeer ( <i>Rangifer tarandus platyrhynchus</i> )
2002 Mariann Sandsund	Dr. scient Zoology	Exercise- and cold-induced asthma. Respiratory and thermoregulatory responses
2002 Dag-Inge Øien	Dr. scient Botany	Dynamics of plant communities and populations in boreal vegetation influenced by scything at Sølendet, Central Norway
2002 Frank Rosell	Dr. scient Zoology	The function of scent marking in beaver ( <i>Castor fiber</i> )
2002 Janne Østvang	Dr. scient Botany	The Role and Regulation of Phospholipase A <sub>2</sub> in Monocytes During Atherosclerosis Development
2002 Terje Thun	Dr.philos Biology	Dendrochronological constructions of Norwegian conifer chronologies providing dating of historical material
2002 Birgit Hafjeld Borgen	Dr. scient Biology	Functional analysis of plant idioblasts (Myrosin cells) and their role in defense, development and growth
2002 Bård Øyvind Solberg	Dr. scient Biology	Effects of climatic change on the growth of dominating tree species along major environmental gradients
2002 Per Winge	Dr. scient Biology	The evolution of small GTP binding proteins in cellular organisms. Studies of RAC GTPases in <i>Arabidopsis thaliana</i> and the Ral GTPase from <i>Drosophila melanogaster</i>
2002 Henrik Jensen	Dr. scient Biology	Causes and consequences of individual variation in fitness-related traits in house sparrows
2003 Jens Rohloff	Dr. philos Biology	Cultivation of herbs and medicinal plants in Norway – Essential oil production and quality control
2003 Åsa Maria O. Espmark Wibe	Dr. scient Biology	Behavioural effects of environmental pollution in threespine stickleback <i>Gasterosteus aculeatus</i> L.
2003 Dagmar Hagen	Dr. scient Biology	Assisted recovery of disturbed arctic and alpine vegetation – an integrated approach
2003 Bjørn Dahle	Dr. scient Biology	Reproductive strategies in Scandinavian brown bears
2003 Cyril Lebogang Taolo	Dr. scient Biology	Population ecology, seasonal movement and habitat use of the African buffalo ( <i>Syncerus caffer</i> ) in Chobe National Park, Botswana
2003 Marit Stranden	Dr.scient Biology	Olfactory receptor neurones specified for the same odorants in three related Heliothine species ( <i>Helicoverpa armigera</i> , <i>Helicoverpa assulta</i> and <i>Heliothis virescens</i> )
2003 Kristian Hassel	Dr.scient Biology	Life history characteristics and genetic variation in an expanding species, <i>Pogonatum dentatum</i>
2003 David Alexander Rae	Dr.scient Biology	Plant- and invertebrate-community responses to species interaction and microclimatic gradients in alpine and Arctic environments
2003 Åsa A Borg	Dr.scient Biology	Sex roles and reproductive behaviour in gobies and guppies: a female perspective
2003 Eldar Åsgard Bendiksen	Dr.scient Biology	Environmental effects on lipid nutrition of farmed Atlantic salmon ( <i>Salmo Salar</i> L.) parr and smolt
2004 Torkild Bakken	Dr.scient Biology	A revision of Nereidinae (Polychaeta, Nereididae)
2004 Ingar Pareliussen	Dr.scient Biology	Natural and Experimental Tree Establishment in a Fragmented Forest, Ambohitantely Forest Reserve, Madagascar

2004 Tore Brembu	Dr.scient Biology	Genetic, molecular and functional studies of RAC GTPases and the WAVE-like regulatory protein complex in <i>Arabidopsis thaliana</i>
2004 Liv S. Nilsen	Dr.scient Biology	Coastal heath vegetation on central Norway; recent past, present state and future possibilities
2004 Hanne T. Skiri	Dr.scient Biology	Olfactory coding and olfactory learning of plant odours in heliothine moths. An anatomical, physiological and behavioural study of three related species ( <i>Heliothis virescens</i> , <i>Helicoverpa armigera</i> and <i>Helicoverpa assulta</i> )
2004 Lene Østby	Dr.scient Biology	Cytochrome P4501A (CYP1A) induction and DNA adducts as biomarkers for organic pollution in the natural environment
2004 Emmanuel J. Gerreta	Dr. philos Biology	The Importance of Water Quality and Quantity in the Tropical Ecosystems, Tanzania
2004 Linda Dalen	Dr.scient Biology	Dynamics of Mountain Birch Treelines in the Scandes Mountain Chain, and Effects of Climate Warming
2004 Lisbeth Mehli	Dr.scient Biology	Polygalacturonase-inhibiting protein (PGIP) in cultivated strawberry ( <i>Fragaria x ananassa</i> ): characterisation and induction of the gene following fruit infection by <i>Botrytis cinerea</i>
2004 Børge Moe	Dr.scient Biology	Energy-Allocation in Avian Nestlings Facing Short-Term Food Shortage
2005 Matilde Skogen Chauton	Dr.scient Biology	Metabolic profiling and species discrimination from High-Resolution Magic Angle Spinning NMR analysis of whole-cell samples
2005 Sten Karlsson	Dr.scient Biology	Dynamics of Genetic Polymorphisms
2005 Terje Bongard	Dr.scient Biology	Life History strategies, mate choice, and parental investment among Norwegians over a 300-year period
2005 Tonette Røstelién	ph.d Biology	Functional characterisation of olfactory receptor neurone types in heliothine moths
2005 Erlend Kristiansen	Dr.scient Biology	Studies on antifreeze proteins
2005 Eugen G. Sørmo	Dr.scient Biology	Organochlorine pollutants in grey seal ( <i>Halichoerus grypus</i> ) pups and their impact on plasma thyroid hormone and vitamin A concentrations
2005 Christian Westad	Dr.scient Biology	Motor control of the upper trapezius
2005 Lasse Mork Olsen	ph.d Biology	Interactions between marine osmo- and phagotrophs in different physicochemical environments
2005 Åslaug Viken	ph.d Biology	Implications of mate choice for the management of small populations
2005 Ariaya Hymete Sahle Dingle	ph.d Biology	Investigation of the biological activities and chemical constituents of selected <i>Echinops</i> spp. growing in Ethiopia
2005 Anders Gravbrøt Finstad	ph.d Biology	Salmonid fishes in a changing climate: The winter challenge
2005 Shimane Washington Makabu	ph.d Biology	Interactions between woody plants, elephants and other browsers in the Chobe Riverfront, Botswana
2005 Kjartan Østbye	Dr.scient Biology	The European whitefish <i>Coregonus lavaretus</i> (L.) species complex: historical contingency and adaptive radiation

2006 Kari Mette Murvoll	ph.d Biology	Levels and effects of persistent organic pollutants (POPs) in seabirds Retinoids and $\alpha$ -tocopherol – potential biomarkers of POPs in birds?
2006 Ivar Herfindal	Dr.scient Biology	Life history consequences of environmental variation along ecological gradients in northern ungulates
2006 Nils Egil Tokle	ph.d Biology	Are the ubiquitous marine copepods limited by food or predation? Experimental and field-based studies with main focus on <i>Calanus finmarchicus</i>
2006 Jan Ove Gjershaug	Dr.philos Biology	Taxonomy and conservation status of some booted eagles in south-east Asia
2006 Jon Kristian Skei	Dr.scient Biology	Conservation biology and acidification problems in the breeding habitat of amphibians in Norway
2006 Johanna Järnegren	ph.d Biology	Acesta Oophaga and Acesta Excavata – a study of hidden biodiversity
2006 Bjørn Henrik Hansen	ph.d Biology	Metal-mediated oxidative stress responses in brown trout ( <i>Salmo trutta</i> ) from mining contaminated rivers in Central Norway
2006 Vidar Grøtan	ph.d Biology	Temporal and spatial effects of climate fluctuations on population dynamics of vertebrates
2006 Jafari R Kideghesho	ph.d Biology	Wildlife conservation and local land use conflicts in western Serengeti, Corridor Tanzania
2006 Anna Maria Billing	ph.d Biology	Reproductive decisions in the sex role reversed pipefish <i>Syngnathus typhle</i> : when and how to invest in reproduction
2006 Henrik Pärn	ph.d Biology	Female ornaments and reproductive biology in the bluethroat
2006 Anders J. Fjellheim	ph.d Biology	Selection and administration of probiotic bacteria to marine fish larvae
2006 P. Andreas Svensson	ph.d Biology	Female coloration, egg carotenoids and reproductive success: gobies as a model system
2007 Sindre A. Pedersen	ph.d Biology	Metal binding proteins and antifreeze proteins in the beetle <i>Tenebrio molitor</i> - a study on possible competition for the semi-essential amino acid cysteine
2007 Kasper Hancke	ph.d Biology	Photosynthetic responses as a function of light and temperature: Field and laboratory studies on marine microalgae
2007 Tomas Holmern	ph.d Biology	Bushmeat hunting in the western Serengeti: Implications for community-based conservation
2007 Kari Jørgensen	ph.d Biology	Functional tracing of gustatory receptor neurons in the CNS and chemosensory learning in the moth <i>Heliothis virescens</i>
2007 Stig Ulland	ph.d Biology	Functional Characterisation of Olfactory Receptor Neurons in the Cabbage Moth, ( <i>Mamestra brassicae</i> L.) (Lepidoptera, Noctuidae). Gas Chromatography Linked to Single Cell Recordings and Mass Spectrometry
2007 Snorre Henriksen	ph.d Biology	Spatial and temporal variation in herbivore resources at northern latitudes
2007 Roelof Frans May	ph.d Biology	Spatial Ecology of Wolverines in Scandinavia
2007 Vedasto Gabriel Ndibalema	ph.d Biology	Demographic variation, distribution and habitat use between wildebeest sub-populations in the Serengeti National Park, Tanzania

2007 Julius William Nyahongo	ph.d Biology	Depredation of Livestock by wild Carnivores and Illegal Utilization of Natural Resources by Humans in the Western Serengeti, Tanzania
2007 Shombe Ntaraluka Hassan	ph.d Biology	Effects of fire on large herbivores and their forage resources in Serengeti, Tanzania
2007 Per-Arvid Wold	ph.d Biology	Functional development and response to dietary treatment in larval Atlantic cod ( <i>Gadus morhua</i> L.) Focus on formulated diets and early weaning
2007 Anne Skjetne Mortensen	ph.d Biology	Toxicogenomics of Aryl Hydrocarbon- and Estrogen Receptor Interactions in Fish: Mechanisms and Profiling of Gene Expression Patterns in Chemical Mixture Exposure Scenarios
2008 Brage Bremset Hansen	ph.d Biology	The Svalbard reindeer ( <i>Rangifer tarandus platyrhynchus</i> ) and its food base: plant-herbivore interactions in a high-arctic ecosystem
2008 Jiska van Dijk	ph.d Biology	Wolverine foraging strategies in a multiple-use landscape
2008 Flora John Magige	ph.d Biology	The ecology and behaviour of the Masai Ostrich ( <i>Struthio camelus massaicus</i> ) in the Serengeti Ecosystem, Tanzania
2008 Bernt Rønning	ph.d Biology	Sources of inter- and intra-individual variation in basal metabolic rate in the zebra finch, ( <i>Taeniopygia guttata</i> )
2008 Solvi Wehn	ph.d Biology	Biodiversity dynamics in semi-natural mountain landscapes. - A study of consequences of changed agricultural practices in Eastern Jotunheimen
2008 Trond Moxness Kortner	ph.d Biology	"The Role of Androgens on previtellogenic oocyte growth in Atlantic cod ( <i>Gadus morhua</i> ): Identification and patterns of differentially expressed genes in relation to Stereological Evaluations"
2008 Katarina Mariann Jørgensen	Dr.Scient Biology	The role of platelet activating factor in activation of growth arrested keratinocytes and re-epithelialisation
2008 Tommy Jørstad	ph.d Biology	Statistical Modelling of Gene Expression Data
2008 Anna Kusnierczyk	ph.d Biology	<i>Arabidopsis thaliana</i> Responses to Aphid Infestation
2008 Jussi Evertsen	ph.d Biology	Herbivore sacoglossans with photosynthetic chloroplasts
2008 John Eilif Hermansen	ph.d Biology	Mediating ecological interests between locals and globals by means of indicators. A study attributed to the asymmetry between stakeholders of tropical forest at Mt. Kilimanjaro, Tanzania
2008 Ragnhild Lyngved	ph.d Biology	Somatic embryogenesis in <i>Cyclamen persicum</i> . Biological investigations and educational aspects of cloning
2008 Line Elisabeth Sundt-Hansen	ph.d Biology	Cost of rapid growth in salmonid fishes
2008 Line Johansen	ph.d Biology	Exploring factors underlying fluctuations in white clover populations – clonal growth, population structure and spatial distribution
2009 Astrid Jullumstrø Feuerherm	ph.d Biology	Elucidation of molecular mechanisms for pro-inflammatory phospholipase A2 in chronic disease

2009 Pål Kvello	ph.d Biology	Neurons forming the network involved in gustatory coding and learning in the moth <i>Heliothis virescens</i> : Physiological and morphological characterisation, and integration into a standard brain atlas
2009 Trygve Devold Kjellsen	ph.d Biology	Extreme Frost Tolerance in Boreal Conifers
2009 Johan Reinert Vikan	ph.d Biology	Coevolutionary interactions between common cuckoos <i>Cuculus canorus</i> and <i>Fringilla</i> finches
2009 Zsolt Volent	ph.d Biology	Remote sensing of marine environment: Applied surveillance with focus on optical properties of phytoplankton, coloured organic matter and suspended matter
2009 Lester Rocha	ph.d Biology	Functional responses of perennial grasses to simulated grazing and resource availability
2009 Dennis Ikanda	ph.d Biology	Dimensions of a Human-lion conflict: Ecology of human predation and persecution of African lions ( <i>Panthera leo</i> ) in Tanzania
2010 Huy Quang Nguyen	ph.d Biology	Egg characteristics and development of larval digestive function of cobia ( <i>Rachycentron canadum</i> ) in response to dietary treatments -Focus on formulated diets
2010 Eli Kvingedal	ph.d Biology	Intraspecific competition in stream salmonids: the impact of environment and phenotype
2010 Sverre Lundemo	ph.d Biology	Molecular studies of genetic structuring and demography in <i>Arabidopsis</i> from Northern Europe
2010 Iddi Mihijai Mfunda	ph.d Biology	Wildlife Conservation and People's livelihoods: Lessons Learnt and Considerations for Improvements. The Case of Serengeti Ecosystem, Tanzania
2010 Anton Tinchov Antonov	ph.d Biology	Why do cuckoos lay strong-shelled eggs? Tests of the puncture resistance hypothesis
2010 Anders Lyngstad	ph.d Biology	Population Ecology of <i>Eriophorum latifolium</i> , a Clonal Species in Rich Fen Vegetation
2010 Hilde Færevik	ph.d Biology	Impact of protective clothing on thermal and cognitive responses
2010 Ingerid Brønne Arbo	ph.d Medical technology	Nutritional lifestyle changes – effects of dietary carbohydrate restriction in healthy obese and overweight humans
2010 Yngvild Vindenes	ph.d Biology	Stochastic modeling of finite populations with individual heterogeneity in vital parameters
2010 Hans-Richard Brattbakk	ph.d Medical technology	The effect of macronutrient composition, insulin stimulation, and genetic variation on leukocyte gene expression and possible health benefits
2011 Geir Hysing Bolstad	ph.d Biology	Evolution of Signals: Genetic Architecture, Natural Selection and Adaptive Accuracy
2011 Karen de Jong	ph.d Biology	Operational sex ratio and reproductive behaviour in the two-spotted goby ( <i>Gobiusculus flavescens</i> )
2011 Ann-Iren Kittang	ph.d Biology	<i>Arabidopsis thaliana</i> L. adaptation mechanisms to microgravity through the EMCS MULTIGEN-2 experiment on the ISS:– The science of space experiment integration and adaptation to simulated microgravity
2011 Aline Magdalena Lee	ph.d Biology	Stochastic modeling of mating systems and their effect on population dynamics and genetics
2011 Christopher Gravningen Sørmo	ph.d Biology	Rho GTPases in Plants: Structural analysis of ROP GTPases; genetic and functional studies of MIRO GTPases in <i>Arabidopsis thaliana</i>

2011 Grethe Robertsen	ph.d Biology	Relative performance of salmonid phenotypes across environments and competitive intensities
2011 Line-Kristin Larsen	ph.d Biology	Life-history trait dynamics in experimental populations of guppy ( <i>Poecilia reticulata</i> ): the role of breeding regime and captive environment
2011 Maxim A. K. Teichert	ph.d Biology	Regulation in Atlantic salmon ( <i>Salmo salar</i> ): The interaction between habitat and density
2011 Torunn Beate Hancke	ph.d Biology	Use of Pulse Amplitude Modulated (PAM) Fluorescence and Bio-optics for Assessing Microalgal Photosynthesis and Physiology
2011 Sajeda Begum	ph.d Biology	Brood Parasitism in Asian Cuckoos: Different Aspects of Interactions between Cuckoos and their Hosts in Bangladesh
2011 Kari J. K. Attramadal	ph.d Biology	Water treatment as an approach to increase microbial control in the culture of cold water marine larvae
2011 Camilla Kalvatn Egset	ph.d Biology	The Evolvability of Static Allometry: A Case Study
2011 AHM Raihan Sarker	ph.d Biology	Conflict over the conservation of the Asian elephant ( <i>Elephas maximus</i> ) in Bangladesh
2011 Gro Dehli Villanger	ph.d Biology	Effects of complex organohalogen contaminant mixtures on thyroid hormone homeostasis in selected arctic marine mammals
2011 Kari Bjørneraas	ph.d Biology	Spatiotemporal variation in resource utilisation by a large herbivore, the moose
2011 John Odden	ph.d Biology	The ecology of a conflict: Eurasian lynx depredation on domestic sheep
2011 Simen Pedersen	ph.d Biology	Effects of native and introduced cervids on small mammals and birds
2011 Mohsen Falahati-Anbaran	ph.d Biology	Evolutionary consequences of seed banks and seed dispersal in <i>Arabidopsis</i>
2012 Jakob Hønborg Hansen	ph.d Biology	Shift work in the offshore vessel fleet: circadian rhythms and cognitive performance
2012 Elin Noreen	ph.d Biology	Consequences of diet quality and age on life-history traits in a small passerine bird
2012 Irja Ida Ratikainen	ph.d Biology	Theoretical and empirical approaches to studying foraging decisions: the past and future of behavioural ecology
2012 Aleksander Handå	ph.d Biology	Cultivation of mussels ( <i>Mytilus edulis</i> ): Feed requirements, storage and integration with salmon ( <i>Salmo salar</i> ) farming
2012 Morten Kraabøl	ph.d Biology	Reproductive and migratory challenges inflicted on migrant brown trout ( <i>Salmo trutta</i> L) in a heavily modified river
2012 Jisca Huisman	ph.d Biology	Gene flow and natural selection in Atlantic salmon
2012 Maria Bergvik	ph.d Biology	Lipid and astaxanthin contents and biochemical post-harvest stability in <i>Calanus finmarchicus</i>
2012 Bjarte Bye Løfaldli	ph.d Biology	Functional and morphological characterization of central olfactory neurons in the model insect <i>Heliothis virescens</i> .
2012 Karen Marie Hammer	ph.d Biology.	Acid-base regulation and metabolite responses in shallow- and deep-living marine invertebrates during environmental hypercapnia
2012 Øystein Nordrum Wiggen	ph.d Biology	Optimal performance in the cold

2012 Robert Dominikus Dr. Philos. Anthropogenic and natural influence on disease  
Fyumagwa prevalence at the human-livestock-wildlife interface in  
the Serengeti ecosystem, Tanzania

