AN ABSTRACT OF THE DISSERTATION OF

Brianna Beechler for the degree of Doctor of Philosophy in Environmental Sciences presented on May 24, 2013.

Title: Rift Valley Fever in African Buffalo (*Syncerus caffer*): Basic epidemiology and the Role of Bovine Tuberculosis Coinfection

Abstract approved:

Anna E Jolles

Rift Valley fever (RVF) is a mosquito-borne zoonotic viral disease native to the African continent. Outbreaks tend to occur in the wet seasons, and can affect numerous mammalian species including African buffalo. It is debated how the virus survives the inter-epidemic period when it is not detected in mammalian populations, either in cryptic wildlife hosts or by vertical transmission in mosquito hosts. In chapters 1 and 2 of this dissertation I show that buffalo do become infected in the inter-epidemic period although that is not sufficient to maintain viral cycling in the system without additional mammalian hosts and high vertical transmission rates.

Bovine tuberculosis is an emerging disease in sub-Saharan Africa, first detected in Kruger National Park buffalo populations in 1990. African buffalo are a maintenance host for BTB in the ecosystem, and there has been detailed research about pathogen provenance and diversity, effects on the host and transmission dynamics. These studies have focused on a single invasive pathogen, BTB – despite the fact that buffalo act as hosts for a multitude of pathogens. Fundamental theory in community ecology and immunology suggests that parasites within a host should interact, by sharing resources, competing for resources or by altering the immune response. In chapter 3 I show that animals with BTB are more likely to become infected with RVF, more likely to show clinical signs and that the presence of BTB increases the size of RVF epidemics in African buffalo. In chapters 4 and 5 I demonstrate that one of the mechanisms underlying this pattern may be immune-mediated whereby animals with BTB have altered susceptibility to RVF. Understanding how emerging diseases, like BTB, may affect native host-pathogen or pathogen - pathogen interactions will help us understand the full impact that emerging diseases may have on an ecosystem.

Rift Valley Fever in African Buffalo (*Syncerus caffer*): Basic Epidemiology and the Role of Bovine Tuberculosis Coinfection

by
Brianna Beechler

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Major Professor, representing Environmental Sciences

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Dean of the Graduate School

I understand that my dissertation will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my dissertation to any reader upon request.

Brianna Beechler, Author

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Chapter 1: Dr. Roy Bengis offered the data from the state veterinary bovine tuberculosis surveys and also assisted in manuscript editing. Dr. Bob Swanepoel offered advice and manuscript editing. Dr. Janusz Paweska, Alan Kemp & Dr. Petrus Jansen van Vuren performed the virus neutralization testing for RVF on all buffalo serum samples in the project.

Chapter 2: Carrie Manore helped design the mathematical model, ran the simulations and wrote the methods/results sections. I also helped design the model, estimated parameters, made the figures and wrote the introduction/discussion sections.

Chapter 3: Bjorn Reninghaus wrote the initial reports from the captive buffalo breeding facility after the outbreak in 2008 and helped obtain the remainder of the breeding facility data. Dr. Carrie Manore helped create the mathematical model used in the manuscript and ran the simulations.

Chapter 4: Heather Broughton and Austin Bell helped perform the labwork during capture periods.

Chapter 5: Erin Gorsich did the bactericidal assay laboratory work. Brian Henrichs was the undergraduate student who ran the lymphocyte proliferation assay samples.

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Rift Valley Fever in African Buffalo (*Syncerus caffer*): Basic Epidemiology and the Role of Bovine Tuberculosis Coinfection

1 – General Introduction

Most free-ranging animal hosts are infected with multiple species of parasites simultaneously; these populations within a host may be regulated by bottom-up effects, such as competition for space or resources or top-down effects via the immune system (Graham, 2008). These parasites can compete for host resources directly inhibiting each other's survival, or by indirectly assisting with each other's survival through the down-regulation of host immune response (Pederson & Fenton, 2006). Using this basic epidemiological and ecological theoretical framework, this study will investigate how an emerging disease, Bovine tuberculosis (BTB) modifies the dynamics of a native infection (RVF) in a competent, free-living host species, African buffalo, in Kruger National Park (KNP). Specifically I will ask 3 main questions.

1) What is the role of African buffalo in Rift Valley fever infection dynamics and what are the basic epidemiological patterns of Rift Valley fever infection in African buffalo (*Syncerus caffer)*? (Chapters 1 and 2)

2) Can bovine tuberculosis alter Rift Valley fever infection dynamics or individual level fitness effects in African buffalo? (Chapter 3)

3) What are the immunological mechanisms by which bovine tuberculosis can alter Rift Valley fever infection? (Chapters 4 and 5)

Study system

The KNP lies between 22.5 and 25.5°S, 31.0 and 31.57° E and covers19,485 km^2. Over the past two decades, the area available to wildlife has effectively doubled by the removal of fences with private nature reserves to the west, and along the border with Mozambique to the east to form the Great Limpopo Transfrontier Park. It has one wet season per year with

summer rainfall (November-April) falling on a north-south gradient from 400-700 mm per year. KNP is located at an average altitude of 250 m (range 200-900 m) above sea level with granitic soils to the west and basaltic soils to the east. The vegetation consists of predominantly mopani (*Colophospermum mopane*) woodland in the north and knobthorn-marula (*Acacia nigrescens-Sclerocarya birrea*) savannah in the south. It has a diverse population of about 200,000 mid and large sized bovids (including impala (*Aepyceros melampus*) and 37,000 African buffalo (*Syncerus caffer caffer*)) and lesser numbers of smaller antelope (Kruger National Park Biodiversity Statistics, 2010-2011).

This longitudinal study involves one of these large bodied ungulates, the African buffalo (Syncerus caffer). Buffalo are gregarious ungulates common throughout sub-Saharan Africa. They are long-lived, becoming reproductive at 4-5 years of age and often living until 15-20 years of age (Prins, 1996). They are considered a suitable host for Rift Valley fever and a reservoir host for bovine tuberculosis. Both of these diseases have a broad host spectrum and significant disease effects in endangered wildlife, livestock and humans. (DeVos et al, 2001; Swanepoel and Coetzer, 2006). To predict disease dynamics and risk to humans and wildlife populations, an understanding of disease dynamics within the reservoir is needed.

RVF is a mosquito-borne, zoonotic viral disease (Phlebovirus Family: Bunyaviridae) that infects numerous mammalian species (Swanepoel & Coetzer, 2006). RVF is native to sub-Saharan Africa (Paweska, 2008), with buffalo acting as possible reservoirs and amplifying hosts (Swanepoel & Coetzer, 2006). Symptoms in buffalo include hepatic necrosis with episodes of abortion following the acute viremic fever event that occurs with infection (Evans et al, 2008). During the period of viremia buffalo can transmit the disease, via a mosquito vector, to other species of wildlife, livestock and humans (Woods et al, 2002; Flick & Bouloy,

2005). Epidemics or outbreaks of RVF are associated with availability of mosquito breeding habitat, and are most common in the rainy spring months and during wet years (Linthicum et al, 1991 & Balkhy & Memish, 2003). Outbreaks have occurred throughout Africa, including South Africa, and have resulted in human deaths and losses of livestock, with mortality rates in livestock as high as 70% in epidemic conditions (Swanepoel & Coetzer, 2006). RVF is present in the buffalo herds in southern KNP at a prevalence of 17% (Joubert honors thesis, 2008). Typically localized outbreaks occur every 4-6 years (Martin et al, 2008); however there is evidence that Rift Valley fever may persist undetected in mosquitoes and mammalian hosts during the "interepidemic period", but the role of buffalo in interepidemic persistence is unknown. I address this topic in chapters 1 and 2.

BTB is a bacterial disease (pathogen: *Mycobacterium bovis*) that causes chronic disease in many mammal species (DeVos et al, 2001 and Swanepoel and Coetzer 2006) and is an emerging problem in human health. Its coinfection with immunosuppressive diseases, such as HIV makes it a significant zoonotic cause of pneumonia. Acute TB, caused by *M. tuberculosis* or *M. bovis*, is the leading proximate cause of death in HIV patients. (Ayele, 2004) Additionally, BTB is a major livestock disease, costing governments, including those of developing nations, millions of dollars annually to monitor and control (Nelson, 1999). BTB is not native to sub-Saharan Africa and is an emerging infection in African wildlife (Michel, 2006). BTB has been spreading south to north within our study area, Kruger National Park (KNP (Cross et al, 2009) since its introduction in the 1960s (Michel, 2009) BTB is present in southern buffalo herds at 40-50% prevalence, 20-30% in the central area and 0-10% in northern buffalo herds (Michel, 2005; Rodwell et al, 2001). In buffalo, BTB is a chronic disease (Swanepoel, 2004) with context-dependent effects that may include declines in body

condition (Caron, 2003 and Jolles, 2008) and reductions in fecundity (Jolles 2005 and 2006) and survival (Jolles, 2005). Although much research has focused on the direct impacts of BTB on host populations (such as buffalo and kudu), little has been done to explore the impacts it may have on other diseases within the system. I address this topic in Chapter 3, exploring whether BTB may have impacts on Rift Valley fever dynamics in African buffalo.

BTB is a dynamic infection, which has changing effects on host immunity throughout the course of infection. Upon initial infection, hosts upregulate their immune response to suppress BTB (Welsh et al, 2005; Thacker et al, 2007), however once infection has occurred they will develop a chronic infection, remaining infected for the remainder of their lives with few clinical signs (deVos et al, 2001). In chronic infections, the immune response often changes, becoming less effective against microparasitic infections due changes in cellular protein expression (Pirson, 2013) and a skew towards a T-helper cell 0 (Th0) or T-helper cell 2 (Th2) phenotype instead of the effective T-helper cell 1 (TH1) response (Welsh et al, 2005). Therefore I investigated how BTB affects the immune response during infection and discuss whether these changes can modify the likelihood of RVF infection within an individual (Chapters 4 and 5).

1- Rift Valley fever in Kruger National Park: Do buffalo play a role in the interepidemic circulation of virus?

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ABSTRACT

Rift Valley fever (RVF) is a zoonotic mosquito-borne virus disease of livestock and wild ruminants that has been identified as a risk for international spread. Typically the disease occurs in geographically limited outbreaks associated with high rainfall events, and can cause massive losses of livestock. It is unclear how RVF virus persists during interepidemic periods but cryptic cycling of the virus in wildlife populations may play a role. We investigated the role that free-living African buffalo (*Syncerus caffer caffer)* might play in interepidemic circulation of the virus, and looked for geographic, age and sex patterns of RVFV infection in African buffalo. Buffalo serum samples were collected (n=1615) in Kruger National Park, South Africa during a period of 1996 to 2007 and tested for antibodies to RVF. We found that older animals were more likely to be seropositive for anti-RVFV antibody than younger animals, but sex was not correlated with the likelihood of being anti-RVFV antibody positive. We also found geographical variation within KNP; herds in the south were more likely to have acquired anti-RVFV antibody than herds farther north – which could be driven by host or vector ecology. In addition, we also conducted a 4 year longitudinal study on 227 initially RVFV seronegative buffalo to look for evidence of seroconversion outside known RVF outbreaks within our study period (2008-2012). In all years of the study between 1996 and 2007 we found young buffalo (under 2 years of age) that were seropositive for anti-RVFV antibody, with prevalence ranging between 0 and 27% each year, indicating probable circulation. In the longitudinal study we found 5 individuals that seroconverted from anti-RVFV antibody negative to anti-RVFV antibody positive, outside of any detected outbreak. Overall, our results provide evidence of long-term undetected circulation of RVFV in the buffalo population.

1.1 INTRODUCTION

Rift Valley fever virus (RVFV; *Bunyaviridae: Phlebovirus*) is a zoonotic pathogen transmitted by mosquitoes and capable of infecting a wide variety of mammals. The virus causes outbreaks of disease in domestic ruminants characterized by death of newborn animals and abortion in pregnant sheep, goats and cattle (Swanepoel and Coetzer, 2004). Humans become infected through contact with the tissues of infected animals or via a mosquito bite. Large outbreaks occur at irregular intervals during years when heavy rains favor breeding of the mosquito vectors (Swanepoel and Coetzer, 2004). The virus was discovered in Kenya in 1930 (Daubney and Hudson, 1931), and was initially recorded only in sub-Saharan Africa, but from 1977-2007 it spread to Egypt, Mauritania, Madagascar, the Arabian Peninsula and the island of Mayotte (Pepin et al 2010). In southern Africa, focal or large-scale epidemics occur in a variable temporal cycle of between 7 and 11 years with these outbreaks usually occurring in the mid- to late summer months when rainfall is at its peak (Swanepoel & Coetzer, 2004). It is unclear how RVF virus persists during the interepidemic periods, but two theories exist, namely long term survival in mosquito eggs infected via vertical transmission and cryptic cycling in as yet undetermined hosts (Chevalier et al, 2010; Chevalier et al, 2004). In 1996, neutralizing antibodies to RVFV were found in the sera of yearling buffalo from the Kruger National Park (KNP) on the northeastern border of South Africa, implying that there had been recent virus activity in the park (PG Howell, University of Pretoria, personal communication, 1996). After heavy rains in January 1999, RVFV was isolated from 6 aborted buffalo fetuses from pens adjacent to Skukuza Camp, KNP, where buffalo free of foot-and-mouth disease virus were being reared, and also from a waterbuck found dead 100 km northwest of Skukuza in Klaserie Nature Reserve, which is not fenced off from the KNP (NICD, unpublished

laboratory records, 1999). The last known occurrence of RVFV on the inland plateau of South Africa had been recorded 23 years previously during a major outbreak in 1974-1976 (Barnard and Botha, 1977), but isolation of the virus from mosquitoes during an inter-epidemic period in 1971 and again from mosquitoes and cattle in a small outbreak on the coast of KwaZulu Natal Province in 1981, suggested that RVFV circulates endemically on the eastern seaboard of the country where the warmer and moister climate is more favorable to mosquitoes (McIntosh, 1972; Jupp et al, 1983).

KNP is contiguous to the coastal plain of Mozambique, with a warm and humid climate that is potentially favorable for RVFV circulation. This prompted us to investigate the persistence of RVFV in the park. Specifically, we used 1,615 serum samples collected opportunistically by the State Veterinary Services in KNP during bovine tuberculosis (BTB) prevalence studies spanning a 10-year period (1996-2007) to estimate RVFV seroprevalence in African buffalo, to evaluate age, sex and geographic patterns of RVFV infection in this species and to seek evidence of interepidemic circulation. We also used a longitudinal dataset from 227 buffalo sampled every six months between 2008-2012 to investigate the incidence of RVFV infection in buffalo over this period.

1.2 Methods

Study area

The KNP lies between 22.5 and 25.5°S, 31.0 and 31.57° E and is 19,485 km^2 in extent, but the area available to wildlife has effectively doubled over the past two decades by the removal of fences with private nature reserves to the west, and along the border with Mozambique to the east to form the Great Limpopo Transfrontier Park. It has one wet season per year with summer rainfall (November-April) ranging north to south from 400-700 mm per

year. Rift Valley fever outbreaks typically occur towards the end of this wet season (Swanepoel and Coetzer, 2004). KNP is located at an average altitude of 250 m (range 200-900 m) above sea level with granitic soils to the west and basaltic soils to the east. The vegetation consists of predominantly mopani (*Colophospermum mopane*) woodland in the north and knobthorn-marula (*Acacia nigrescens-Sclerocarya birrea*) savannah in the south. It has a population of about 200,000 bovids the size of impala (*Aepyceros melampus*) and larger, including 37,000 African buffalo (*Syncerus caffer caffer*), and lesser numbers of smaller antelope (Kruger National Park Biodiversity Statistics, 2010-2011). BTB seroprevalence studies were conducted throughout the park, but the longitudinal portion of this study was restricted to the southern portion of KNP, in the Crocodile Bridge and Lower Sabie sections. The Crocodile Bridge section is along the southeastern boundary of the park, and much of it lies along the Crocodile River. The Lower Sabie section is just north of the Crocodile Bridge section and lies around the Sabie River.

Sample collection

Blood samples were collected from 1,615 buffalo in lethal (sacrificial) and non-lethal bovine tuberculosis prevalence surveys in 1996, 1998, 1999, 2005, 2006 and 2007, with a large fraction of the buffalo (590) captured in 1998 (see Table 1 for sample sizes). Capture locations were targeted (not randomly selected) for the tuberculosis survey (Figure 1; Table 1), but animals captured each day were randomly selected. Sex and age were determined for each animal. Age was estimated based on emergence of incisor teeth and horn size (Sinclair, 1977). The date and GPS coordinates were also recorded at each capture event. In addition to the survey data, serum samples from 227 radio-collared female buffalo from the southern portion of the park (initial mean age 3.5 years), which had been immobilized and re-bled every

six months from 2008-2012, were used to monitor incidence of RVF infection. Any animal that died during the study period was replaced by a similarly aged animal to maintain a constant sample size of 200 individuals at each recapture. Over the 4-year period 227 initially seronegative buffalo were monitored. Serum harvested from clotted blood was stored at -70C until tested for neutralizing antibody to RVFV as described previously (Paweska et al, 2003). The virus neutralization test used here is considered to be the gold standard for Rift Valley fever serodiagnostics; therefore it has a presumed sensitivity and specificity of near 100% (Swanepoel & Coetzer, 2004; Pepin et al, 2010). In fact when 955 bovids and 1473 sheep from RVF free countries were tested in an unrelated study there were no false positives identified (NICD, unpublished data).

Statistical analysis

Only the 1998 parkwide survey data, and not the data for other years with more limited distributions, were used to evaluate whether RVF seroprevalence was correlated with geographic location (Figure 1). For geographic location analyses, the park was divided into 3 broad areas noted as south, central and north to represent the rainfall gradient seen in the park with rainfall higher in the south than north (Venter et al, 2003). These areas were divided for analysis by the large permanent rivers of the region, which conveniently divide the park into 3 regions and somewhat restrict buffalo movement. Data was assessed for normality and equal variance using a Shapiro-Wilks normality test. The relationship between herd-level prevalence of antibody to RVF in the 1998 survey and geographic location was evaluated using an ANOVA with a Tukey's post-hoc test. A generalized linear model (GLM), with binomial distribution and logit link function, was used to evaluate whether site of capture (Crocodile Bridge or Lower Sabie) was correlated with an individual's likelihood of being seropositive

for RVF in the longitudinal study. Age at capture was included as a covariate in the GLM to account for the potential effects of varying age distributions on any geographical pattern.

To evaluate whether demographic traits of 1486 buffalo sampled between 1996-2007, was associated with anti-RVFV antibody status, we used a GLM to test the effect of age and sex on individual serostatus. Year and location of capture were included as covariates. Since our data on year and capture location are non-independent the variables were included on the model solely to account for their potential effects on individual serostatus. To differentiate between undetected large-scale epidemics or small-scale interepidemic cycling, a similar model was used to assess whether year/geographic location was associated with anti-RVFV antibody status of animals between 0.5 and 1 year of age, using all calf samples collected in all regions between 1996-2007. Buffalo <6 months old were excluded, as it is possible that antibody in this age group could be maternally derived. For calves of female animals naturally infected with RVFV virus maternal antibody usually persists until 3-4 months of age (Geering et al, 2002), and in sheep it wanes by 2 months (Zeller et al, 1997). There is no data for buffalo, but maternal immunity to other viral infections lasted to a median of 3-5 months of age and no longer than 7 months at the extreme high end (Singh et al, 1967; Thomson; 1996;Hamblin and Hedger, 1978).

All statistical analyses were performed using the computer package R (R Core Development Team). For all analyses a p-value of less than 0.05 was considered statistically significant. Maps of the capture locations and herd RVFV serological status were prepared using ArcView GIS 9 (Figure 1).

Ethics Statement

This project was registered with the Scientific Services Projects Committee of the South African National Parks Board (Sanparks), and received ethical clearance from the Sanparks Animal Use and Care Committee. As an official project of the State Veterinary Services, it was automatically cleared in terms of the requirements of Section 20 of the Animal Diseases Act No. 35 of 1984. The Animal Care and Use Committees at Oregon State University and University of Georgia also approved the portion of the study involving the longitudinal monitoring of buffalo.

1.3 RESULTS

A larger percentage of herds in the south and central regions were seropositive for antibodies to RVFV than herds in the North (Figure 1), and based on the 1998 parkwide data, herd level prevalence differed significantly among the 3 regions of the KNP ($F(2,25)=3.386$, $p=0.05$; Figure 2). A Tukey's post hoc test showed that prevalence was lower in the North than in the South or Central regions (South vs. Central no difference; South vs. North mean difference 7.45%, $p<0.05$; Central vs. North mean difference 6.94%, $p<0.05$).

In examining factors associated with individual RVFV serological status across all buffalo captured between 1996-2007, and accounting for variation in sample year and geographic location, it was found that there was no correlation with sex of the animals (estimate=10.18, $F(1300)=0.019$, $p=0.9848$), while age was positively associated with serostatus, such that older animals were more likely to have antibody to RVFV (estimate=0.15, $F(1300)=5.6$, $p<0.0001$) (Figure 3). In examining the dataset for calves <1 year of age to look for evidence of recent infection, it was found that animals between 0.5 and 1 year of age were infected at each survey time point except during the 2006 and 2007 surveys in the North (Figure 4). However, neither geographic location (North vs. Central, Estimate=-

0.32, (F (83)=-0.26, p=0.796; South vs. North, estimate=0.02,F (83)=0.02, p=0.984), nor year (estimate=-0.05, F (83)=0.641,p=0.648) were correlated with RVFV serological status in calves.

During the 2008-2012 longitudinal study, five of 227 seronegative buffalo seroconverted, for a total incidence rate of 2.2%, or an annual incidence rate of 1-3%. Of the five seroconverters, three occurred in the 2008/2009 wet season, and one each in the following two wet seasons (2009/10 and 2010/11). At initial capture, 76 animals were anti-RVFV antibody positive, and were not included in the longitudinal study. However of these 76 RVFV positive individuals, there was significant geographic variation in the South, with individuals in the Crocodile Bridge area having a higher probability of being seropositive at initial capture than animals in the Lower Sabie area (F=17.34, p<0.0001). These differences were also seen at the herd level with overall prevalence higher at Crocodile Bridge (36.3%) than at Lower Sabie (10.3%).

1.4 DISCUSSION

Geographic patterns

The geographic differences in prevalence e of anti-RVFV antibody in the KNP were pronounced in the parkwide sample year of 1998. Herd seroprevalence was significantly higher in the South and Central regions. One possible explanation for the lower seroprevalence in the North is a lower density of competent mosquito vectors as a consequence of lower rainfall (Venter et al, 2003), or landscape and vegetation differences. RVFV transmission occurs principally through mosquito vectors (Pepin et al, 2010), and surveys have shown that suitable *Aedes* and *Culex* mosquito species are common in the KNP (Jupp, 1996; Alan Kemp, personal communication), but little is known about vector

distribution and density in the different regions. Another unlikely possible cause for the geographical variation is reduced host population density in the northern part of the park (Owen-Smith & Ogotu, 2003), although mosquito-borne diseases are thought to be relatively unresponsive to host population changes because vector-biting behavior is largely independent of host population density (Anderson & May, 1986).

The possibility that buffalo in the north region are less susceptible to RVFV even if exposed at the same rate is also unlikely. Buffalo from the north are not isolated from the south and central regions, and despite the rivers used to define the geographic regions, buffalo from populations in all three regions occasionally intermix (Cross et al 2009). There is limited data on immune function or genetic profile of buffalo in KNP or elsewhere, however it has been suggested that there may be variation in innate immunity between herds in the South (Beechler et al, 2012), as well as in RVFV seroprevalence though this has not been linked to disease susceptibility. There is circumstantial evidence in livestock of breed differences in susceptibility to RVFV, and inbred strains of rats vary in the outcome of exposure to infection, but there is no evidence that susceptible species can become refractive to the extent that they fail to develop a detectable immune response to infection (Swanepoel and Coetzer 2004). Within the south region of the KNP, the 2008-2010 data show that individual buffalo in the Crocodile Bridge area had a higher probability of being seropositive for RVFV than individuals in the Lower Sabie area. The home range of these two buffalo herds overlap two separate river systems, and in 2008 an outbreak of RVFV was detected in an intensive buffalo breeding facility, and in livestock and humans outside the KNP along the Crocodile River that forms the southern boundary of the park (Archer et al, 2011; Grobbelaar et al, 2011). The Crocodile Bridge area of the KNP lies on the boundary where the outbreak occurred, and

hence it is likely that the difference in overall serostatus between Lower Sabie and Crocodile Bridge is a result of the localized outbreak of RVFV on the Crocodile River boundary in 2008.

Age and sex patterns

In evaluating the entire data set for 1996-2007, it is evident that older buffalo are more likely to test positive for neutralizing antibody to RVFV. Circulation of neutralizing antibody to RVFV in domestic ruminants is long lasting, possibly lifelong (Swanepoel & Coetzer, 2004), and hence it is logical that there is a cumulative prevalence of antibody in buffalo with increasing age. There was no evidence of gender bias in seropositivity.

Evidence for interepidemic infection

The main objective of this study was to seek evidence that buffalo were being infected and seroconverting during interepidemic periods. In most years some young buffalo tested seropositive (Figure 4), despite no known outbreaks in Kruger National Park. The only survey years in which young buffalo did not test RVF seropositive were 2006 and 2007, both of which were restricted to the Northern region of KNP where RVF seroprevalence is significantly lower. In the Central and South regions seropositivity in animals between 0.5 years and 1 year of age occurred nearly annually; however these data were temporally and geographically scattered, making it unlikely that infection was associated with large-scale undetected epidemics. Additionally, five seroconversions were recorded in the Crocodile Bridge and Lower Sabie areas during the wet seasons of 2008/9, 2009/10, and 2010/11, and no known RVF outbreaks occurred in or within 50 miles of Kruger National Park during these periods. These results provide evidence for undetected circulation of RVFV in buffalo and possibly other wildlife species during interepidemic periods. Similar conclusions were reached by LaBeaud et al (2011) who found African buffalo seroconverting outside any known

outbreak of RVF. However, the results presented here make an even stronger case for interepidemic transmission of RVFV in buffalo, since the diagnostic assay we used (virus neutralization) is more specific for RVFV than the hemagglutination-inhibition assay used in LaBeaud et al (2011). The hemagglutination-inhibition assay (HAI) may produce false positive results if sera contain antibody to phleboviruses that are antigenically similar to RVFV. Moreover, a diagnostic cut-off value for HAI, indicating RVFV infection has not been established for buffalo making LaBeaud's results challenging to interpret. On the other hand, virus neutralization is the gold standard for detecting RVFV antibodies (McIntosh, 1980; Swanepoel et al, 1986), with specificity near 100% (NCID, unpublished data).

Antibodies to RVFV have been found in many other wildlife species, including, but not limited to, impala, kudu, Thomson gazelle, gerenuk, bushbuck, waterbuck, white and black rhinoceroses, and elephant (Davies, 1975; Anderson and Rowe, 1998; Fischer-Tenhagen et al., 2000; Paweska et al., 2005; Evans et al., 2008; Paweska et al., 2008; Paweska at al., 2010), and the disease was confirmed in a waterbuck found dead in the Klaserie Nature Reserve in 1999 (NICD, unpublished laboratory records, 1999). It is notable that the genetic lineage of RVFV found in aborted buffalo fetuses in Skukuza and in the dead waterbuck in Klaserie in 1999, was the same as that which appeared in captive buffalo, farm animals and humans along the Crocodile River outside the KNP in the wet season of 2008, and also in captive buffalo outside the KNP to the north of Klaserie in the same year (Grobbelaar et al, 2011). However to the authors' knowledge no extensive surveys of RVF incidence in livestock surrounding KNP have been performed. The same virus spread to farming areas in the northeast of South Africa in 2008, representing the first occasion on which RVFV had been recorded on the interior plateau since 1976, and in the following year it appeared to the

south in KwaZulu-Natal Province (Grobbelaar et al, 2011). In 2009-2011, a different lineage of RVFV, which had first been encountered in the Caprivi Strip of Namibia in 2004, spread widely in the central interior of South Africa during a succession of exceptionally wet years (Grobbelaar et al, 2011). Thus, not only was there evidence of protracted circulation of RVFV in a major wildlife conservation areas, but also presumptive evidence of spread of the virus to adjacent farming regions. This emphasizes the importance of understanding RVFV interepidemic cycling in wildlife populations, and investigating what other wildlife species may be involved in the sylvatic cycle. Although we demonstrate that there is likely undetected interepidemic cycling of RVF within buffalo populations, it is at a very low rate. Further investigations into whether this level of transmission in buffalo is sufficient to maintain RVF during interepidemic periods are necessary. Further studies examining whether hosts or vectors drive the geographical patterns of this disease are also needed to fully understand RVFV ecology in Kruger National Park.

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Table 1.1 Capture table by year for the BTB seroprevalence study between 1996-2007.

Year Sampled	Geographic Area of Focus	Number of Herds Sampled	Number of buffalo captured	Number of anti-RVFV positive herds
1996	Northern	6	110	5
1998	Parkwide	29	588	18
1999	Northern	12	171	10
2005	Southern	12	245	9
2006	Northern	10	133	3
2007	Northern	13	229	5
Total		82	1476	50

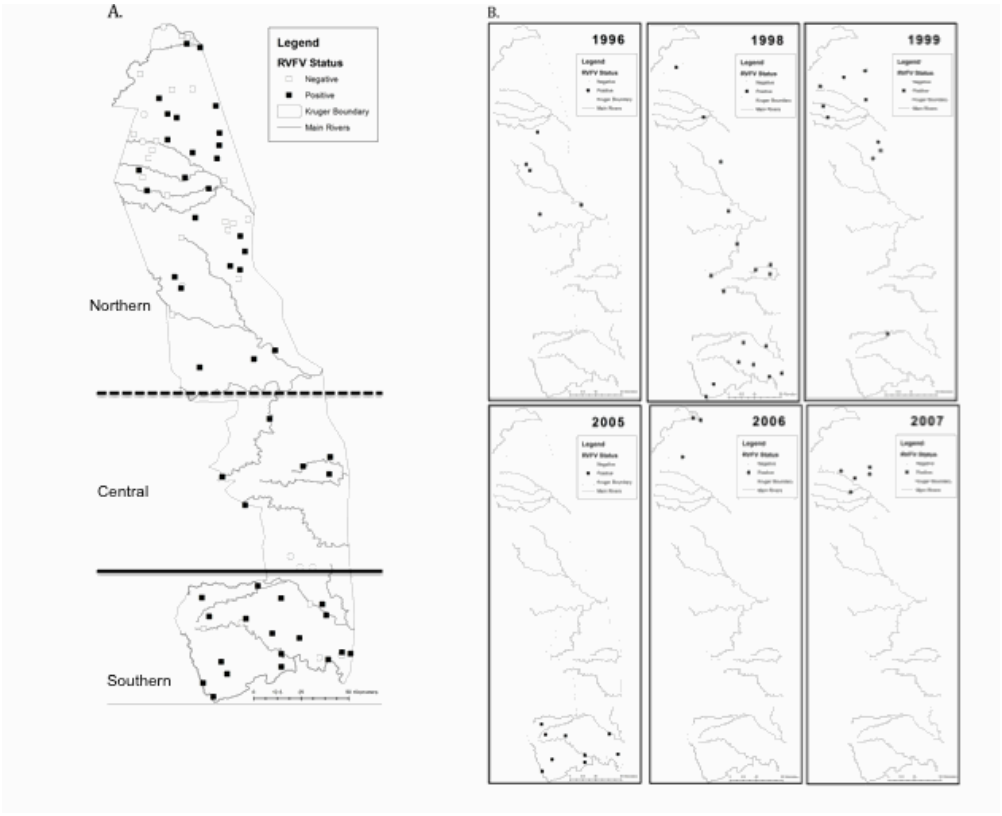


Figure 1.1: Serostatus of buffalo herds captured between 1996-2012 by geographic lo
(A) and year (B). Filled in squares denote sample sites where at least one buffalo was
seropositive, whereas open squares indicate sample sites where no buffalo were RVF
positive. For geographic analysis the park was divided into 3 regions, Southern, Cer
and Northern as denoted on panel A. The southern portion of the park was any site b
the solid line, the central any site between the solid and dotted line and the north any
above the dotted line. These divisions were drawn along the large permanent rivers a
represent a significant difference in rainfall in Kruger National Park.

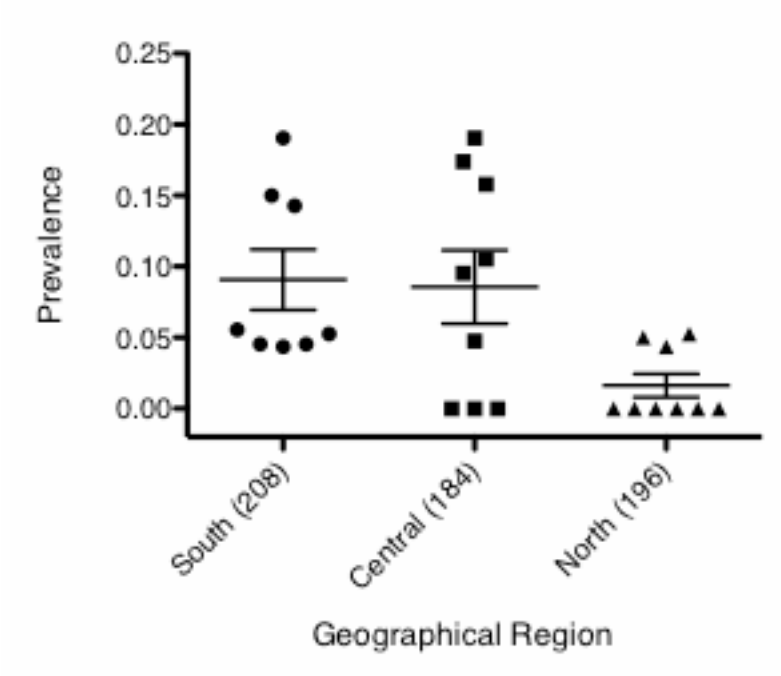


Figure 1.2: 1998 park-wide survey data showing that seroprevalence to RVF in buffalo herds varied by region. Each point is a herd, with prevalence noted on the y-axis. The centerline denotes median prevalence with standard error bars. The x-axis denotes the geographical region with the total number of buffalo sampled in that region in parentheses. The significant difference (p=0.05) between herds appears to be driven primarily by the North region having significantly lower herd level prevalence.

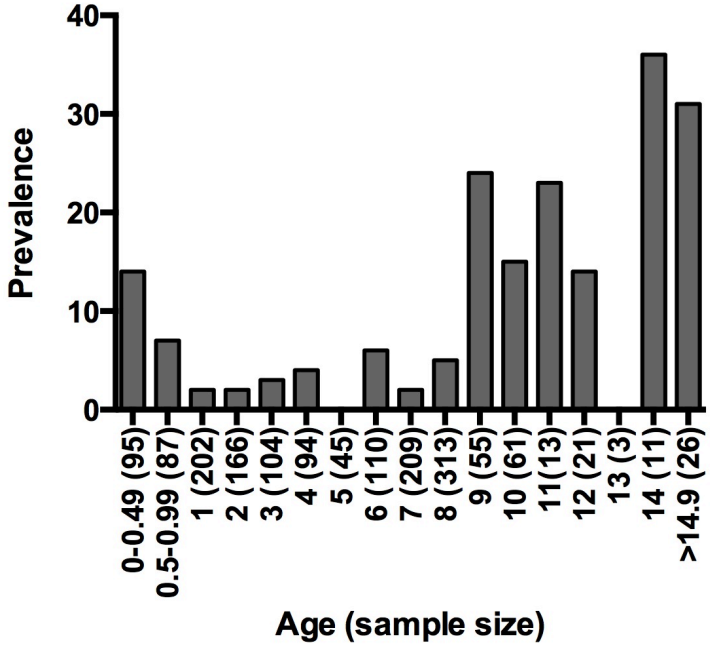
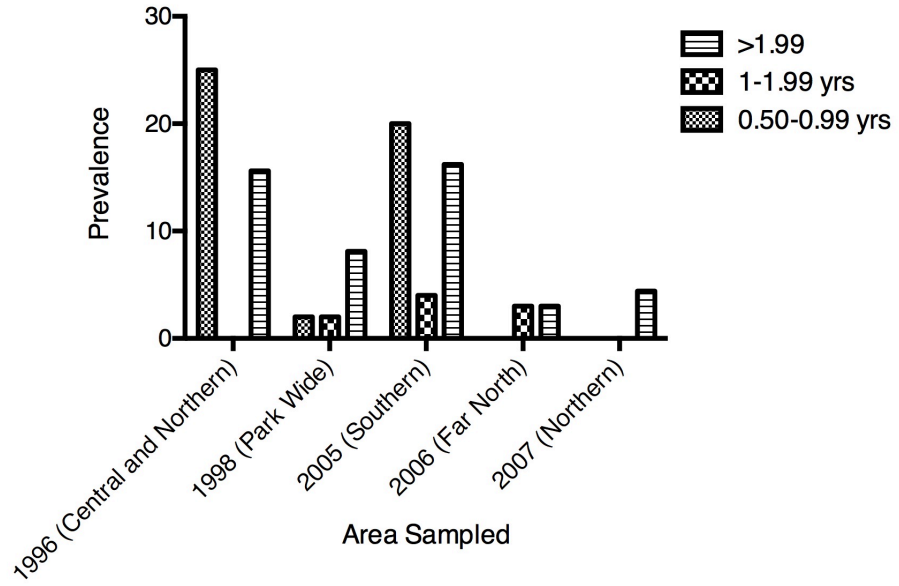


Figure 1.3: RVF Seroprevalence by age of buffalo. Age is shown on the x-axis and prevalence for that age category on the y-axis. Generally seroprevalence increases with age ($p<0.0001$), however note the high prevalence in young calves (under 0.5 yrs of age) and older calves (between 0.5 and 1 year of age). The high prevalence in very young calves (under 0.5 years of age) is likely due to colostral transfer of antibody from their mother's at birth, where as the increased prevalence in calves older than 0.5 months indicates potential interepidemic circulation.



	Sample Size (0.50-0.99)	Sample Size (1-1.99)	Sample Size (>1.99)
1996 (Central and Northern)	11	12	4
1998 (Park Wide)	61	46	104
2005 (Southern)	16	10	23
2006 (Far North)	0	2	30
2007 (Northern)	7	12	41

Figure 1.4: Seroprevalence by year and age category. Sample sizes are noted in a table below the image. Seroprevalence is noted on the y-axis with the x-axis being year and region of data collection. There is no data for 1999 because age classes were not quantified. In 1996, 1998 and 2005 there are calves between 0.5-1 years of age that are RVF seropositive, indicating they must have been exposed within that year of life.

2 - Inter-epidemic and Between-Season Persistence of Rift Valley fever: Vertical Transmission or Cryptic Cycling

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ABSTRACT:

Rift Valley fever (RVF) is an emerging zoonotic mosquito-borne infectious disease that has been identified as a risk for spread to other continents and can cause mass livestock mortality. In equatorial Africa, outbreaks of RVF are associated with high rainfall, when vector populations are at their highest. It is, however, unclear how RVF virus persists during the inter-epidemic periods and between seasons. Understanding inter-epidemic persistence as well as the role of vectors and hosts is paramount to creating effective management programmes for RVF control. We created a mathematical model for the spread of RVF and used the model to explore different scenarios of persistence including vertical transmission and alternate wildlife hosts, with a case study on buffalo in Kruger National Park, South Africa. Our results suggest that RVF persistence is a delicate balance between numerous species of susceptible hosts, mosquito species, vertical transmission and environmental stochasticity. Further investigations should not focus on a single species, but should instead consider a myriad of susceptible host species when seeking to understand disease dynamics.

2.1 INTRODUCTION

Rift Valley fever (RVF) is an emerging infectious disease that has been identified as a risk for international spread by the Office of International Epizootics (OIE), Center for Disease Control (CDC) and World Health Organization (WHO). Large epizootics have occurred through sub-Saharan Africa and have a significant impact on livestock production, inducing up to 90% mortality of juvenile small livestock (sheep and goats) and episodes of abortion in cattle, sheep and goats (Swanepoel & Coetzer, 2004). Rift Valley Fever virus (RVFV) is a mosquito-borne, zoonotic pathogen (*Phlebovirus* Family: *Bunyaviridae*) that infects numerous mammalian species (Swanepoel & Coetzer, 2004) including domestic and

wild ruminants. Since its discovery in the 1930's (Daubney & Hudson, 1931) RVF was confined to Sub-Saharan Africa and Madagascar until 2000, when it was detected on the Arabian Peninsula (confirmed in by the CDC in a MMWR report, 2000; reviewed in Balkhy & Memish, 2003).

In Southern Africa, focal or large-scale epidemics occur in a variable temporal cycle of between 7 and 11 years. Generally these outbreaks occur in the mid- to late summer months with above average rainfall, when vector abundance has peaked (Swanepoel & Coetzer, 2004). It is unclear how RVF virus persists during the interepidemic periods. Furthermore, in Kruger National Park, distinct wet and dry seasons result in peaking mosquito populations during the wet season, but during the dry season mosquito density is close to zero. This suggests that transmission of RVF likely does not occur during the average dry season. Since there are no known long-term mammalian carriers of RVF, persistence from wet season to wet season is also a mystery. One possible mode of between season and between epidemic persistence is vertical transmission with desiccation resistant mosquito eggs that are viable for several years (Chevalier, 2010; Chevalier et al, 2004). As many as 23 mosquito genera have been found to be capable of RVF virus transmission worldwide, however, mosquitoes in the genera Aedes may be particularly important for virus maintenance in the interepidemic period (Pepin, 2010; Turell et al, 2008). Aedine mosquitoes are able to transmit RVF transovarially and RVF outbreaks are associated with increased activity of these mosquitoes during years with elevated rainfall when many Aedine mosquitoes emerge (Linthicum et al, 1991; Balkhy & Memish, 2003; Martin et al, 2008). Aedine mosquitoes lay their eggs on the soil or vegetation on the verges of low-lying depressions and pans filled with water (Becker, 1989). These eggs must undergo a period of

dehydration followed by wetting before hatching (Gargan, 1998) and can lie dormant for many years, hatching when seasonal pools refill with water during heavy rainfall (Gargan, 1998). During periods of high rainfall, infected Aedes eggs, laid as many as 8 years prior, can hatch and transmit RVF virus to susceptible hosts (Gargan, 1998; Linthicum et al 1983). For these reasons, mosquitoes in the genus Aedes are widely believed to be important as an endemic maintenance vector of RVF between seasons and during inter-epidemic periods, however whether they are sufficient as a maintenance mechanism for RVF during inter-epidemic periods is unclear.

It has also been suggested that wildlife may play a role in interepidemic maintenance of RVF. Studies in Kenya (Rostal et al, 2010) and in South Africa (Labeaud et al, 2010) have found that wildlife species convert from seronegative to seropositive outside of any known outbreak and antibodies to RVFV have been found in many wildlife species, including, but not limited to, impala, white rhinoceroses, bushbuck and waterbuck (Davies, 1975; Anderson and Rowe, 1998; Fischer-Tenhagen et al, 2000; Paweska et al, 2005; Evans et al, 2008; Paweska et al, 2008). One species of significant concern is African buffaloes (*Syncerus caffer)* (Swanepoel & Coetzer, 2004; Labeaud et al, 2011), which are water dependent social bovids. As with most ruminants, viremic buffalo may transmit the disease, via a mosquito vector, to livestock, to other wild ruminants and to humans (Woods et al, 2002; Flick & Bouloy, 2005). Due to the large populations of buffalo in game parks, their propensity to mingle with livestock and humans on the borders and their known competence as RVF hosts, there has been special interest in whether they could serve as an interepidemic host for RVF.

Mathematical models can compare different mechanisms for persistence of RVF. Gaff et al (2007) and Gaff et al (2012) modeled RVF without seasonality in order to explore methods of control once an outbreak is occurring. The Xue et al, 2011 model of RVF in South Africa focused on livestock and human cases, while Anyamba et al, 2006 worked on predicting risk of RVF outbreaks in East Africa based on weather data. Although wildlife is considered to play a part in RVF persistence between epidemics, it is has been largely ignored or included implicitly in previous models. We explicitly include wildlife in our model, including African buffalo and other potential reservoir species. Unlike previous authors, in this paper we focus on the inter-epidemic persistence of RVF by adapting the Chitnis et al, 2013 model for RVF to include marked seasonality and to store infected eggs during the dry season.

We used this adapted mathematical model of the spread of RVF to study different scenarios of persistence, starting with an emphasis on African buffalo as a possible wildlife reservoir host. We use Kruger National Park (KNP) as a model system, but the conclusions are generalizable to other savannah ecosystems in sub-Saharan Africa and offer insights to the spread of RVF into previously uninfected areas. We first tested if vertical transmission alone can result in interepidemic persistence of RVF in a herd of buffalo in KNP. We then included an alternate "cryptic" host in the model to test if the presence of multiple hosts could account for the inter-epidemic persistence of RVF. We propose that rather than one or the other, the combination of vertical transmission and cryptic cycling in other hosts is the most likely mechanism for persistence of RVF during interepidemic periods. Lastly we considered how host immunity affected persistence.

2.2 Materials and Methods

The underlying transmission model

Our model adapted from Chitnis et al, 2013 divides African buffalo into three distinct compartments: susceptible S_h, infectious I_h, and recovered R_h. The total buffalo population is then $N_h=S_h+I_h+R_h$. Buffalo enter the susceptible class through a per-capita birth rate, b_h, and leave all compartments through a natural per-capita death rate, d_h where $1/d_h$ is the average lifespan of a buffalo, about 15 years. To simplify the model, we assume that the buffalo herd is at a stable carrying capacity, H_0. We considered a geographically restricted area with the "herd" consisting of a reasonable number of buffalo likely to come into contact with mosquitoes at a given water hole. A susceptible buffalo can be infected by the bite of an infected mosquito with probability γ_h and is subsequently moved into the infectious compartment, I_h. The incubation period in buffalo is ignored here since it is relatively short (1-3 days). The buffalo recover and are moved into the recovered compartment R_h at a rate γ_h where $1/\gamma_h$ is the average time spent infectious. We assume buffalo remain immune for life once recovered. Infected buffalo, mostly the young, die from infection at a low rate, δ_h (Swanepoel & Coetzer, 2004). Although some horizontal transmission may take place due to contact with aborted fetuses, it is not likely to play a role in inter-epidemic persistence, so is ignored here. See Table 1 for a list of all parameters, variables, and their units.

Adult female mosquitoes are divided into three compartments: susceptible, S_v, those in the extrinsic incubation period, E_v, and infectious, I_v. The total adult female mosquito population is then N_v. For the purpose of understanding long term persistence via vertical transmission,

we include only Aedes mosquitoes in our model since they are the only mosquito genera known to exhibit vertical transmission between infected adults and eggs for Rift Valley fever (Pepin, 2010). Adult female mosquitoes enter the susceptible compartment through the hatching and emergence of susceptible eggs/larvae/pupae. Mosquito eggs and larvae are divided into two compartments: susceptible aquatic mosquitoes S_e, and infectious aquatic mosquitoes I_e. Mosquitoes enter the susceptible aquatic mosquito compartment through a per-capita natural birth rate b_v. The birth term for mosquitoes accounts for the egg-laying rate of mosquitoes, the survival and hatching rate of eggs, and the survival and emergence rate of larvae. Mosquitoes hatch at a per-capita rate $\bullet_e$ where$1/\ \bullet_e$ is the average time it takes to move from egg to emerging adult mosquito. The total number of aquatic stage mosquitoes is N_e. Adult mosquitoes leave all compartments via a constant per-capita death rate d_v where $1/d_v$ is the average lifespan of an adult female mosquito. To simplify, we assume that during the wet season the mosquito population quickly reaches a stable size, V_0 since once eggs are submerged, adult mosquitoes emerge within days. Since adult female mosquito populations drive transmission, there are enough eggs available for the mosquitoes to reach carrying capacity every wet season. These assumptions ignore some of the more complex aspects of mosquito ecology, such as predation and competition in larvae in favor of simplicity and better known parameters.

An adult female mosquito is infected by biting an infectious buffalo with probability ℓ_v and move into the incubating compartment E_v. Although many epidemic models assume that the contact rate, and thus transmission, is either frequency dependent or density dependent (Wonham et al, 2006), we use a contact rate that falls between the two and depends on both the density of mosquitoes and available hosts as outlined in the Chitnis et

al, 2013 model (Appendix 1). Incubating mosquitoes enter the infectious compartment I_v at rate ϕ_v where $1/\phi_v$ is the average time spent in the extrinsic incubation period. Once a mosquito is infectious, it remains so for the rest of its life. An adult female mosquito can also be born infected. Since infectious Aedes mosquitoes can transmit RVF virus transovarially, we assume that a proportion of infectious mosquitoes, $\succ_v$ (referred to here as the 'vertical transmission rate'), will produce at least one infectious egg that survives to adulthood and emerges as an infectious female. See Appendix 1 for a full description of the model and associated differential equations.

Seasonality

Since Kruger National Park, and the majority of sub-Saharan Africa, has distinct wet and dry seasons that significantly affect the mosquito populations, we include seasonality in our model by alternating wet season and dry season parameters. See Table 2 for the parameter values used in the wet and dry seasons. Figure 2 shows a typical mosquito population in our model. The general shape remains the same regardless of mosquito carrying capacity and disease status. We make the assumption that the wet and dry season parameters do not vary from year to year so the same amount of rain falls every wet season. This is a simplifying assumption but allows us to consider the 'best case scenario' from the perspective of RVF and to consistently compare different scenarios. Additionally, directly correlating absolute mosquito population size with weather data is not straightforward.

During the wet season, some of the eggs that Aedes mosquitoes lay do not hatch until the subsequent wet season. To include this in our model, we computed the number of infectious mosquitoes for the last 30 days of the peak of the wet season, let a proportion ($\succ_v$) of them lay at least one infectious egg that will survive the dry season and result in an

emerging infectious adult female mosquito during the next wet season. If the total number of infectious eggs that survive the dry season and emerge is below 1, then we say RVF has died out. During the dry season, we assume there are no mosquitoes. This assumption could also be easily relaxed for areas that do not have as distinct wet and dry seasons.

Although eggs have been known to remain viable and infected with RVF for up to 7 years after being laid, very little data exists on the rate at which eggs die or how long they live, so for simplicity we assume for most runs that eggs do not hatch more than one year after being laid. We explored the impact of this assumption by allowing eggs to survive for up to three years for several scenarios and found that multiple year egg survival only had an impact for the lowest vertical transmission and vector-to-host ratios and for introduction of RVF into a naïve herd. As more data is made available about mosquito dynamics this assumption can be reevaluated.

Simulation scenarios

To test the first hypothesis that vertical transmission alone can allow for long-term persistence of RVF in buffalo, we ran the model for values of $\succ_v$ ranging from 0-10%. We also varied the vector-to-host ratio and the transmission rates to determine how sensitive our results are to those values. We chose to vary these parameters based on sensitivity analysis of a similar model in Chitnis et al, 2013, indicating that the basic reproduction number is sensitive to transmission probability from mosquito to host, vector to host ratio, the mosquito biting rate, and mosquito lifespan. Female Aedes mosquitoes typically do not disperse more than 500 meters from their natal habitat to find a host, so we assumed mosquito migration is negligible and that most female mosquitoes will encounter only the animals that water near where they hatched. A herd of buffalo will typically return to the

same watering holes in their home range, so it is also unlikely that a female Aedes mosquito will encounter buffalo from another herd. Thus we assume the mosquitoes will only be exposed to the buffalo herd that frequents the natal watering hole. Since herds of buffalo in Kruger average about 1000 individuals, we use 1000 buffalo as our baseline value for H_0 but also run the simulations for 500 and 1500 individuals . We considered the *minimum vector to host ratio* (MVH) necessary to maintain persistence under various vertical transmission rates. Remember that here 'vector' means female Aedes mosquitoes.

To test the second hypothesis that the primary means of persistence is through circulating in an alternate host in addition to buffalo, we simulate the system with an alternate host. Since we do not know what that host may be, we simply assumed that there are some additional susceptible hosts every year that can acquire and transmit RVF to the resident mosquitoes. We assumed that the alternate host has an equal or shorter lifespan, or herd turnover, to buffalo since buffalo are among the most long-lived ungulates in the park that are susceptible to RVF. Since buffalo live for 15 years on average, we vary the alternate host lifespan from 3-15 years. We vary the force of infection from alternate host to mosquito, ℓ_r to determine what level of infection in the alternate host is necessary to maintain RVF in the system, and in particular buffalo, over long periods of time (see Appendix 1 for description of ℓ_r).

Finally, we combined both vertical transmission and an alternate host. We ran the model for varying levels of infection in the reservoir species, varying values of vertical transmission and various host-to-vector ratios and transmission rates. We did not consider any scenarios in which no vertical transmission occurs, and transmission is dependent on mammalian hosts alone. In order for RVF to survive the dry season without any vertical

transmission, there must be transmission during the dry season. Since there are very few mosquitoes during the dry season, RVF does not persist through the dry season even with an alternate host in our simulations. Between-season persistence depends entirely on vertical transmission (or, alternatively, on consistent introduction of the virus from outside the system, which we are not considering here).

2.3 Results

Buffalo as the main mammalian interepidemic host

We started by considering the case where buffalo are the only host in order to determine if RVF can persist locally in a buffalo herd with reasonable vertical transmission rates for mosquitoes. Currently, most buffalo herds in Kruger have some level of immunity from previous outbreaks (Beechler et al, accepted pending revision). We therefore considered scenarios where 15% of the buffalo herd is immune to RVF from previous exposure (Figure 3a). RVF can persist at 1% vertical transmission rates in the larger herd sizes and with very large minimum vector to host ratios (MVHs), while persistence does not occur for the smaller herd of 500 buffalo. We found that 6-10% vertical transmission allows for persistence in the widest range of scenarios. Still, an MVH of at least 8:1 is required every rainy season even for high vertical transmission rates.

Since Aedes mosquito eggs are desiccation resistant and can potentially survive for several years, we let the eggs last for up to 3 years in the scenario with 1000 buffalo with 15% initial immunity. Letting the eggs survive longer only had a large impact on the MVH needed for 1% vertical transmission, reducing it from 86:1 to 34:1. It appears that with steady yearly rainfall assumed in this model, multiple year survival for eggs does not have a big impact on the results so we assume for the rest of the scenarios that eggs survive only

one year. This assumption would need to be revisited for varying rainfall or when new data becomes available about egg survival across seasons.

Other mammalian interepidemic hosts?

After considering buffalo alone as the primary hosts of RVF, we added a local alternate host (waterbuck, impala, etc.) that is also susceptible to RVF. This alternate host adds an additional transmission term to the model, representing the force of infection to mosquitoes from the alternate host(s) and to the alternate hosts from the mosquitoes. We ran the simulations for varying levels of vertical transmission and alternate host scenarios. Figures 3b and 3c show the alternate host transmission rates, vertical transmission, and minimum vector to host ratios (MVHs) needed for RVF to persist long term. We found with both an alternate host and vertical transmission, RVF can persist long term at 1-3% vertical transmission rates when the host transmits RVF relatively well. We found that the smaller the lifespan of the alternate host, the better the chance of persistence, since susceptibles become available sooner (Figure 4). We let the alternate host transmission rates (both to and from the vectors) vary from half that of buffalo (.5B) to 1.5 times that of buffalo (1.5B). When the alternate host is half as good at transmitting RVF (.5B), they have a dilution effect and RVF is less likely to persist in our simulations. For this case, RVF does not persist for 1-2% vertical transmission, but can persist at high MVHs for 3% vertical transmission with 1000-1500 buffalo and 1000-1500 alternate hosts. When the alternate host transmits as well as buffalo (1B), RVF is more likely to persist than with buffalo alone due to availability of more susceptible hosts and the shorter lifespan of the alternate hosts (Figure 3b). Finally, when the alternate host transmits better than buffalo (1.5B), RVF becomes much more likely to persist under a wide range of scenarios for low vertical transmission (Figure 3c).

Scenarios considering both MVH and number of infected eggs surviving

In addition to evaluating minimum vector to host ratio we also evaluated the model output to determine the number of eggs persisting in each scenario. Due to environmental stochasticity, scenarios where persistence is dependent on to 1 or 2 infected eggs for multiple seasons is very unlikely. At the MVH needed for persistence, the average number of eggs surviving every year is quite low (usually less than 5) so we computed the average number of infected eggs surviving the dry season for various V:H ratios and vertical transmission rates (Figure 5). For 1000 buffalo alone (no alternate host) and for 2% vertical transmission, there needs to be 2 times the MVH in order for the average number of infected eggs surviving the dry season to be above 10 per year. For 3% vertical transmission, there has to be at least 1.5 times the MVH to have average infected eggs surviving the dry season above 10 eggs per year. In scenarios where alternate hosts are present and where the alternate hosts are the same or superior transmitters, the MVH ratio needed for at least 10 eggs surviving per year is reduced. So, the presence of alternate hosts decreases the vector to host ratio needed for a more reasonable level of egg survival (Figure 5 and Figure A2).

Outbreaks without host immunity

Last we considered an RVF outbreak in a completely susceptible herd of buffalo. These results are applicable to invasion of RVF in geographical areas where it was not previously present. In this case, RVF does not persist long term for any scenario with a 3% vertical transmission or less (see Figure 6). For 4% vertical transmission, RVF will persist in larger herds (1000-1500) with very high vector to host ratios, while for smaller herds (500 animals), RVF still does not persist. The initial outbreak in a susceptible herd is large, thus herd immunity is high, and since buffalo are quite long-lived, RVF must survive in eggs and with very low level transmission for 10-20 years. If the V:H ratio is too large, then RVF no

longer persists either, as it spreads too quickly initially, leaving too few susceptible buffalo for subsequent outbreaks. This results in a fairly narrow range across which RVF will persist for 4% vertical transmission. When the vertical transmission reaches 6%, RVF can persist for all three herd sizes. The MVH remains high for 500 and 1000 individuals, but is lower for 1500 individuals (about 10:1). For 8-10% vertical transmission, persistence is much more likely for all scenarios. Figure 6 shows MVHs needed for persistence with a fully susceptible herd.

2.4 Discussion

Buffalo as the main interepidemic mammalian hosts

If RVF is already established in a buffalo population and herd immunity is about 15% then RVF can persist at 1-3% vertical transmission, but requires consistent and high minimum V:H ratios (MVHs) and the number of eggs persisting from season to season is low (1-5 eggs). Although there is no consensus on what typical vector to host ratios are in KNP, evidence so far suggests that a 2:1-20:1 ratio of female mosquitoes to available hosts is reasonable. Our model output suggests that dropping the minimum VH ratio below 20:1 require at least 3% consistent vertical transmission and at least 1000 buffalo present. Here, 3% vertical transmission means that 3% of the infected female mosquitoes living in the last month of the wet season will lay at least one infected egg that will survive the dry season, hatch, and emerge as an infected adult female the next wet season. For this scenario, the average number of infected eggs surviving and continuing transmission are 1-3 eggs per dry season.

Models considering both MVH and number of infected eggs surviving

In addition to the V:H ratio we also considered surviving eggs as an important variable. We show that although the minimum V:H ratio (MVH) results in persistence, it is often with only 1-5 infected eggs surviving each dry season. Environmental stochasticity minimizes the likelihood that a small number of eggs will consistently survive the dry season and re-infect the hosts. In order for the number of surviving infected eggs to have a minimum of 5 per dry season while keeping the V:H ratio at 20:1, a vertical transmission rate of 12% with a population of 1000 buffalo is required. This vertical transmission rate is unreasonably high, and even at a herd size of 1500 buffalo the vertical transmission rate required to maintain infection and a reasonable number of infected eggs (a minimum of 5 each year) at a 20:1 V:H ratio is 10%. We believe these rates of vertical transmission are ecologically unrealistic, with reasonable vertical transmission rates in Aedes mosquitoes for other infections (ex. Lutomiah et al, 2007; Diallo et al, 2007; Baqar et al, 1993) ranging between 1-6% with 5-6% being high but within the realm of possibility. Therefore, given the uncertainty of environmental stochasticity and the high vertical transmission rates or high host population required, it is unlikely that RVF can persist in buffalo alone as the predominant mammalian host.

As more data is made available, these conclusions may be modified. Since the results of our model are sensitive to vector to host ratios, vertical transmission rates, and egg survival over dry seasons, it is important that more be learned about these aspects of mosquito biology. Considering the impact of environmental stochasticity on egg survival for more than 1 year may also play an important role in RVF virus maintenance emphasizing the importance of data elucidating the probability of long term survival of eggs. If the vertical transmission rates our model suggests are necessary for persistence are not viable,

then focus can be shifted to the importance of unknown additional hosts. When we incorporate these cryptic hosts, the vertical transmission rate necessary and the minimum V:H ratio (MVH) are decreased to more reasonable levels. A 20:1 V:H ratio required only a 2.5% percent vertical transmission rate for 1000 buffalo and 1000 alternate hosts with average lifespan of 7 years. This 2.5% vertical transmission rate results in a minimum of 5 infected eggs and average of greater than 10 eggs surviving each dry season. With more infected eggs surviving, the virus is more likely to persist through stochastic events.

Other mammalian interepidemic hosts

In general, more infected eggs survive with higher V:H ratios and with more available hosts in our simulations. This suggests that the most likely mode of persistence apart from consistent re-introduction is a combination of buffalo and alternate competent hosts with high vector to host ratios in the wet season. When we explored how transmission efficiency and lifespan (herd turnover) of the cryptic alternate host affect the likelihood of persistence, we found that transmission efficiency plays a much larger role. Possible host species are numerous, as many ungulates have been shown to be susceptible to RVF. Antibodies to RVFV have been found in many wildlife species, including, but not limited to, impala, white rhinoceroses, bushbuck and waterbuck (Davies, 1975; Anderson & Rowe, 1998; Fischer-Tenhagen et al, 2000; Paweska et al, 2005; Evans et al, 2008; Paweska et al, 2008), but very little data exists on transmission efficiency for these species. Further studies are required to determine which species are most likely to be efficient transmitters of RVF in the inter-epidemic period.

Outbreaks without host immunity

We also used this model to briefly explore how considering a naïve population of mammalian hosts would affect the maintenance of the virus in the interepidemic period. We

set initial herd immunity to 0, so the entire population of buffalo is susceptible to infection. We found that the initial outbreak in a naïve herd leads to so many infected animals that there are no susceptibles for future generations of RVF. In this case, persistence without re-introduction of either an infected host or more susceptibles, depends on high (6-10%) vertical transmission rates. This affects the invasion potential of the disease and the need for susceptible hosts with high turnover rates for it to successfully invade into and persist new areas as is feared in Europe (Chevalier et al, 2010) and North America (Kasari et al, 2008).

Possible model extensions and model refinement

Missing data about parameters such as the vertical transmission rates, how many Aedes (and Culex) mosquitoes are present in the wet season on average, reasonable vector to host ratios, the variation in number of mosquitoes present as weather varies, and transmission rates from alternate hosts increase the level of uncertainty in models such as ours. Acquisition and analysis of such data would not only greatly increase our understanding of Rift Valley fever, but would give us important insight into various other zoonotic mosquito born infectious diseases such as chikungunya, West Nile virus, and dengue among many others. With more and better data, researchers together with veterinarians, doctors, and public health colleagues, can better predict risk, good surveillance methods, total consequence, and optimal methods of control and prevention. This lack of data about mosquito biology and ecology as well as transmission rates for various species extends across most mosquito-borne diseases and will become more important with climate change and increased globalization.

In addition to benefiting from more data about mosquitoes and alternate host species, a reasonable next step is to include environmental stochasticity in the model via

varying rainfall and mosquito carrying capacities, mosquito and egg survival, and vertical transmission rates. As we learn more about species that are competent hosts for RVF, we can include specific species information in the model instead of a general 'alternate host'. How immunity plays a role is also important and the effect of waning immunity over time or maternal immunity should be considered. Our current model gives qualitative insights into the system, showing that a combination of mammalian hosts and vertical transmission are necessary to maintain the disease in the interepidemic period. But as we learn more about RVF and acquire better data, we can move toward developing models that can provide more accurate risk of outbreaks over time.

Table 2.1: The parameters and variables for the RVF model and their dimensions

Symbol	Description	Dimensions
bh/dh	Buffalo per-capita birth/death rates	Day-1
bv/dv	Mosquito per-capita birth/death rates	Day-1
H0	Stable buffalo population in absence of disease	Animals
V0	Stable adult female mosquito population	Animals
γe	Mosquito hatch and emergence rate	Day-1
λv	Contact/transmission rate for mosquitoes	Day-1
λh	Contact/transmission rate for buffalo	Day-1
γh	Rate buffalo move from infectious to recovered	Day-1
δh	Rate at which buffalo die from RVF	Day-1
νv	Rate mosquitoes move from incubating to infectious	Day-1
φv	Proportion of infectious mosquitoes that lay infectious eggs	Dimensionless
Sh	Susceptible buffalo	Animals
Ih	Infectious buffalo	Animals
Rh	Recovered and immune buffalo	Animals
Se	Susceptible eggs/larvae	Animals
Ie	Infectious eggs/larvae	Animals
Sv	Susceptible adult female mosquitoes	Animals
Ev	Incubating, or exposed, adult female mosquitoes	Animals
Iv	Infectious adult female mosquitoes	Animals

Table 2.2: Parameter values for wet and dry seasons (see Chitnis et al, 2013 for references)

Parameter	Wet Season	Dry Season
1/dh	15 Years	15 Years
1/dv	20 Days	14 Days
$1/\gamma e$	10 Days	1000 Days
σv	0.33 Day-1	0.25 Day-1
σh	19 Day-1	19 Day-1
βhv	0.12	0.12
βvh	0.38	0.38
$1/\eth h$	4 Days	4 Days
δh	0.01 Day-1	0.01 Day-1
$1/\nu v$	14 Days	14 Days

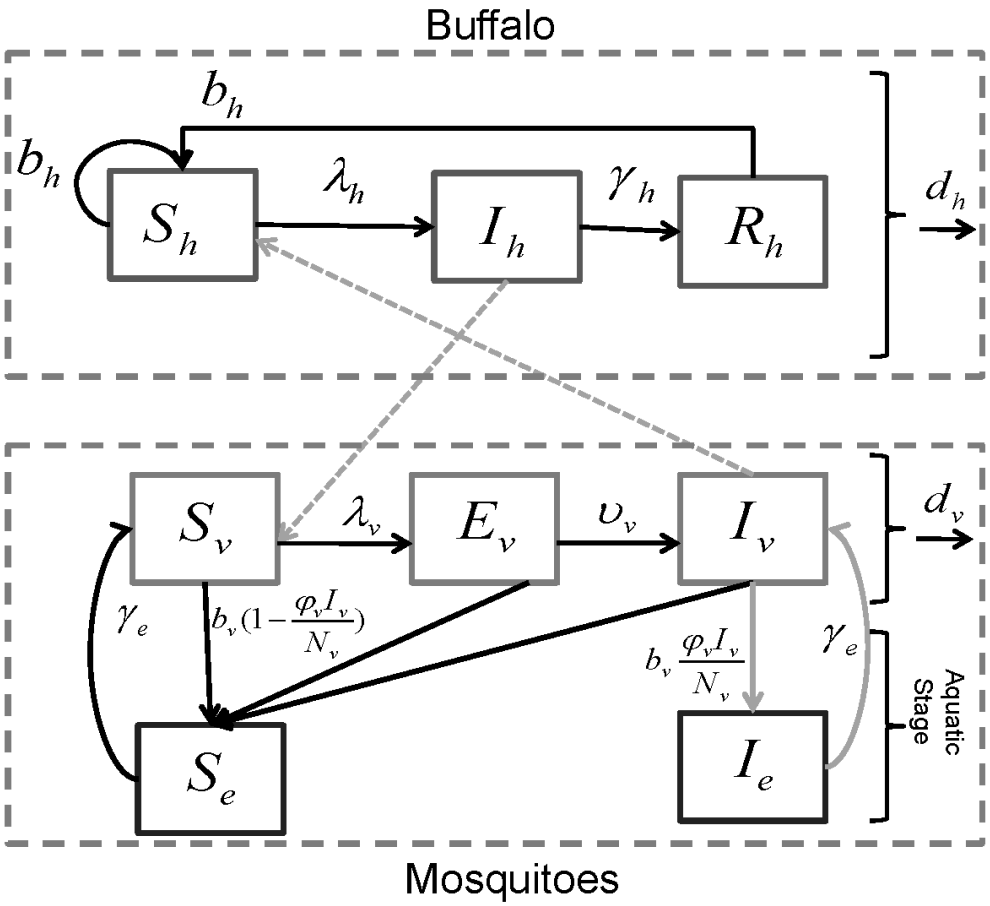

Figure 2.1: Diagram for the disease transmission model. Arrows represent flow in or out of a compartment and letters above the arrows represent the rate of flow. The light grey arrows highlight where vertical transmission in mosquitoes occurs and mosquito-buffalo contact (i.e. a successful bite).

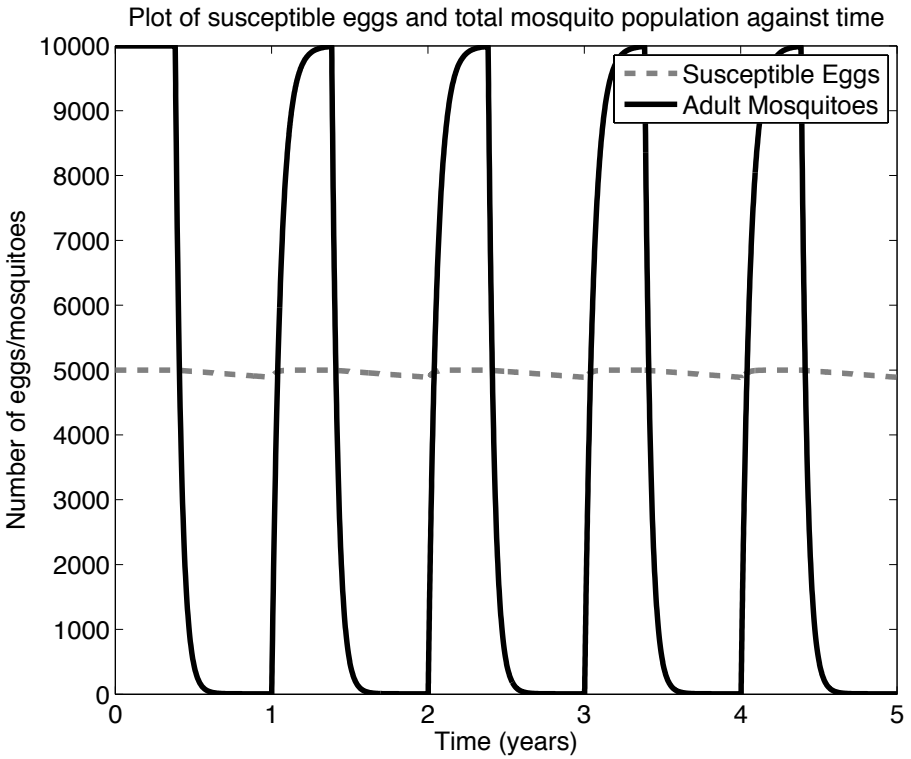

Figure 2.2: A typical time series of mosquito populations over 5 years. Half of the year is a wet season, characterized by many mosquitoes and half of the year is the dry season with very few or no mosquitoes present. We assume eggs are available all year long so if a flooding even occurs, some mosquitoes will hatch.

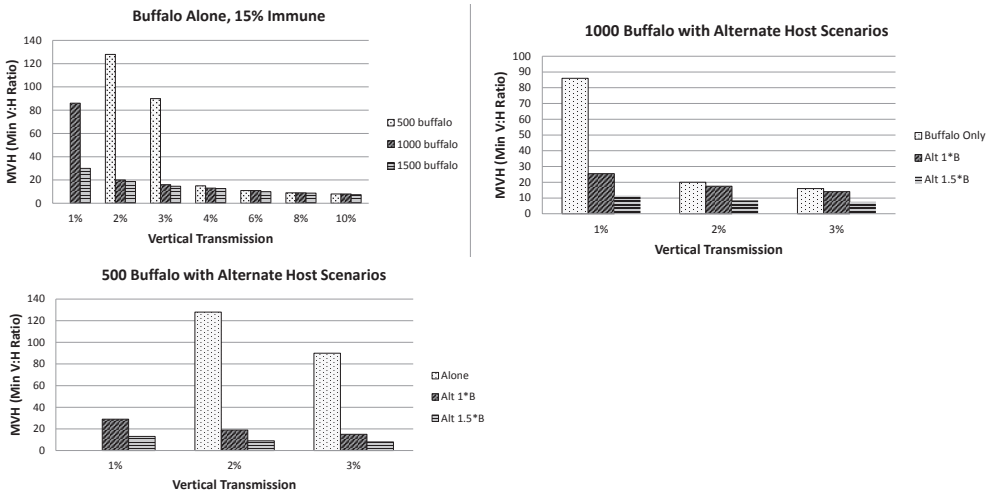

Figure 2.3: The minimum vector to host (MVH) ratio needed for long term persistence of RVF with 15% initial immunity. Plot (a) is for 500, 1000, or 1500 buffalo without an alternate host present. Persistence is not possible at 1% vertical transmission with only 500 buffalo present. Plot (b) is the MVH needed for persistence with 1000 buffalo and 1000 alternate hosts. The alternate hosts have either the same transmission rates as buffalo (1B) or 1.5 times the transmission rates of buffalo (1.5B). Plot (c) is the MVH needed for persistence with 500 buffalo and 500 alternate hosts with the same transmission rate as buffalo and with 1.5 times the transmission rate of buffalo. Adding hosts that are as good or better transmitters than buffalo decreases the MVH needed for persistence.

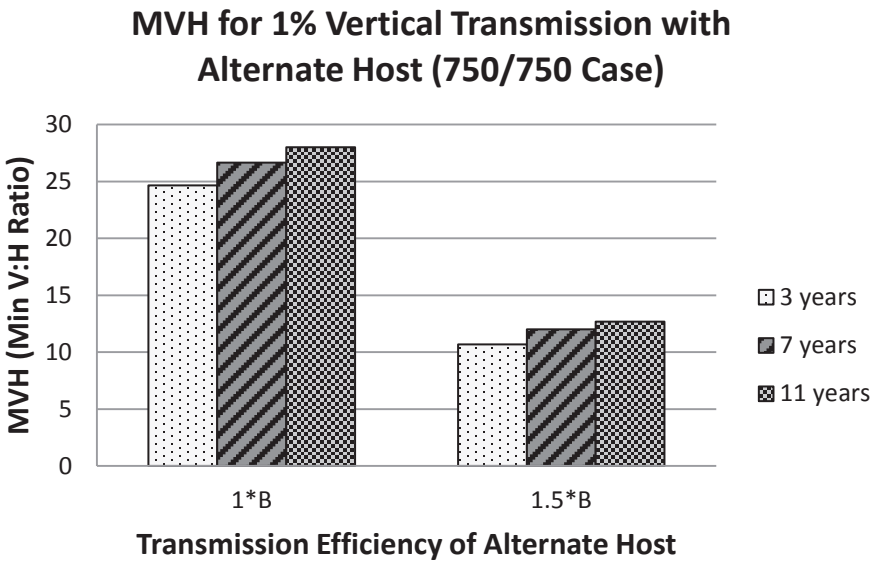

Figure 2.4: The effect of alternate host lifespan and transmission competency on the minimum V:H ratio (MVH) needed for persistence in a herd of 750 buffalo/750 alternate hosts. Although lifespan does have an effect, the transmission competency and not lifespan (herd turnover) is most important as seen in Figure 3.

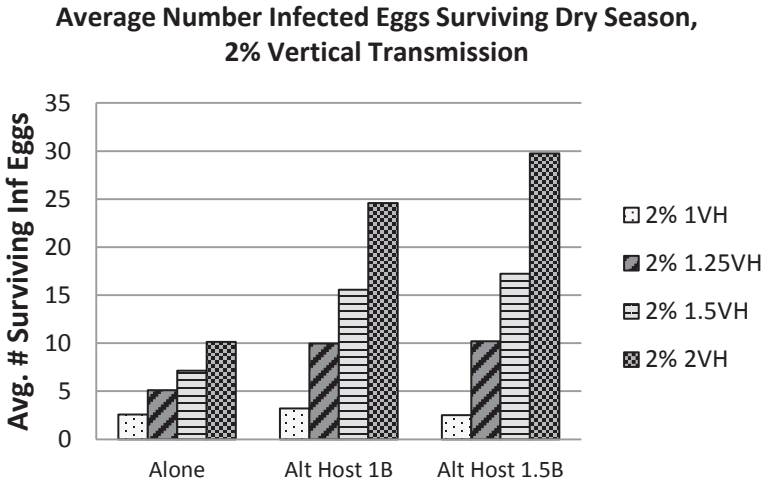

Figure 2.5: The average number of infected eggs surviving the dry season for 2% vertical transmission rates with various V:H ratios. Both 1000 buffalo alone and 1000 buffalo with 1000 alternate hosts are considered. Alternate hosts either have the same transmission as buffalo (1B) or 1.5 times the transmission rates of buffalo (1.5B). The first bar is the average number of infected eggs surviving each dry season at the MVH (denoted 1VH). The second bar is the average number of infected eggs surviving for 1.25 times the MVH (1.25VH), the third for 1.5 times the MVH (1.5VH), and the fourth for 2 times the MVH (2VH). Figure A2 in Appendix 1 shows the 1% and 3% scenarios.

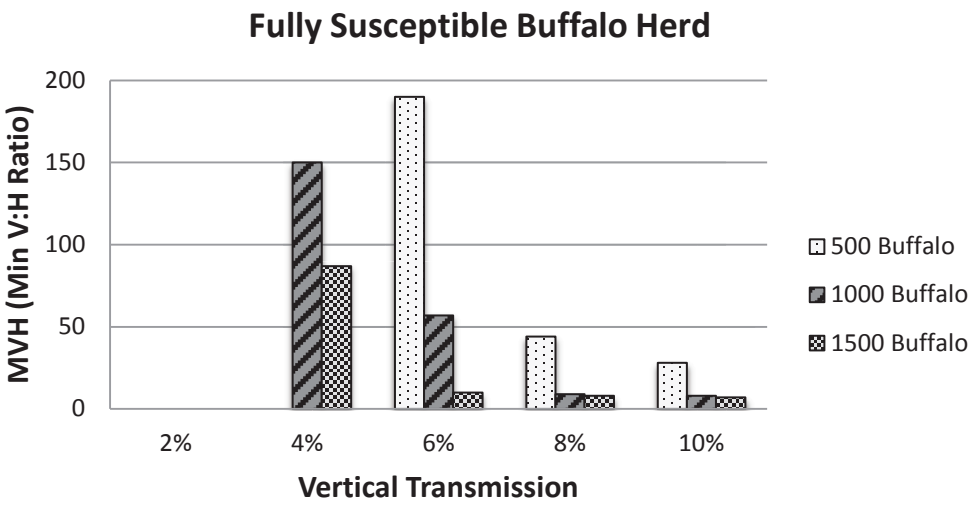

Figure 2.6: Minimum vector to host ratio (MVH) needed for persistence when RVF is introduced to a fully susceptible or naïve buffalo herd. No persistence occurs below 4% vertical transmission. It seems that either high vertical transmission rates or re-introduction of RVF from an outside source is necessary for persistence in a naïve herd.

2.5 APPENDIX

Description of the Model

Let $\leftarrow_v$ be the number of times a mosquito would bite a buffalo per unit time given the opportunity, $\leftarrow_h$ be the maximum number of bites a buffalo sustains per unit time, and $\updownarrow_{hv}$ be the probability that when an infectious mosquito bites a buffalo it successfully transmits RVF to the buffalo. The value of $\leftarrow_v$ depends on the mosquito's gonotrophic cycle and preference, if any, for biting buffalo (see Chitnis et al, 2006 and Chitnis et al, 2013 for a complete description of the contact rate and its properties). The value of $\leftarrow_h$ depends upon the size of buffalo, time spent near the mosquitoes, and evasive techniques such as switching the tail. When mosquito populations are very high, the contact rate is limited by availability of hosts; at lower to medium-high mosquito populations, the contact rate is dominated by the density of mosquitoes. The force of infection from mosquitoes to buffalo is

$$\lambda_h = \frac{\sigma_v N_v \sigma_h}{\sigma_v N_v + \sigma_h N_h} \cdot \frac{I_v}{N_v} \cdot \beta_{hv}$$

or, in words, the product of (number of bites that one buffalo receives per unit time)*(probability that the biting mosquito is infectious)*(probability of transmission given an infected bite). Similarly, if we let $\updownarrow_{vh}$ be the probability that if a susceptible mosquito bites an infectious buffalo it will be infected, then the force of infection from buffalo to mosquitoes is $\lambda_v = \frac{\sigma_v \sigma_h N_h}{\sigma_v N_v + \sigma_h N_h} \cdot \frac{I_h}{N_h} \cdot \beta_{vh}$.

Under these assumptions, the differential equations that model the system are:

Buffalo **Mosquitoes**

$$\frac{dS_h}{dt} = b_h H_0 - d_h S_h - \lambda_h S_h, \qquad \frac{dS_e}{dt} = b_v V_0\left(1 - \frac{\varphi_v I_v}{N_v}\right) - \gamma_e S_e \qquad \frac{dS_v}{dt} = \gamma_e S_e - d_v S_v - \lambda_v S_v$$

$$\frac{dI_h}{dt} = \lambda_h S_h - d_h I_h - \gamma_h I_h - \delta_h I_h \qquad \frac{dI_e}{dt} = b_v V_0\left(\frac{\varphi_v I_v}{N_v}\right) - \gamma_e I_e \qquad \frac{dE_v}{dt} = \lambda_v S_v - d_v E_v - \nu_v E_v$$

$$\frac{dR_h}{dt} = \gamma_h I_h - d_h R_h \qquad \frac{dI_v}{dt} = \nu_v E_v - d_v I_v$$

See Figure 1 for a transfer diagram of the model. If seasonality and disease are ignored and if it is assumed both mosquitoes and buffalo are at carrying capacity, then the disease free equilibrium is $N_v = V_0$, $N_e = b_v V_0 / \bullet_e$, and $N_h = H_0$.

Seasons are implemented by adjusting V_0, the carrying capacity of mosquitoes, and other mosquito-related parameters through time. The number of infected eggs stored during the dry season is determined by multiplying $\succ_v$ times the number of infectious mosquitoes in the last month of the wet season.

The alternate reservoir host changes the susceptible and incubating mosquitoes equations to:

$$\frac{dS_v}{dt} = \gamma_e S_e - d_v S_v - (\lambda_v + \lambda_r) S_v$$

$$\frac{dE_v}{dt} = (\lambda_v + \lambda_r) S_v - \nu_v E_v - d_v E_v$$

where ℓ_r is the force of infection from some reservoir host species to the mosquitoes, following the same format as $\leftarrow_h$ distributing mosquito bites evenly between the alternate hosts and the buffalo.

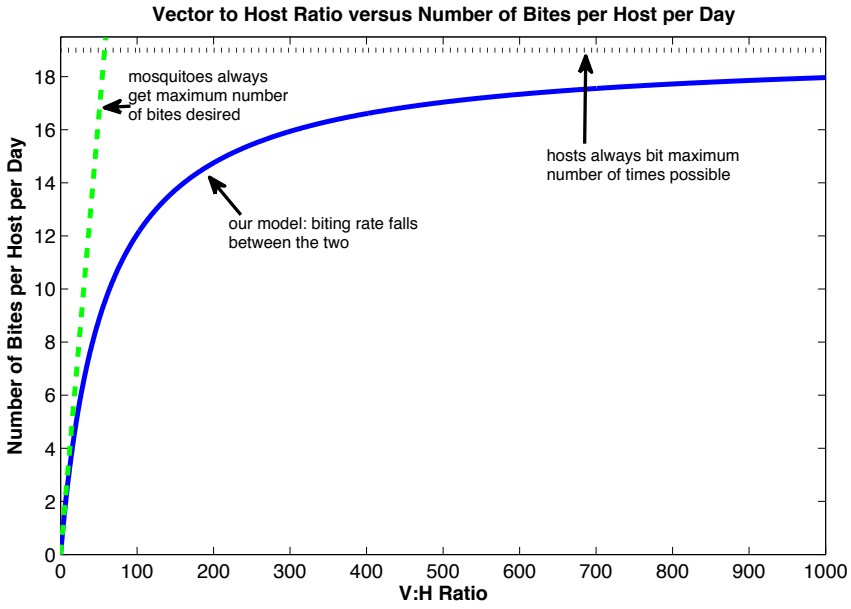

Figure A2.1: Plot of the number of bites per host per day as a function of the vector to host ratio.

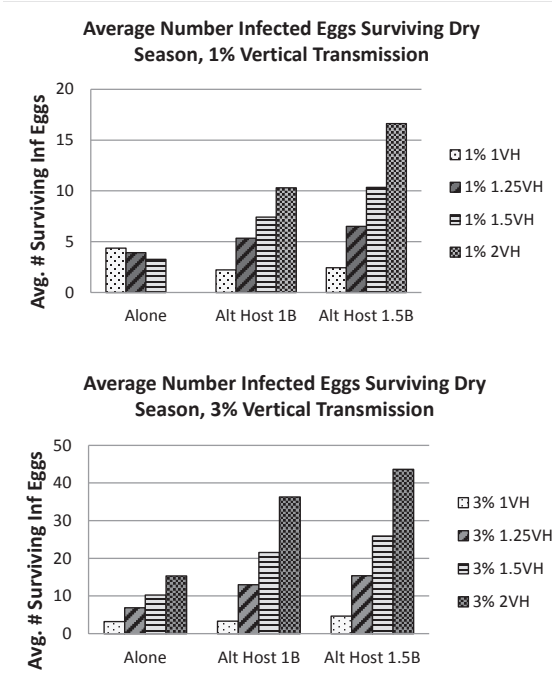

Figure A2.2: As in Figure 5, the average number of infected eggs surviving each dry season for (a) 1% and (b) 3% vertical transmission with varying host composition and varying vector to host ratios.

3- Enemies and Turn-Coats: The Emerging Disease Bovine Tuberculosis exposes pathogenic potential of Rift Valley fever virus in a native host

Brianna Beechler, Carrie Manore, Vanessa Ezenwa, Bjorn Renighaus, Anna Jolles

Ecology Letters
In Prep

ABSTRACT

The ubiquity and importance of parasite co-infections in populations of free-living animals is beginning to be recognized but few studies have demonstrated differential fitness effects of single versus co-infection in free-living populations. We investigated interactions between the invasive bacterial disease bovine tuberculosis (BTB) and the native viral disease Rift Valley fever (RVF) in a competent reservoir host, African buffalo, combining data from a natural outbreak of RVF in an buffalo breeding facility in 2008 with data collected from a neighboring free-living herd of African buffalo in Kruger National Park. African Buffalo infected with BTB were 1.7 times as likely to acquire RVF in the captive population and 2.3 times more likely to be seropositive for RVF in the natural population than BTB negative buffalo, demonstrating the facilitation of one pathogen by another. A mathematical model demonstrated that this facilitation led to altered RVF epidemic intensity and length in the presence of BTB in buffalo populations. In addition coinfected buffalo suffered direct fitness consequences of infection, by aborting their fetuses, whereas the risk of abortion in buffalo with only one infection (BTB or RVF) did not differ from that observed in uninfected buffalo. The data indicate that the consequences of emerging infections are not limited to direct effects of the emerging infection, but can potentially alter the dynamics and fitness effects of native infectious diseases in free-ranging wildlife populations.

3.1 INTRODUCTION

Emerging infectious disease events have increased in the last 50 years due to global changes such as climate alterations, increased contact between wildlife and livestock, and increased travel; with the majority of events originating from wildlife species (Jones et al, 2008). These emerging diseases can cause cascading effects through the host population,

such as population declines (McCallum et al, 2009; Vredenburg et al, 2010, Thorne and Williams, 1998) due to increased mortality such as rinderpest in subsaharan Africa (Plowright, 1982) or decreased fecundity such as experience by koala bears infected with chlamydia (Augustine, 1988). Not only do these emerging diseases have direct effects on host populations they may also alter the fitness effects of native diseases within the ecosystem, perhaps due to increased mortality of doubly infected individuals (Jolles et al, 2008) or decreased fecundity of doubly infected individuals (Johnson and Hoverman, 2012; Joly & Messier, 2005), thereby altering traditional host-parasite dynamics.

In addition to reducing host fecundity and survival, an emerging disease may alter the dynamics of previously existing diseases, potentially resulting in increased spread of the pre-existing native disease. Recent literature has considered that the native pathogen community may alter the success of an invading infectious disease (Telfer et al, 2012), for example within host parasite community affects the likelihood of invasion of the swim bladder nematode in European eels where eels with increased microparasite and macroparasite richness are more likely to be infected by the invading pathogen (Martínez-Carrasco et al, 2011). A parasite specific interaction is described in African Buffalo where native nematodes facilitate the invasion of BTB into the host population (Ezenwa et al, 2010). However, very little work has been performed investigating how the presence of an emerging pathogen may alter the dynamics of previously existing native infections.
An emerging pathogen may erode host defenses against native infections, increasing transmission risk of the native infection in infected individuals (Ezenwa, et al, 2011). Alternatively, the emerging infection may remove susceptible animals from the pool by cross-protective immune response (Graham, 2008) or mortality (Jolles et al, 2008) reducing

the transmission events of a native infection. We sought to investigate whether an emerging pathogen, Mycobacterium tuberculosis, alters the likelihood of an animal acquiring a native pathogen, Rift Valley fever, in a common host species, African Buffalo in Kruger National Park.

BTB is a bacterial disease caused by Mycobacterium bovis and is an emerging infection in African wildlife (Michel et al, 2006) and human populations (DeVos et al, 2001). It is not native to sub-Saharan Africa and was first found in the late 1980's inside Kruger National Park (DeVos et al, 2001; Swanepoel & Coetzer, 2006) with likely introduction to South Africa in the 60's from imported European cattle (Michel et al, 2009). BTB has been spreading south to north within KNP since its introduction in the 1960s and is present in southern herds at 40-50% prevalence, 20-30% in the central area and 0-10% in northern herds (Michel et al, 2006) and has recently been found crossing the northern boundary of KNP into buffalo in Zimbabwe's Gonarezhou National Park (deGarine-Wichatitsky et al, 2010). In buffalo, BTB is a chronic lifelong infection, (Cross et al, 2009) with context-dependent effects that may include declines in body condition (Caron et al, 2003 & Jolles et al, 2008) and reductions in fecundity (Jolles et al, 2005 & Jolles et al, 2006) and survival. (Jolles, 2005) While the direct effects of BTB in buffalo populations have been evaluated, no work has been performed asking how BTB might affect other diseases within the system, such as RVF.

RVF is a mosquito-borne, zoonotic viral disease (Phlebovirus Family: Bunyaviridae) that infects numerous mammalian species and can cause mass mortalities in livestock during epidemic events that occur every 4-6 years in Southern Africa (Swanepoel & Coetzer, 2006). RVF is native to sub-Saharan Africa (Paweska, 2008), and infects African

buffalo in Kruger National park (LaBeaud et al, 2011; Beechler et al, 2013). Once infected African buffalo can exhibit clinical signs, including abortion (Skukuza State Veterinary Reports, 1998; Davies and Karstad, 1981) and mortality, especially in young animals (Pepin et al, 2010) due to increased susceptibility in target cells (Pepin et al, 2010) and altered immune response (Zhao et al, 2008).

We used this study to investigate the role of bovine tuberculosis in a natural RVF outbreak in both a free-ranging and captive population of African buffalo. For the captive population we asked whether animals with BTB were more or less likely to become infected with RVF during the outbreak, and were more or less likely to abort. We then looked to see if these patterns found in the captive African buffalo population were mirrored in a free-ranging population within Kruger National Park. Finally we used a mathematical model to determine whether these alterations in individual susceptibility could scale up to alter population level patterns of disease transmission.

3.2 METHODS

RVF outbreak in the captive population

In 2008 an outbreak of RVF occurred in and around Kruger National Park (Archer et al, 2012). We collected data from a buffalo breeding facility on the southern boundary of Kruger National Park, the Nkomazi area, on RVF infection prior to, during and post outbreak. The outbreak was first noted on the facility on January 14, 2008 and the entire herd was vaccinated between the 13th and 26th of February. Prior to vaccination blood was collected from each individual and was serologically tested for RVF using a hemagglutination-inhibition (HAI) titration assay at Onderstepoort Veterinary Institute in Pretoria, South Africa (LaBeaud et al, 2011; Scott et al, 1986). This breeding facility had

both BTB + buffalo and BTB - buffalo, with disease designations made by the farm officials. Animals were assigned BTB status based on the results of multiple skin tests prior to the outbreak, This assay is described in the OIE terrestrial manual (2012) and has been used in African buffalo (Munang'andu et al, 2011; Jolles et al, 2008). Briefly, animals are intradermally injected with bovine tuberculin and the swelling response measured 72 hours later with animals having a swelling response greater than 2 mm considered positive. Animals listed as BTB negative were certified disease free based on the results of a prior caudal fold skin tests BTB test, as concordant with the regulations for disease free buffalo breeding facilities. The sensitivity and specificity of caudal fold skin BTB tests is 80-91% and 95-100% in cattle (Lilenbaum et al, 1999; Llamazares et al. 1999, Ameni et al. 2000); 80.9% and 90.2% in African buffalo (J.P. Raath, unpublished data). Animals listed as BTB positive were positive on a skin test and on subsequent follow-up tests. These animals were maintained in a close by and ecologically similar boma, but had no direct contact with BTB negative animals.

To determine the cause of mortality in the juvenile and adult buffalo state veterinarians performed full necropsies and noted the presence of lesions concordant with Rift Valley fever infection, such as liver necrosis. Infection was confirmed with immunohistochemical (Van der Lugt et al, 1996) staining at the Onderstepoort Veterinary Institue. Aborted fetuses were also collected and state veterinarians performed full necropsies, finding postmortem lesions concordant with Rift Valley fever infection, such as liver necrosis and RVF infection was confirmed with immunohistochemical staining at the University of Pretoria Diagnostic Laboratory and RT-PCR (Espach et al, 2002) performed on fetal blood samples at Onderstepoort Veterinary Institute. Previous non-outbreak years

pregnancy and birthing data was used to determine an intercalving interval on the farm of 462 days (from 1999-2007, n=756) and an average pregnancy rate of 73% in adult female cows. When calculating abortion rates in populations we used a denominator of 73% of the total reproductive females with and without RVF. We then assessed whether abortion rates were different between the 4 disease groups (coinfected, single RVF infection, single BTB infection, uninfected) using a Kruskal Wallis ANOVA with Dunn multiple comparisons. To assess if BTB alters the individual likelihood of an animal acquiring RVF, we used a fishers exact test comparing the likelihood of seroconversion between adult female BTB - buffalo and adult female BTB + buffalo.

RVF outbreak in the free-ranging population

To evaluate whether BTB/RVF coinfection patterns found in the buffalo breeding facility were mirrored in a free living population we sampled 96 free-living young female buffalo in the southern portion of Kruger National Park near the buffalo breeding facility in October 2008 (approximately 7 months after the outbreak of RVF) as part of a larger bovine tuberculosis study. Animals were chemically immobilized with M99 (etorphine hydrochloride), azaperone and ketamine by darting from a helicopter. After immobilization animal age was determined and blood was collected via jugular venipuncture into lithium-heparinized tubes for disease diagnostics. Animal ages were assessed from incisor emergence patterns for buffalo 2–5 years old and from tooth wear of the first incisor for buffalo 6 years of age and older (Jolles 2007). Following data collection, immobilization was reversed using M5050 (diprenorphine), animals were chemically restrained for no longer than 60 minutes. All immobilizations were performed by South African National Parks (SANParks) veterinarians and game capture staff, and all procedures were approved

by Oregon State University, University of Montana, and SANParks Institutional Animal Care and Use Committees.

We determined RVF serostatus with the virus neutralization tests (Paweska et al, 2008; Beechler et al 2013). Tuberculosis infection status was determined using a standard whole-blood gamma interferon assay protocol (Wood and Jones 2001; Schiller et al. 2009). In brief, this assay is performed by comparing the in vitro IFNg response to *Mycobacterium bovis* antigen (bovine tuberculin) to the IFNg response to an avian tuberculin antigen and background IFNg levels in the absence of antigenic stimulation. This assay has been optimized for use in African buffalo (Michel et al. 2011), and blood cells from buffalo infected with *M. bovis* show a pronounced spike in IFNg production in response to bovine but not avian tuberculin, whereas bovine tuberculin challenge does not induce IFNg production in the blood of unexposed animals (Michel et al. 2011). We implemented the gamma interferon assay with the BOVIGAM enzyme-linked immunosorbent assay kit (Prionics), which has a sensitivity of 86% and a specificity of 92% in African buffalo (Michel et al. 2011).

We performed a Fishers exact test to determine whether animals with BTB were more likely to also be seropositive for RVF than their BTB negative counterparts in the free-ranging population. The majority of these RVF positive animals likely converted in the 2008 outbreak, so a relative risk was calculated for acquiring RVF with and without BTB. To further evaluate the correlation between BTB and RVF we performed a generalized linear model with binomial distribution to evaluate whether BTB status correlated with RVF status, after accounting for buffalo age, body condition and pregnancy (Table 2) in the free-ranging population.

Effect of BTB on RVF dynamics - the mathematical model

We then altered a mathematical model (Manore & Beechler, 2012) to explore how the altered risk of RVF infection in an individual infected with BTB may alter epidemic dynamics of RVF in buffalo. We used the model (Figure 1) for spread of Rift Valley fever in buffalo during one rainy season to explore the effects of BTB prevalence on the timing and magnitude of an RVF outbreak. While keeping the general form of the model and parameter values the same, we altered the Manore & Beechler (2012) model to account for BTB presence in a herd in the following ways. We considered an RVF outbreak in a single rainy season, so did not explicitly model BTB transmission. A proportion of the buffalo, F_{TB}, are BTB positive, while the remaining buffalo are BTB negative. We assumed that initial immunity to RVF was the same across BTB positive and negative buffalo. For the purposes of this study, we assumed that death due to disease is equal for both BTB+ and BTB- buffalo. The probability that a bite from an infectious mosquito will result in RVF infection for a BTB- buffalo is b_{RV} while BTB+ buffalo are considered to have a higher probability, $c_{TB}b_{RV}$, of being infected by a bite from an infectious mosquito. We assume the probability of a susceptible mosquito acquiring the virus after biting an infectious buffalo is the same for both BTB+ and BTB- buffalo, namely b^v. Although this may not necessarily be the case, we do not have sufficient evidence to assume otherwise.

Since we only considered one season, we assumed that the buffalo population is constant (1000 buffalo) and ignored birth and death processes. However, the mosquitos live on a much faster time scale, so birth and death are modeled for the mosquito populations with a wet season carrying capacity resulting in a vector to host ratio of 15:1. All runs begin after the mosquito populations have had a chance to ramp up to carrying capacity and the

outbreak is started with one exposed and one infectious mosquito. Mosquitoes are at peak numbers for 45 days, after which the carrying capacity is reduced to zero and the mosquitoes slowly die out so that very few mosquitoes are left after 90 days of simulation. In this scenario, RVF transmission is generally slowed not by loss of susceptible hosts, but by loss of vector populations at the end of the rainy season.

3.3 RESULTS

Of the total 371 buffalo located on the breeding facility, 235 had known RVF statuses before and after the epidemic. Of these 235 buffalo, 60 were calves under 1 year of age, 156 were adult cows and the remaining 19 were adult bulls. There was a total seroconversion rate of 34.9% due to the outbreak., Table 1 shows the breakdown by sex and age. In the free-ranging population of buffalo 39.59% (38/96) were RVF seropositive. Clinical signs associated with RVF infection were noted during the outbreak. One adult female buffalo and one young calf died from RVF (verified by lesions during post-mortem and RT-PCR of liver tissue). Eight female buffalo aborted with characteristic RVF lesions found in the aborted fetuses, and infection confirmed with RT-PCR of fetal blood and immunohistochemical staining of the liver. Of the eight female buffalo, two individuals aborted a 10 month old fetus, three aborted a 4-5 month old fetus, one a 3-4 month old fetus, and the age of the fetus was not recorded for the other two abortions.

Coinfected buffalo were more likely to abort than uninfected or singly infected with BTB or RVF (Kruskal Wallis statistic=50.36, $p<0.00001$, Figure 2). While 7% (2/29) of the pregnant buffalo infected with only RVF aborted, 46% (6/14) of the coinfected animals aborted, so the relative risk of abortion was 6.57 times greater in coinfected individuals than those infected only with RVF. No buffalo infected with only BTB aborted. In previous

years there was no difference between abortion rates in the BTB+ and BTB – individuals. Unfortunately we were unable to assess whether coinfected animals in the free-ranging population were more likely to abort than singly infected individuals, as we demonstrated in the captive population. The population of buffalo captured was primarily pre-reproductive and it is also likely that any animal that did abort due to RVF was pregnant 7 months later at this capture.

In the farmed population buffalo that are BTB + have a relative risk of acquiring RVF that is 1.744 (CI 1.171 to 2.596) times higher than their BTB - counterparts (Fisher exact test, p=0.0147). Where as 56.25% of the BTB+ adult female buffalo seroconverted during a natural outbreak in a buffalo breeding facility, only 32.26% of the BTB - adult female buffalo seroconverted (Figure 1a). In the free ranging population, buffalo that were BTB + have a relative risk of being seropositive for RVF that is 2.326 (CI 0.89 to 6.056) times higher than their BTB - counterparts (Fisher exact test, p=0.03) (Figure 1b). Neither age or buffalo body condition correlated with RVF serostatus, nor altered the direction and magnitude of the correlation between RVF serostatus and BTB infection (Table 2).

We used a mathematical model to determine whether these individual changes in the likelihood of acquiring RVF could affect the size of RVF epidemics. We ran the simulations while varying three key parameters: the additional RVF transmission factor for BTB positive buffalo, the prevalence of TB in the herd, and initial immunity to RVF. We varied the increased risk of RVF for BTB positive animals, c_{TB}, from 1.0-4.4 times the risk for BTB negative buffalo. We varied BTB prevalence, F_{TB}, from 0 to 1 and varied initial herd immunity to RVF from 0-20%. As anticipated, RVF prevalence in BTB positive buffalo was higher than in BTB negative. However, we found that increasing BTB prevalence within a

herd increases the magnitude of an RVF outbreak, dependent upon the "increased risk" of acquiring RVF infection due to BTB (Figure 3a), resulting in increased prevalence in both BTB+ and BTB - individuals (Figure 3c). Although the percent of buffalo initially immune reduces the size of outbreak, there is still an increase in magnitude due to BTB; it is simply reduced compared to assuming no initial immunity (Figures 3b and 3d). Not only did BTB increase the intensity of RVF outbreaks, it also altered the length of RVF outbreaks, increasing the length of the epidemic at low to moderate increased individual risk but decreasing the length of the epidemic at higher individual risk (Figure 4a and 4b). Although the relative effect of BTB prevalence on RVF transmission varied across the parameter ranges explored, the general pattern of increased magnitude and longer outbreaks for RVF remained consistent.

3.4 DISCUSSION

We show that the effects of an emerging pathogen, such as BTB, are not limited to direct effects on the host, but may also alter the infection patterns of diseases previously existing within the ecosystem or host species. BTB increases the likelihood of an animal acquiring RVF, worsens the clinical signs and also increases the intensity and length of RVF outbreaks at a population level.

BTB had dramatic impacts on the expected fitness consequences of infection with RVF, whereby animals with BTB were 6 times more likely to abort due to RVF than those uninfected with BTB. So while BTB may have minor population level effects on the buffalo population as a single disease (Jolles et al, 2008, Cross et al, 2008), it may exacerbate the effects of other diseases such as RVF, which could cause dramatic effects on host population dynamics. This effect may be of a greater magnitude because Rift Valley fever outbreaks

typically occur in the wettest years (Martin et al, 2008), which are also the years that buffalo populations tend to increase due to decreased mortality and increased calf survival (Mills et al, 1995; Owen-Smith & Ogotu, 2003; Seydack et al, 2011), allowing them to compensate for the higher mortality and decreased calf survival in the dry years. Future work should focus on understanding whether the alteration of individual level buffalo-RVF interactions scales up to affect buffalo population dynamics.

Buffalo in both the free-ranging and captive population were approximately twice as likely to acquire RVF when previously infected with BTB, providing strong evidence that BTB may affect host susceptibility to other pathogens, such as RVF. BTB may directly increase susceptibility to RVF by altering the host's immune response, increasing the risk of acquiring a microparasite. BTB in cattle causes dynamic alterations to the host immune response over time (Widdison et al, 2006), whereby animals with older infections have reduced ability to respond in ways appropriate to fight microparasites such as RVF (Welsh et al, 2005; Pirson, 2012). Alternatively, there may be a genetic or early age developmental predisposition whereby animals that acquire BTB have a weak immune response to microparasites (Allen & Maizels, 2011; Ezenwa et al, 2011, Driscoll et al, 2011; Allen et al, 2010) thereby increasing the chance they get infected with other microparasites such as RVF. Future work will need to elucidate whether there is evidence for immunological interactions between these two pathogens in African buffalo and whether genetic profile plays a significant role. If altered immune response due to BTB increases an animal's susceptibility to other parasites, then evaluation of the effects of BTB in the ecosystem should not be limited to direct effects of the BTB itself but should include the indirect effects of BTB on host-native parasite interactions.

We used a mathematical model to show that individual changes in microparasite susceptibility due to BTB infection could increase the intensity of RVF epidemics in African buffalo. As the prevalence of BTB increased, the size of an RVF epidemic in buffalo increased, with more disease occurring not only in buffalo with BTB, but also in BTB negative buffalo. Increasing the transmission of RVF by a factor of 3.4 in BTB positive buffalo best replicated the pattern we saw in free-ranging and captive buffalo; where approximately twice as many BTB+ buffalo acquired RVF than BTB- buffalo. At this factor a BTB prevalence of only 20% increases outbreak size, a biologically relevant parameter as BTB prevalence range from 0% in the northern portion of the park where BTB has not yet fully invaded to 50% in the southern portion of the park where BTB first was found (Michel et al, 2006) As BTB invades into Kruger National Park, and moves north (de Garine-Wichatisky et al, 2010), RVF epidemics may worsen in severity in African Buffalo. Whether this increase in infected buffalo will increase the risk of outbreaks extending into humans, domestic livestock or free-ranging ruminants needs to be investigated. Not only did the number of buffalo infected in the outbreak increase, the time of the outbreak also lengthens unless the outbreak occurs at very high BTB prevalence with a large increase in transmission factor for BTB+ buffalo. Then RVF sweeps through the population at a quicker pace, shortening the outbreak time. However, in this model we considered only buffalo in a closed population, so the supply of other competent hosts may dampen this shortened effect in the ecosystem.

This study indicates that the effects of an emerging pathogen, such as BTB, are not limited to direct effects on the host, but may also alter the infection patterns of diseases previously existing within the ecosystem or host species. BTB increases the likelihood of an

animal acquiring RVF, worsens fitness effects in coinfected animals and increases the intensity and length of RVF outbreaks. This could have an impact on human, wildlife and livestock health throughout the region as BTB emerges. This study also emphasizes that researchers must consider both the host and disease community into which an emerging disease invades because the emerging disease may alter parasite-parasite interactions, resulting in altered parasite-native host interactions and change population level disease transmission patterns.

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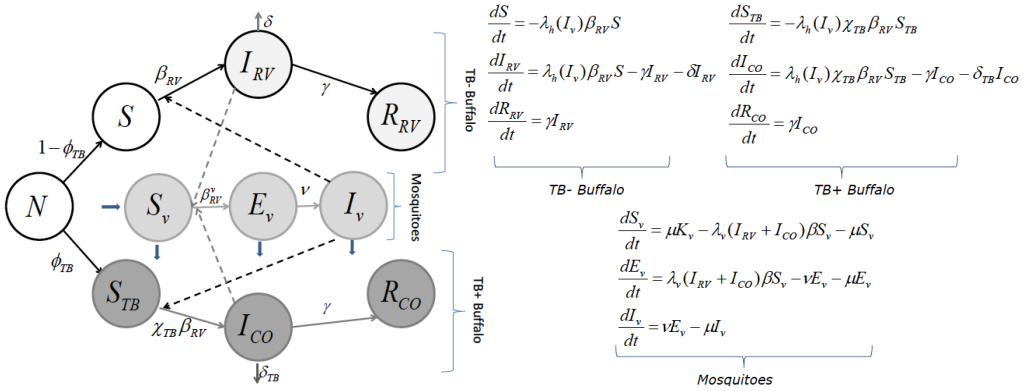


Figure 3.1: Diagram of RVF/BTB model and equations. Note that the transmission factor (beta) for BTB+ buffalo is multiplied by a constant (1.4, 2.4, 3.4 or 4.4) to represent the increase in transmission. A constant transmission increase factor of 3.4 best represented the approximately 2 times greater prevalence in BTB+ than BTB- we saw in our free-ranging and captive populations for the parameter values chosen.

Table 3.1: Age and sex patterns of RVF seroconversion of disease free buffalo during a natural outbreak.

	Number seroconverted	Total number tested	Percent Seroconverted
Adult Cows	40	124	32.26%
Adult Bulls	3	19	15.79%
Calves under 1 year	21	26	80.77%

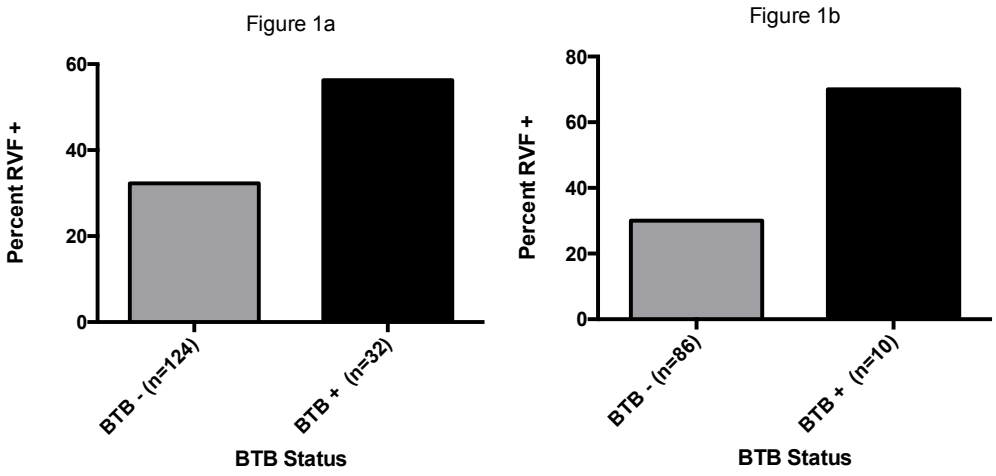
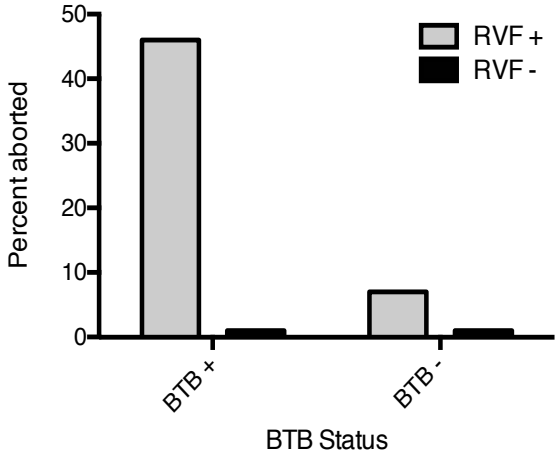


Figure 3.2: BTB + buffalo were more likely to acquire RVF infection (Fisher exact test, p=0.0147) during an outbreak in a buffalo breeding facility (Figure 1a) and are more likely to be seropositive (Fisher exact test, p=0.03) in a free-ranging herd (Figure 1b).

Table 3.2: A generalized linear model was performed to further evaluate the correlation between BTB status and RVF seropositivity in free-ranging African buffalo. Age, pregnancy status, and overall body condition did not alter the positive association between BTB and RVF.

	Estimate	SE	P value
Age	0.11	0.10	0.298
BTB Status (Positive)*	1.45	0.73	0.049*
Pregnancy Status (Yes)	0.13	0.71	0.856
Body Condition	0.03	0.36	0.927



	Mean Rank Difference	P value
BTB+/RVF+ vs. RVF only*	29.85	P<0.0001
BTB+/RVF+ vs. BTB only*	35.57	P<0.0001
BTB+/RVF+ vs. Uninfected*	35.57	P<0.0001
BTB only vs. uninfected	0	NS
RVF only vs. uninfected	5.724	NS
RVF only vs. BTB only	5.724	NS

Figure 3.3: Adult female buffalo with both BTB and RVF were more likely to abort than those singly infected with RVF or BTB (Kruskal Wallis ANOVA with Dunn multiple comparisons).

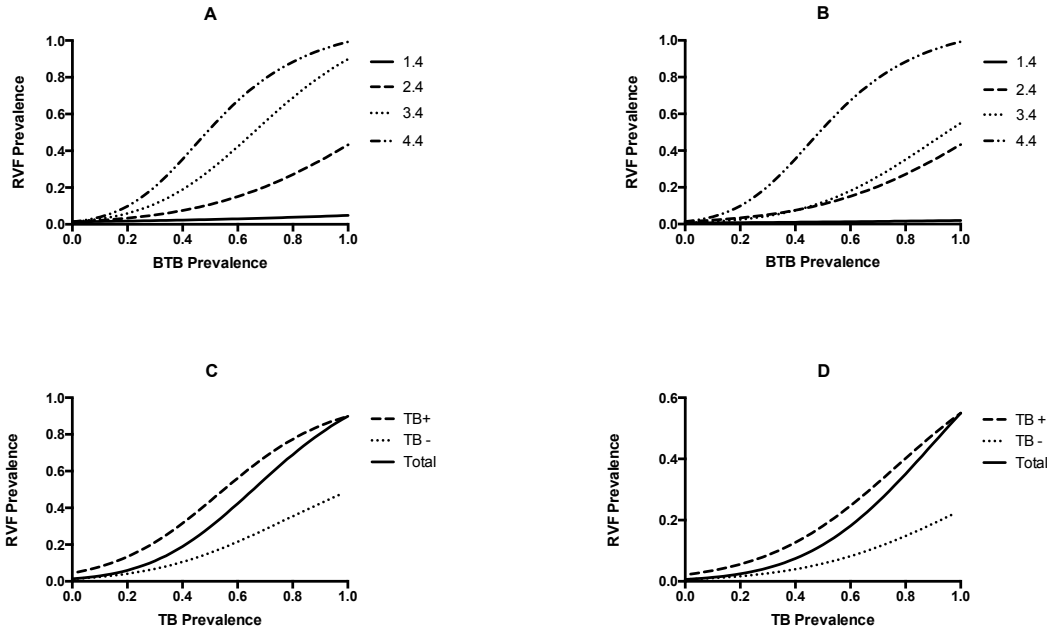


Figure 3.4: Panels A (no prior RVF immunity) and B (20% prior immunity to RVF) show that as BTB prevalence increases so does the total RVF outbreak size, but the extent of the increase depends on the factor by which transmission is increased due to BTB (transmission factors 1.4, 2.4, 3.4 and 4.4 are shown in the figure). Panels C (no prior RVF immunity) and D (20% prior RVF immunity) show that not only does total RVF outbreak size increase, but that the increase in infected animals occurs in both BTB+ and BTB- for an transmission factor of 3.4.

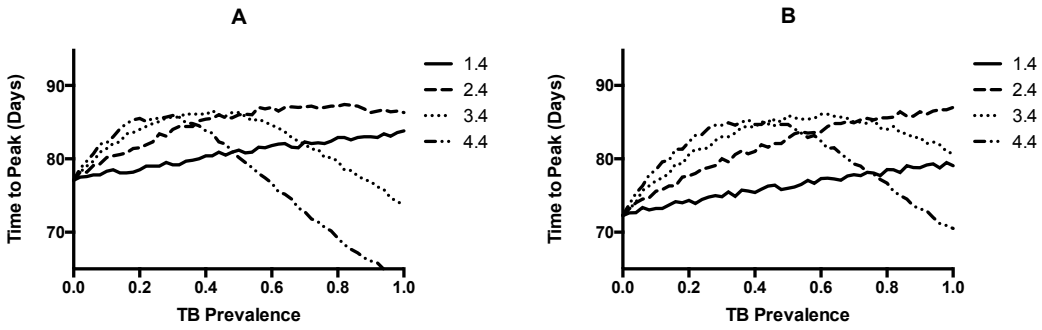


Figure 3.5: The presence of TB can cause an epidemic to last longer in populations with no immunity (A) and 20% immunity (B), for various transmission factors, unless the increase in susceptibility is so great the epidemic runs out of susceptible individuals.

4 – Innate immunity in free-ranging African buffalo (*Syncerus caffer*): associations with parasite infection and white blood cell counts.

Brianna Beechler, Heather Broughton, Austin Bell, Vanessa Ezenwa, Anna Jolles

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ABSTRACT

Mammalian immunology has been studied in great detail in laboratory animals, but few of the tools, and less of the insight derived from these studies have been placed in the context of natural, outbred wildlife populations subject to variable environments. We investigated patterns of innate immunity in free-ranging African buffalo in relation to host traits (age, reproductive status, body condition, white blood cell counts) and disease status (bovine tuberculosis (BTB), gastrointestinal nematodes, coccidia, ticks). We evaluated and used an in-vitro assay measuring bactericidal competence of blood to assess a component of innate immunity in 200 female buffalo captured at Kruger National Park, South Africa, in June/July and October 2008. Animals with BTB had higher bactericidal competence of blood. Animals with higher neutrophil counts had higher bactericidal competence, whereas animals with lower lymphocyte counts had higher bactericidal competence. This pattern was driven by animals captured at the end of the dry season (October) and may be evidence of immune polarization, whereby individuals are unable to up-regulate multiple components of immunity simultaneously. Bactericidal competence did not vary with host pregnancy status, body condition, age, lactation, tick infestation, nematode egg count, or coccidia oocyst count. Overall, we demonstrate that the bactericidal competence assay is practical and informative for field-based studies in wild bovids. Our results also show a correlation between bactericidal competence and bovine tuberculosis infection, and reveal possible functional polarizations between different types of immune response in a free-ranging mammal.

4.1 INTRODUCTION

Parasites and pathogens affect the fitness of most free-living organisms, and as such represent a major selection pressure on their hosts (Grenfell and Dobson 1995). As a result, hosts must protect themselves against parasitic exploitation by investing resources in immune defenses. Ecoimmunology investigates the costs of immunity, optimal strategies for effective immune defense in the face of multiple demands on host resources, and plasticity in these strategies due to variable host environments. Despite lively interest in this emerging field (Demas et al 2011; Martin et al 2011; Schulenberg et al 2009; Martin et al 2008; Fenton et al 2008; Martin et al 2006) there is still a lack of knowledge about variation in immunological capabilities in wild mammalian populations (Sadd and Schmid-Hempel 2009). Empirical studies in ecoimmunology have focused largely on birds (e.g see Wilcoxen et al 2010; Lee et al 2008; Friedl and Edler 2005; Alonso-Alvarez and Tella 2001; Grasman 2002) and invertebrates (e.g. Cremer and Sixt 2009; Moret & Siva-Jothy 2003; Moret & Schmid Hempel 2001), extending strong foundations in life history theory in these taxa. Transferring these approaches to free-living mammal species has only recently begun (for example see Ezenwa et al 2012; Jackson et al 2011; Graham 2010; Allen et al 2009).

The immune response is a complex, interdependent web of factors often divided into innate and acquired arms of immunity. Innate immunity provides an immediate initial line of defense against invading pathogens, and directs subsequent acquired immune responses (Tizard 2004). Innate immunity is thought to be developmentally less costly than adaptive immunity, but its up-regulation carries substantial energetic and immunopathological costs (Martin et al 2008; Klasing 2004). Innate immunity is mediated by a number of serological

and cytological effector mechanisms. The main serological components are complement mediated killing, natural antibodies, antimicrobial peptides, soluble acute phase proteins and lysozyme activity; whereas cytological components include neutrophil, macrophage, and natural killer cell mediated killing of pathogens (Tizard 2004). Previous studies have looked at patterns of innate immunity in free-ranging wildlife, predominantly in birds, using a variety of assays including the bactericidal competence assay (reviewed in Boughton et al 2011; Demas et al 2011). Several studies have demonstrated individual variation in bactericidal capability related to environmental parameters (Allen et al 2009; Martin et al 2008). For instance, bats in caves had decreased bactericidal ability compared to bats roosting under bridges (Allen et al 2009), while body condition had no effect on bactericidal competence. Although innate immune variation is often associated with environmental and demographic variables, genetic variation has also been shown to be an important predictor of innate immune capability (Ardia et al 2011; Wilcoxen et al 2010; Forsman et al 2009). Several studies have also noted seasonal patterns in innate immune capability in a range of species (reviewed in Martin et al 2008). Other studies have found interspecific variation in bactericidal ability (Millet et al 2007; Martin et al 2007; Matson et al 2006), sometimes along a life history gradient (Tieleman et al 2005).

Here, we build on this information, using the bactericidal competence assay to evaluate the degree to which host traits and infection status are correlated with innate immune function in a free-ranging bovid population. Before assessing the ecological questions we first evaluated the bactericidal assay for use in African buffalo. We asked, first, how bactericidal competence of blood and plasma compare since plasma contains only serological effectors of the innate immune response, whereas whole blood contains both

cellular and serological components; second, whether there is evidence for a dose-dependent bactericidal effect of buffalo blood and plasma; and third, whether individual buffalo varied in innate immunity as measured by the assay. The assay was adapted for field-based use in birds by Tieleman et al (2005), and has subsequently been used by numerous other field studies of birds (e.g. Forsman et al 2008; Hasselquist 2007; Millet et al 2007; Matson et al 2006) and mammals (e.g. Allen et al 2009) and is reviewed in Demas et al (2011) and Boughton et al (2011). The in-vitro bactericidal assay trialed here measures the ability of whole blood (i.e. serological and cytological innate effectors) or plasma (i.e. serological components only) to kill bacterial cells. As such, the assay provides an easily interpreted functional measure of an innate immune response to a specified bacterial pathogen; hosts with high in-vitro bactericidal ability should be better able to limit infection by the microorganisms used in the assay.

Using the bactericidal competence assay, we investigated patterns of innate immunity in relation to host traits (age, reproductive status, body condition) and disease status (bovine tuberculosis (BTB), gastrointestinal nematodes, coccidia, ticks). The importance of connecting disease profiles to immune profiles has recently been emphasized in the literature (Hawley and Altizer 2011), but data from free-living host populations are sparse. We also looked for evidence of within-immune system polarization, comparing lymphocyte counts to bactericidal competence. Lymphocytes are a white blood cell that functions within the adaptive immune system, helping to mount antibody mediated (B cell) and cell mediated (T cell) responses (Tizard, 2004). Because hosts subject to variable environments may exhibit plasticity in their allocation to different immune components – e.g. innate vs. adaptive, constitutive vs. inducible – according to their costs and effectiveness

in combating different infectious challenges (Martin et al 2008; Bradley and Jackson 2008; Fenton et al 2008; Houston et al 2007; Klasing 2004; Lochmiller and Deerenberg 2000), we expected that hosts with limited resources may allocate their resources to different forms of immune response.

4.2 METHODS

Study Site and population:

Kruger National Park (KNP) is located in northeastern South Africa and comprises almost 19,000 km^2, with a buffalo population of approximately 30,000 animals (Cross et al 2009). Adult female African buffalo (*Syncerus caffer)* were captured in the southern portion of KNP, as part of a larger study on parasite interactions in free-ranging buffalo. Young (age 2-5yrs) female buffalo were targeted for capture because of the needs of the larger study. The first 100 buffalo were captured at the end of the wet season between June 23 and July 5 2008 (Lower Sabie herd). The second 100 buffalo were captured at the end of the dry season between October 1 and October 8 2008 (Crocodile Bridge herd). Animals were chemically immobilized with M99 (etorphine hydrochloride) and ketamine by darting from a helicopter. Following data collection, immobilization was reversed using M5050 (diprenorphine). All immobilizations were performed by South African National Parks (SANParks) veterinarians and game capture staff; and all procedures were approved by Oregon State University, University of Montana, and SANParks Institutional Animal Care and Use Committees (IACUC).

Demographic parameter assessment:

After immobilization, demographic data were collected including: age, body condition, pregnancy and lactation status. Pregnancy was assessed by rectal palpation, which

has a nearly 100% sensitivity rate after 51 days of gestation in Egyptian buffalo (*Bos bubalis*; Aly et al 2011), while lactation was evaluated via manual milking of all 4 teats (Jolles et al 2005). Age was assessed from incisor emergence patterns for buffalo 2-5 years old animals, and from tooth wear of incisor one for buffalo 6 years and older (Jolles 2007). Body condition was measured by visually inspecting and palpating four areas on the animal where fat is stored in buffalo: ribs, spine, hips and base of tail. Each area was scored from 1 (very poor) to 5 (excellent) and a body condition score calculated as the average of all four areas. This index is correlated with the kidney fat index (Ezenwa et al 2009), and similar body condition indices have been used in other studies of African buffalo (Caron et al 2003; Prins 1996).

Sample collection

Blood was taken from each animal via jugular venipuncture between 30-60 minutes after darting and collected into sterile EDTA, and lithium heparinized tubes for white blood cell counts and differentials, and BTB diagnostics and bactericidal competence assays, respectively. All samples were placed on ice within 5 minutes of collection in a cooler for transportation back to the laboratory. Plasma was collected from heparinized tubes after centrifugation for 10 minutes at 5000g to ensure the separation of cytological components. Plasma (100ul) was pipetted from the top of the tube for use in the bactericidal assay. Fecal samples were collected rectally from each animal and placed on ice within 5 minutes for transport back to the laboratory. Total time between sample collection and sample testing or storage in appropriate conditions was never greater than 8 hours, and typically ranged between 4-6 hours.

Infection status assessment

Fecal egg and oocyst counts were used to assess gastrointestinal nematode and coccidia prevalence and load. Counts were performed using a modified McMaster method (Ezenwa 2003), and all fecal samples were processed within 8 hours of collection. In livestock, nematode fecal egg counts can reflect both the number of worms in the host and the fecundity of those worms (Cabaret et al 1998; Stear et al 1994), however this has not been investigated in African buffalo so we cautiously refer only to fecal egg counts when discussing the results pertaining to worms. Tuberculosis infection status was determined using a standard whole blood gamma interferon assay protocol (Schiller et al. 2009; Wood and Jones 2001). . In brief, this assay is performed by comparing the *in vitro* IFN γ esponse to *M. bovis* antigen (bovine tuberculin) to the IFN γ response to an avian tuberculin antigen and background IFN γ levels in the absence of antigenic stimulation. This assay has been optimized for use in African buffalo (Michel et al 2011; Grobler et al. 2002), and blood cells from buffalo infected with *M. bovis* show a pronounced spike in IFN γ production in response to bovine, but not avian tuberculin; whereas bovine tuberculin challenge does not induce IFN γ production in the blood of unexposed animals (Michel et al 2011). We implemented the gamma interferon assay with the BOVIGAM enzyme linked immunosorbent assay kit (Prionics, Switzerland), which has a sensitivity of 86% and a specificity of 92% in African buffalo (Michel et al 2011). Tick burden was assessed by counting the number of adult and nymphal ticks located in three body areas where tick density is highest in buffalo - inguinal, axillary and perianal regions (Anderson et al. unpublished data).

White Blood Cell Counts

White blood cell counts were performed on whole blood on the day of capture, within 8 hours of blood collection, using an automated impedance cell counter (Model ABC-VET) to determine the number of white blood cells, red blood cells and platelets in each sample. Differential counts of white blood cells to determine the fractions of each type of white blood cell (eosinophils, neutrophils, basophils, monocytes and lymphocytes) were performed manually from a blood smear by a single trained observer. All counts are reported as cells/ml.

Bactericidal Competence Assay

We tested bactericidal competence of whole blood (June and October) and plasma (June only). In all cases, testing was done on the day of sample collection and within 8 hours. Following methods similar to those outlined in Matson et al. (2006), we diluted whole blood (5 μL, 10 μL, and 20 μL) or plasma samples (5 μL, 10 μL, and 20 μL) from each individual in a broth containing CO2-independent media (Gibco-Invitrogen, Carlsbad, CA), 4mM L-glutamine and 5% heat-inactivated fetal calf serum for a total volume of 220 μL. No components of the media contained any mediators that result in death of bacteria. A 20 μL aliquot containing 600 colony-forming units (CFUs) of non-pathogenic *E. coli* (ATCC 8739) was added to each diluted plasma and blood sample. The bacterial culture was prepared from lyophilized pellets (3.1×10^7 CFUs per pellet; Epower Microorganisms no. 0483E7, MicroBioLogics, St. Cloud, MN), which were reconstituted according to the manufacturer's instructions. The *E. coli* strain we used is a laboratory strain that the buffalo have not encountered in the wild, therefore previous exposure is unlikely and acquired immunity should not play a significant role in killing the bacteria.

The resulting mixtures of bacteria, media and diluted whole blood or plasma (220 μL total) were vortexed for three seconds and incubated at room temperature for 30 min. This period allowed for the bacteria and blood components to interact. Samples were again briefly vortexed to establish a uniform mixture of the suspension. Following this, 75 μL aliquots were pipetted onto tryptic soy agar (TSA) plates and the mixture was spread uniformly over the surface of the agar. All samples were plated in duplicate. In addition, each day, five controls were plated containing only bacteria and broth on TSA, from which we established reference numbers of colonies for the corresponding samples. Plates were allowed to dry for a period of 20 min, after which they were inverted and placed in an incubator at 37ºC. After 24 hrs, the number of viable colonies was counted and plate quality was assessed by checking for contamination, desiccation, or poor spreading of sample.

Statistical Analysis

Only plates with the quality rating of good (i.e. not desiccated, contaminated or poorly spread) were included in analyses. The proportion of colonies killed was calculated as:

$$\frac{\mu(\text{\# colonies on control plates}) - \mu(\text{\# colonies on the experimental plates})}{\mu\ (\text{\# of colonies on control plates})}$$

The mean proportion of colonies killed was calculated from replicate plates. The average number of colonies on the control plates was used as an offset term to account for daily variation in control colony number, since the number of bacterial colonies added to samples was not consistent from one day to the next. Ratio data was arcsine transformed, but for ease of visualization graphs are presented with the original ratios. To evaluate the differences between sample types (plasma versus blood) paired t-tests with a Bonferroni

correction were performed between similar concentrations of plasma and blood. In addition, we assessed the pairs for equal variance using a Levene's test. To assess whether increasing concentrations of blood and plasma had an effect on the proportion of colonies killed a one-way ANOVA was performed. To assess whether bactericidal competence of whole blood correlated with environmental, demographic, or disease parameters, a generalized linear model using a quasipoisson distribution and log link function was performed with the untransformed data. The quasipoisson distribution is appropriate for independent variables representing a difference in counts, when those counts are overdispersed as was the case here.

We included sample processing day in the model to statistically account for daily variation such as laboratory conditions and time to sample processing. Our full model included the following main effects: day, season / herd, age, condition, pregnancy, lactation, BTB status, fecal egg count (worms), fecal oocyst count (coccidia), tick burden, neutrophil count/ml, lymphocyte count/ml, eosinophil count/ml, monocyte count/ml, as well as all two-way interaction terms with season (e.g. season*age, season*condition, etc) and age (age*condition, age*pregnancy, etc). Backward selection was performed by eliminating any variable that did not cause a drop in deviance of greater than 5% from the full model to the reduced model. This method is similar to using Akaike's Information Criteria (AIC), but a drop in deviance criteria is a more appropriate method for overdispersed count data than AIC.

4.3 RESULTS

Assay Optimization: Sample type Comparison

For a given sample volume, bactericidal competence of plasma was higher than that of whole blood (Figure 1) for June captures (n=90). However, when exploring our data for associations between host traits and innate immunity, we found that variability in blood bactericidal competence, but not plasma bactericidal competence was explained by some of the host traits we measured (Table 1 for blood; no significant associations for plasma). Plasma bactericidal competence was thus less informative than bactericidal competence of whole blood in the context of the host traits examined here. This difference between sample types was not due to higher variance in blood bactericidal competence overall, as compared to plasma (Levene's test, $F=2.191$, $p=0.14$). In our October sampling we therefore used whole blood samples only; and below we present results for whole blood bactericidal competence rather than plasma.

Assay Optimization: Dose dependence of bactericidal blood activity

The proportion of colonies killed by buffalo blood was dose-dependent: the more blood added to the bacterial broth, the fewer colonies were able to establish (one-way ANOVA: $n=186$; $F=2346.189$, $p<0.0001$; Figure 2). Samples containing 20 µl of blood showed very low bacterial growth, with an average of 76.3% of colonies killed compared to control samples containing no blood, limiting variability in bactericidal competence between individual buffalo. This reduced variability made the 20 µl concentration less informative for evaluating demographic and disease patterns. Samples containing 5 µl of blood resulted in highly variable bactericidal competence, with many sample plates not differing from, or

even exceeding control plates in bacterial growth. Samples treated with 10 μl of blood showed intermediate variability in bactericidal competence and when we tested initial associations between bactericidal ability and host traits we found the 10μl concentration to be the most informative for detecting demographic and disease patterns. Thus, for all further analyses, the 10 μl blood volume was used as the response variable.

Host traits and disease status as predictors of bactericidal competence

We assessed whether host age, reproductive status (pregnancy and lactation), body condition, white blood cell counts, and disease status (GI nematodes, coccidia, TB status, tick burden) were correlated with bactericidal competence (see Table 2 for summary data on the dependent variables). For all analyses we controlled for capture period (June / July – Lower Sabie herd or October – Crocodile Bridge herd) and day. Capture period had a significant effect on bactericidal competence; with competence being higher in the October samples than the June / July samples (Table 1). We also detected day-to-day variation in bactericidal competence (ANOVA, $F=7.88$, $p=<0.0001$). This day-to-day variation was due primarily to variation in the number of colony forming units per pellet and was accounted for statistically in all further analyses (see appendix).

Of the host traits and infection parameters examined, tuberculosis status, neutrophils and lymphocytes showed significant associations with innate immunity as measured by bactericidal competence (Table 1). Animals with BTB tended to have higher bactericidal blood competence. Animals with higher neutrophil counts had higher blood bactericidal competence, but we detected a negative correlation between lymphocyte counts and blood bactericidal competence driven by the October sampling of animals (Figure 3 and interaction effects in Table 1). Lactation, age, body condition, nematode fecal egg counts, coccidia

oocyte counts, tick burden, reproductive status, total white blood cell counts, basophils, eosinophils, and monocytes did not predict blood bactericidal competence.

4.4 DISCUSSION

Assay Optimization: Sample type Comparison

Plasma was more effective at killing bacteria than the same amount of whole blood suggesting that the serological components of the innate response are important in the killing response. We attribute the difference in bactericidal competence between blood and plasma to a greater concentration of immunologically active components (such as complement) in plasma as compared to whole blood, which contains numerous non-effector cells such as red blood cells (Tizard 2004). This is similar to the findings of Matson et al (2006), where the killing ability of 20 μl of blood was equivalent to 10 μl plasma. An alternative explanation, is that the strain of *E. coli* used (ATCC 8739) is more susceptible to the serological components than the cytological components, since typically bacterial killing of the ATCC 8739 strain is considered complement dependent (Millet et al 2007). Despite the increased killing ability of plasma, no host traits or infection variables accounted for the observed variation in percentage of bacteria killed, whereas some of these variables were predictive of whole blood bactericidal competence. One could argue that this is confounded by capture period, such that all correlations observed between whole blood and bactericidal ability were driven by the October captures, and the lack of associations for plasma could be due to the fact that these samples were collected in the June/July capture session. However, although the white blood cell correlations with bactericidal competence were driven by the October captures, the correlation between BTB and whole blood bactericidal ability was present for both capture periods. Previous studies have used whole blood (e.g. Buehler et al 2008;

Millet et al 2007; Tieleman et al 2005) or plasma (Forsman et al 2008), but only Matson et al (2006) evaluated the use of plasma and blood in the same study. In contrast to our results, Matson et al (2006) found that both plasma and blood varied in the same direction with their parameters; however, they were comparing bactericidal ability among species rather than among individuals of a single species as in our study.

One limitation of the data presented here is that they are cross-sectional. We are thus unable to tease apart variation among individuals due to their current circumstances (e.g. reproductive status, condition, infections, the season when they were captured, etc), as opposed to variation due to individual quality *per se*. It is possible that individual buffalo maintain relatively constant levels of complement and other proteins involved in plasma bactericidal activity, but that each buffalo has a different set point for these relatively inexpensive defense compounds. By contrast, buffalo may regulate cytological components of innate immunity, which are more costly energetically and in terms of immunopathology (Buehler et al 2008; Tizard 2004; Klasing 2004), according to available resources and concurrent energy demands. Longitudinal and genetic data on innate immunity in buffalo would help clarify why we are observing this difference between blood and plasma bactericidal competence. It would also be helpful to use different pathogens, in addition to *E. coli,* which may preferentially be targeted by different components of the innate immune response (serological vs. cytological), to further explore potential reasons for observed differences between blood and plasma bactericidal competence in buffalo.

Host traits and disease status as predictors of bactericidal competence

Capture period, tuberculosis status, neutrophils, and lymphocytes showed significant associations with innate host immunity in our study population of free-ranging African

buffalo. Capture period was strongly correlated with bactericidal competence; with animals in the October capture having higher competence than those in the June/July capture. Unfortunately this was confounded by geographical location of capture, as we captured in the Lower Sabie herd in June/July and the Crocodile Bridge herd in October. We are thus unable to distinguish whether this variability in BKA is attributable to seasonal shifts in immunity, or to differences in immunity between the two buffalo herds, (e.g. based on resource availability, parasite exposure or genetic background). For example, there is a striking difference in infection profile (see Table 2) between the two seasons, with tick burden and nematode egg count being more prevalent in the October capture period (Crocodile Bridge herd), although neither tick burden nor nematode egg count correlated with bactericidal ability in this study. Tuberculosis status was weakly and positively correlated with bactericidal competence, such that those animals that were BTB positive had increased bactericidal competence. There is evidence that animals with acute BTB infection have up-regulated immune responses, which may include innate immune responsiveness (Pollock et al 2006). By contrast, chronically infected animals would be expected to suffer immune-suppression, resulting in anergy (Cross et al 2009; Raja 2004). Most buffalo acquire BTB infection between the ages of 2-5 (Cross et al 2009), and the majority of our buffalo are young, with the range of BTB infected animals ranging from 1 year to 11 years of age and a median of 4 years of age (entire population ranges are reported in table 2), so most of the BTB infections in these animals are recent. Concordantly the immune system stimulation caused by BTB infection may result in an up-regulated bactericidal competence in the BTB positive animals in this study.

There was a positive correlation between neutrophil count and bactericidal competence of whole blood. Neutrophils are one of the main effector cells of bacterial killing ability of whole blood, the other main cellular component being macrophages (Tizard, 2004). The importance of the cytological component of blood in bactericidal competence has been demonstrated in several species including mice (Hanski et al 1991) and rats (Davies et al 1981). Neutrophils had a stronger correlation with bactericidal competence in the October capture period, than in the June/July capture period as evidenced by the interaction term between neutrophil count and the season of capture (Table1). Bactericidal competence was higher overall in animals captured in October than in July, perhaps increasing detectability of the effects of individual immune components on bactericidal competence. Alternatively, seasonal and / or herd variability in immune functioning may be causing the observed difference in the role of neutrophils in bactericidal activity.

There was a negative association between lymphocyte count and bactericidal competence of whole blood, suggesting possible immune polarization, with individual animals that have high bactericidal competence having lower lymphocyte counts. Lymphocytes are typically associated with an acquired immune response, whereas bactericidal competence is primarily mediated by an innate immune response. Our data are thus suggestive of polarization between innate and acquired immune responses; but additional functional measures of the acquired and innate response are needed to explore this concept.

Polarizations within the immune system can be caused by genetic or environmental factors, can be inherited (genetic or epigenetic) or functional (Ardia et al 2011, Betelli et al

2007; Graham et al 2005). There are no published accounts of polarization between bactericidal and any other immune competence measure in mammals, however other types of within-immune system polarizations have been published. For example, many studies in mammals have found evidence suggesting polarization between two types of lymphocytes of the acquired immune system (TH1 vs. TH2) (Ezenwa et al 2011; Graham 2008; Jolles et al 2008; Morel and Oriss, 1998). Animals tend to be able to mount an effective TH1 or TH2 response, but not both simultaneously, because messenger molecules that up-regulate TH1 immunity simultaneously down-regulate TH2 pathways, and vice versa (Mosmann and Sadd 1996; Abbas et al, 1996). In invertebrates, polarizations between bactericidal ability and other immune responses have been documented. For instance, in a study of *Trichoplusia ni* larvae there was polarization between bactericidal competence of hemolymph and hemocyte (a phagoytic immune cell) numbers (Freitak et al, 2007), however whether this was due to a functional polarization or energetic constraints is unknown. Similarly there are published accounts of polarizations between phenyloxidase activity and bactericidal competence in bumblebees (Moret & Siva-Jothy, 2003). Once again, however, the mechanism behind this polarization is unclear.

The polarization we observed between lymphocyte count and bactericidal ability was driven by the animals captured in October; if we examine each capture period separately, polarization is detectable in the October sample, but not the June/July sample (Figure 3). Our October 2008 capture fell towards the end of the dry season in Kruger National Park, when food resources for herbivores are sparse and grazers are likely to have used up much of their fat reserves (Bengis et al 2006; duToit 2003;). Accordingly, the buffalo we sampled in October were in much worse body condition than those sampled in

June / July (mean body condition, Oct = 2.9; June/July = 4.2; 2-tailed t-test, $p<0.001$). Perhaps buffalo under severe resource limitation are unable to maintain high bactericidal competence and high lymphocyte numbers simultaneously. Different types of immune response incur different energetic costs, pathological costs, and have differential spectrums of effect (Klasing, 2004) so animals under resource restriction may respond to an immunological challenge differently than animals not under resource restriction. Indeed, there is accumulating evidence that the degree of TH1-TH2 polarization in mammals, for example, may vary with resource levels (Long and Nanthakumar 2004; Jolles et al. 2008; Ezenwa and Jolles, 2011). Alternatively, pathogen exposure (Hawley and Altizer 2010) or genetic differences (Ardia et al 2011) between buffalo from the Crocodile Bridge and Lower Sabie herds may be responsible for this immune polarization being detectable in one group but not the other. Longitudinal data on both groups of animals will be needed to clarify how herd membership and / or season relate to immune polarization. Nevertheless, this study reveals a striking negative association between lymphocyte count and bactericidal competence in a wild mammal population, which is suggestive of immune polarization. We also found evidence for modulation of innate immunity by concurrent BTB infection. Future work, focusing on longitudinal patterns of immunity holds promise for disentangling host and environmental factors driving the immune variability we observed in this study system.

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Table 4.1: Effect of demographic, environmental and disease variables on bactericidal capability of blood. * indicates $p < 0.05$.

Variable	Estimate	F value	Standard Error	p-value
Day	-0.148	-3.537	.042	0.0005*
Capture period (July)	-1.523	-4.017	0.380	<0.0001*
Tuberculosis (Positive)	0.286	2.003	0.143	0.047*
Neutrophil count	0.152	2.464	0.062	0.015*
Lymphocyte count and Capture Period Interaction	-0.117	-2.129	0.055	0.035*
Lymphocyte count and Capture Period Interaction	0.148	1.722	0.086	0.087
Neutrophil count and Capture Period Interaction	-0.349	-1.942	0.180	0.054

Table 4.2: Summary information on 200 buffalo broken down by herd.

	Lower Sabie (June/July capture period)	Crocodile Bridge (October capture period)
Pregnancy Status	50.7% pregnant	63.1% pregnant
Lactation	20% lactating	3% lactating
Age (in years)	Median: 2, Mean: 3.7	Median: 4.7, Mean:4
Body Condition	Mean of 4.2	Mean of 2.9
Nematode egg count	30% infected with a mean burden of 2.07 eggs/gram	69% infected with a mean burden of 4.44 eggs/gram
Tick burden	100% infected with a mean of 136 ticks per individual	100% infected with a mean of 774 ticks per individual
Coccidia oocyst count	27% infected with a mean of 137 OPG	44% infected with a mean of 133 OPG.
BTB Status	10% infected	14% infected

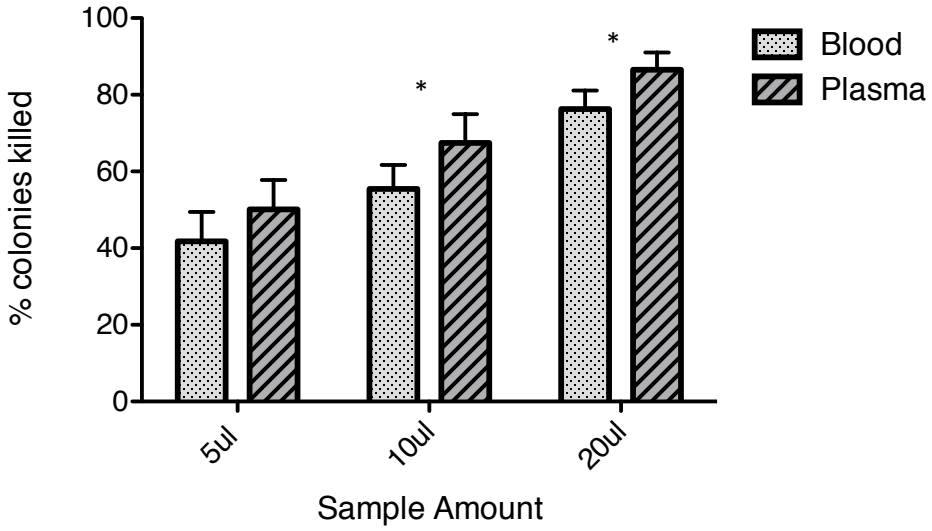

Figure 4.1: Mean percentage of colonies killed with standard error by sample type and concentration for June captures only (n=90). Blood concentration ("blood 5", "blood 10", "blood 20") is given according to the volume of blood (5, 10 or 20 µl , respectively) contained in each 220 µl of bacterial broth. Significant differences were seen between blood 10 and plasma 10 (paired t-test: t =2.54, p=0.0119,); blood 20 and plasma 20(t = 2.79, p=0.0059, t-test), but not between blood 5 and plasma 5 (p=0.119, t-test). Asterisks indicate significant differences.

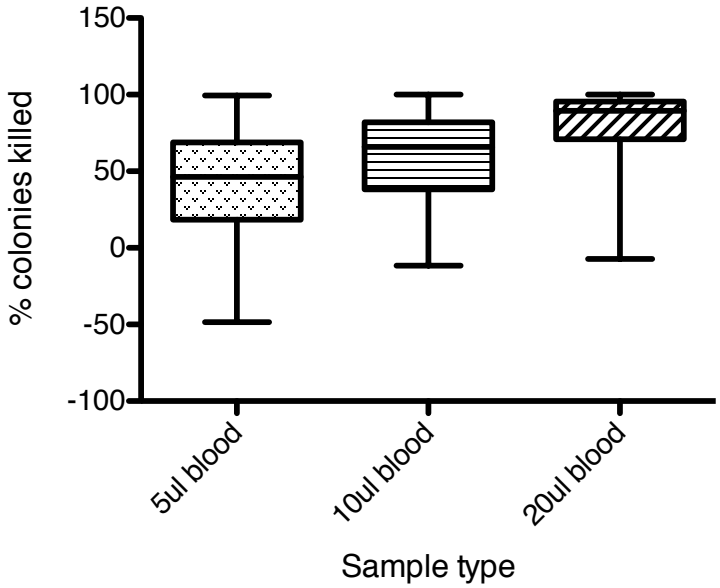

Figure 4.2: A box plot showing dose dependence in bactericidal activity of buffalo blood. Means are denoted with a solid line, the box shows the 75th percentile of data points, and the range is denoted with the lines extending from the box. The percentage of bacteria colonies killed increased with blood concentration. Blood concentration ("blood 5", "blood 10", "blood 20") is given according to the volume of blood (5, 10 or 20 µl , respectively) contained in each 220 µl of bacterial broth.

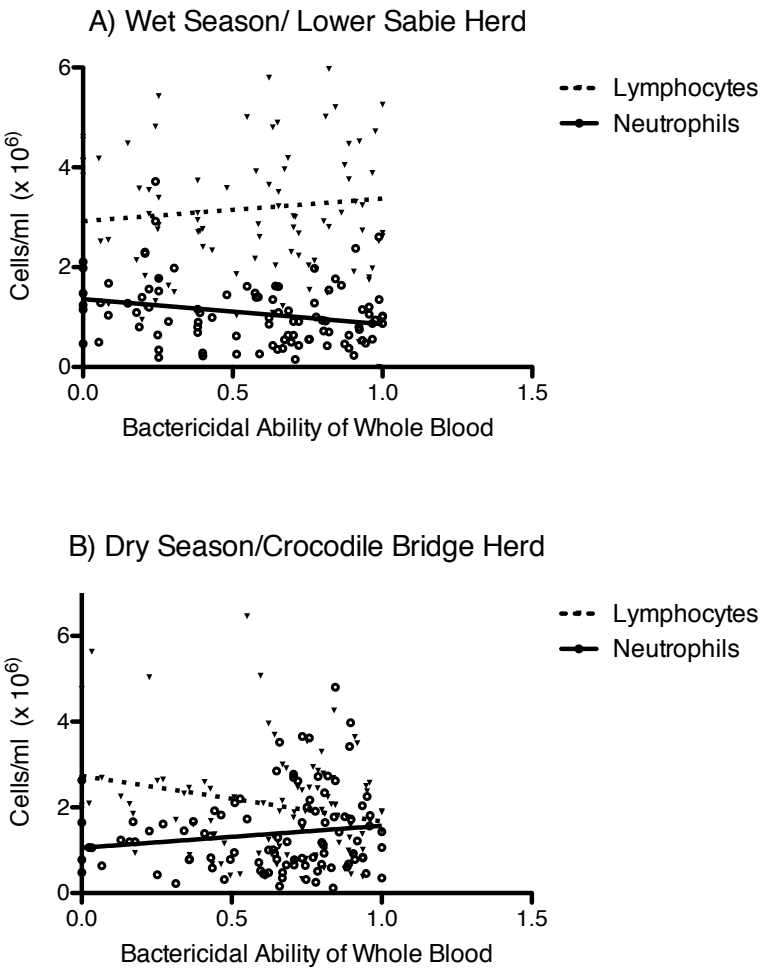

Figure 4.3: Correlation between lymphocytes/ml, neutrophils/ml and bactericidal ability (% colonies killed) by season/herd. Figure A shows that there was no significant correlations between lymphocytes or neutrophils and bactericidal ablity (BKA) in the June/July Capture period. However, figure B shows that lymphocytes were negatively correlated with BKA and neutrophils positively in the October capture period.

4.5 APPENDIX

We recaptured 57 of the female buffalo in the Lower Sabie herd in Kruger National Park between June and July of 2009, to investigate (and subsequently mitigate) the causes of the high day to day variation observed in our 2008 samples. We hypothesized that some of the day-to-day variability in bacterial growth may be due to different numbers of colony-forming units present in each *E. coli* pellet (the pellets are certified to contain 1.1-9.9 x 10^7 CFU of E. coli)

We therefore performed a the bactericidal assay using E. coli colonies maintained in the laboratory, instead of using a new E. coli pellet each day. To prepare the actively growing E. coli colonies, we reconstituted the pellet as above and plated 75 μl of bacteria broth on TSA plate, then incubated for 24h at 37 C. These cultures were used to create TSA slant cultures of bacteria that were maintained in the refrigerator. The day before an experiment was set to be run, refrigerated colonies of the two bacterial populations were used to inoculate individual fresh TSA plates and allowed to incubate overnight for use in the following day's bactericidal assay. Refrigerated colonies on slants were replaced weekly. In order to perform the assay, all bacterial dilutions were then created from the incubated plate using the BD BBL Prompt Inoculation System (Cat# 226306) and following manufacturer instructions, which is utilized in bacteriology laboratories to acquire a constant number of colony forming units per ml. We mixed 100 colony forming units of *E. coli* with the buffalo blood and plated as described above. Data were analyzed the same as the 2008 data.

The samples collected in the summer of 2009 with the modified BKA protocol showed no day to day variation (ANOVA, $F=0.869$, $p=0.61$) in the number of colonies on the control plates, or in bactericidal activity of buffalo blood, supporting our hypothesis that variability in the concentration of colony-forming units between lyophilized *E. coli* pellets was a causal factor. For our 2008 samples our best option remains to control for day-to-day variation in bacterial growth and BKA statistically, by including the number of colonies on control plates, and day as variables in our statistical models. The 2009 experiments serve to explain this unwanted variability, and have allowed us to modify our protocol in our subsequent work.

5 – The effect of Bovine Tuberculosis on host defenses against intracellular microparasitic diseases in African buffalo

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Functional Ecology
In Prep

ABSTRACT

Most free-living animals are infected with multiple parasites that can interact within the host to alter disease progression and transmission. Parasites can interact directly by competition for resources or indirectly via the immune system. It has recently been suggested that bovine tuberculosis may alter the host immune system in bovids, reducing its ability to react to an invading pathogen (Piron et al, 2013). We looked for evidence of alterations to immunity due to bovine tuberculosis (BTB) infection in a population of free-living African buffalo that were being monitored every 6 months in Kruger National Park. We found that BTB had strong effects on the immune system of the host, and that these effects were detectable in population level patterns. Buffalo recently infected with BTB had upregulated IL12 production in response to invitro stimulation with two mitogens (a general mitogen and a disease specific mitogen) and had increased lymphocyte production in response to in vitro stimulation. We also found that animals with BTB had reduced innate immune capability as measured by bactericidal ability of whole blood. This suppressed innate immune response may result in BTB increasing individual host susceptibility to another pathogen. This study demonstrates a need for a better understanding of immune mediated disease interactions and the role they may play in disease invasion.

INTRODUCTION

The unprecedented increase of emerging infectious diseases in conjunction with anthropogenically induced range shifts of endemic pathogens exposes hosts to novel parasite combinations, lending urgency to research on disease dynamics in wildlife systems (Jones et al, 2008). Most free-ranging animals are simultaneously infected with multiple species of parasites. These parasites can interact within a host in a competitive manner, inhibiting each other's survival, or a facilitative manner, promoting each other's survival (Pederson & Fenton, 2006). Understanding the disease dynamics of each parasite requires that researchers consider the impacts of other infections in the system. One of the main ways in which parasites can interact is via the host immune system (Graham, 2008). If the immune response mounted to one parasite is cross protective to another, then infection with one parasite can prevent the other from establishing. In contrast, immune responses may be mutually antagonistic (Bordes & Morand, 2009) where an immune response to one type of parasite may allow infection of another parasite by preventing an appropriate immune response (Fenton & Graham, 2008), creating a facilitative effect of one parasite species on another.

We sought to investigate the role of immune interactions in mediating infection dynamics in natural populations using two, ecologically important diseases, Rift Valley fever and Bovine tuberculosis in African buffalo. Previously we found that animals with bovine tuberculosis are twice as likely to acquire Rift Valley fever as animals without bovine tuberculosis in natural outbreaks (Beechler et al, 2013). We propose that one hypothesis to explain this pattern is an immune mediated interaction whereby animals with

BTB are less able to mount and appropriate immune response. In cattle and buffalo, BTB is a chronic disease (Swanepoel, 2004, DeVos et al, 2001) that modifies the immune system of hosts in dynamic ways to ensure its survival within the host (Waters et al, 2011; Pollock, 2006). Initially animals mount a strong immune response (Beechler et al, 2012; Thacker et al, 2007) that may be cross protective against RVF (and other intracellular microparasites), but later in infection BTB causes an altered immune response that may have a facilitative effect on RVF infection (Piron et al, 2005).

Rift Valley fever (RVF) is an intracellular microparasitic viral disease that requires the host to mount a strong innate immune response to protect against infection (Biron, 1999; Flick and Bouloy, 2005, Pepin et al, 2010). Cattle initially infected with BTB mount a strong innate immune response to fight infection (Pirson et al, 2013, Vordermeier et al, 2012) but this innate immune response may be altered or suppressed later in infection (Pirson et al, 2013).

In addition to an altered innate immune response, BTB affects the cell-mediated acquired immune system. Animals mount a T helper cell 1 (Th1) IFNy type response within the first month of BTB infection (Pollock et al, 2006), which may be cross protective against RVF, but later in infection cattle have a suppressed TH1 response with a skew to a Th0 profile (Jiao et al, 2003; Welsh et al, 2005; Pollock et al, 2006) that may have a facilitative effect on RVF infection.

We used a longitudinal study of free-ranging African buffalo to understand how BTB infection alters the host's ability to mount an immune response to another intracellular microparasitic pathogen, such as RVF. We hypothesized that animals with BTB would initially show increased immune response in measured parameters (table 5.1) relevant to

intracellular microparasites, followed by suppressed immune response in the measured parameters later in the course of an infection. We use 3 measures of immunity relevant to intracellular microparasite defense to investigate how BTB alters the acquired immune response (IL12 production and lymphocyte proliferation) and the innate immune response (IL12 production and bactericidal ability of whole blood). We first investigated how the host's ability to mount an IL12 response to a nonspecific mitogen (pokeweed) and a specific mitogen (RVF vaccine) change with BTB status. IL12 is a cytokine important in immunity to intracellular pathogens (Biron, 1999), by participating in the acquired immune response causing Th0 t-cells to develop into Th1 t-cells, while in innate immunity it has a large role in natural killer cell activity (Tizard, 2004). As a functional measure of acquired immunity we then ask how the host's ability to mount a lymphocyte response to nonspecific mitogens (pokeweed and LPS) and a disease specific mitogen (RVF vaccine) change with BTB Status. Finally, as a measure of innate immunity, we ask how bactericidal ability of whole blood changes with BTB status.

METHODS

Study Site & Buffalo Capture Technique

Kruger National Park (KNP) is located in northeastern South Africa and comprises almost 19,000 km^2, with a buffalo population of approximately 30,000 animals (Cross et al 2009). We sampled 100 free-living young female buffalo in the southern portion of Kruger National Park in 2008 as part of a larger bovine tuberculosis study. Animals were chemically immobilized with M99 (etorphine hydrochloride), azaperone and ketamine by darting from a helicopter. After immobilization, each individual was radio-collared, demographic data collected (age and body condition) and blood samples collected by jugular

venipuncture into appropriate tubes for disease diagnostics and immune profile work (Table 1). Blood samples were stored on ice and transported back the lab within 8 hours. Following data collection, immobilization was reversed using M5050 (diprenorphine) and naltrexone, animals were chemically restrained for no longer than 60 minutes. Time of capture, and amount of anesthesia was used was used initially included in all statistical models but was never found to be an important predictor. Licensed veterinarians and technicians performed all immobilizations and Oregon State University, University of Georgia, and South African National Parks Institutional Animal Care and Use Committees approved all procedures.

Each radio-collared individual was recaptured every 6 months for 4 years (2008-2012) using a similar technique as above. Any animal that died during the study period was replaced by a similarly aged animal to maintain a constant sample size of 100 individuals at each recapture. At each capture period the same data was collected as described below in the data collection section.

Disease and Demographic data collection

Animal ages were assessed from incisor emergence patterns for buffalo 2–5 years old and from tooth wear of the first incisor for buffalo 6 years of age and older (Jolles 2007). Body condition was measured by visually inspecting and palpating four areas on the animal where fat is stored in buffalo: ribs, spine, hips and base of tail. Each area was scored from 1 (very poor) to 5 (excellent) and a body condition score calculated as the average of all four areas. This index is correlated with the kidney fat index (Ezenwa et al 2009), and similar

body condition indices have been used in other studies of African buffalo (Caron et al 2003; Prins 1996).

Tuberculosis infection status was determined using a standard whole-blood gamma interferon assay protocol (Wood and Jones 2001; Schiller et al. 2009). In brief, this assay is performed by comparing the in vitro IFNg response to *Mycobacterium bovis* antigen (bovine tuberculin) to the IFNg response to an avian tuberculin antigen and background IFNg levels in the absence of antigenic stimulation. This assay has been optimized for use in African buffalo (Michel et al. 2011), and blood cells from buffalo infected with *M. bovis* show a pronounced spike in IFNg production in response to bovine but not avian tuberculin, whereas bovine tuberculin challenge does not induce IFNg production in the blood of unexposed animals (Michel et al. 2011). We implemented the gamma interferon assay with the BOVIGAM enzyme-linked immunosorbent assay kit (Prionics), which has a sensitivity of 86% and a specificity of 92% in African buffalo (Michel et al. 2011).

Immune Parameter data collection

IL12 Assessment

Cytokines are immunologically active proteins, that aid in cell signaling during a host immune response and have been proposed as an excellent way to simplistically and realistically describe the immune profiles for the purpose of understanding within-host parasite interactions (Graham et al, 2007). We assessed IL12 response production in whole blood in response to in vitro stimulation with two mitogens, pokeweed and the Smithburn strain of live Rift Valley fever virus. Pokeweed is a general immune stimulant that is often

used to induce cytokine and cell proliferation and the strain for Rift Valley fever we used was a modified live strain used in vaccines (Smithburn strain).

After return from the field whole blood in lithium heparinized tubes was subsetted into 1.5 ml aliquots. Into each aliquot we added 50 ul of mitogen (30,000 live RVF virus units from Onderstepoort Biologicals or 15ug of pokeweed (Sigma L9379, rehydrated in PBS) into experimental tubes and 50ul of PBS into control tubes. Whole blood and mitogen (PBS for controls) were incubated at 37C for 24 hours. After 24 hours the plasma was pipetted off the top of the tube, placed in cryovials and stored at -20C until analysis. The quantity of IL12 in each sample was measured using a sandwich ELISA validated in African buffalo (O'Neal et al, in prep) with each sample tested in duplicate. The difference in IL12 detected between control and experimental tubes was used as the independent variable in statistical analyses. However for graphical purposes we always graph the ratio of IL12 change, which is calculated as:

$$\frac{\mu(\text{IL12 in tubes with mitogen}) - \mu(\text{IL12 in control tubes})}{\mu\ (\text{IL12 in control tubes})}$$

Lymphocyte Proliferation Assay

We used the lymphocyte proliferation assay tests the functional ability of lymphocytes to multiply in response to in vitro stimulation with mitogens (Demas et al, 2011). We used 2 mitogens; pokeweed is a nonspecific stimulant causing proliferation of both B and T lymphocytes while LPS stimulates primarily B cells (Grasman et al, 2002). An alamar blue based lymphocyte proliferation assay was adapted from Gogal et al (1997) to assess lymphocyte response. Mononuclear cells were isolated from whole blood using

Novamed Uni-Sep tubes with medium added (product U-04) according to manufacturer instructions. Isolated mononuclear cells were immediately mixed with 500ul of complete AIM-V media with bovine albumin (Invitrogen, product 31035). Lymphocyte concentration was adjusted to 4000 cells/ul with complete media after counting live cells (using trypan blue) on a hemacytometer. Cells (600,000 cells per well, 150 ul of media mixture) and mitogen (or PBS for controls) were plated in duplicate on a 96 well flat bottomed plate and incubated for 48 hours at 37C (RVF & pokeweed) or 72 hours at 37C(LPS) at 5% CO2. Mitogens used were 10 ug pokeweed (Sigma L9379), which stimulates the division of all lymphocytes, and 1 ug LPS, (Sigma, L4391) which stimulates primarily B cell division. In addition the Smithburn strain of RVF vaccine (3000 virus units) was used to stimulate lymphocyte division in response to a disease specific mitogen. After 48 hours or 72 hours 30 ul of alamar blue (invitrogen, DAL1025) was added and it was incubated for another 24 hours. At a final time of 72 or 96 hours the sample absorbance was determined on a plate reader at two wavelengths, 600 nm and 570 nm. Absorbance was calculated by subtracting the reading at 600 from the reading at 570nm. Proliferation was then calculated as

$$\frac{\mu(\text{final absorbance in mitogen wells}) - \mu(\text{final absorbance in control wells})}{\mu\ (\text{final absorbance in control wells})}.$$

Bactericidal Ability of Whole Blood

This assay is an approximate measure of innate immune capability and was performed as described in Beechler et al (2012) with replicate plates. Briefly, for experimental tubes whole blood and bacteria are mixed together and incubated for 30 minutes. For control tubes the same quantity of bacteria and PBS are mixed. After 30 minutes the mixture is

plated onto agar and the bacteria allowed to grow at 37C for 12 hours. After 12 hours the number of bacteria colonies on each plate are counted. The number of colonies killed by whole blood is determined by subtracting the number of colonies on the experimental plate from the control plate. This is used as the independent variable in statistical analyses, and we account for day-to-day variation in growth by including the number of colonies on the control plates as an offset term in all statistical models (see Beechler et al, 2012 for complete description). For all graphs we graph the proportion killed, which is calculated as

$$\frac{\mu(\text{\# colonies on control plates}) - \mu(\text{\# colonies on the experimental plates})}{\mu\ (\text{\# of colonies on control plates})}.$$

Data Analysis

Question 1: How does the hosts ability to mount an IL12 response to a nonspecific mitogen (pokeweed) and a specific mitogen (RVF) change with BTB Status?

We first asked if animals with BTB differed from those without BTB in IL12 production after stimulation with the nonspecific mitogen (pokeweed). To do this we performed a generalized linear mixed model (Gaussian family, log link, independent variable of the difference between IL12 in the stimulated samples and IL12 in the nonstimulated sample) on 118 individual buffalo that we had repeated IL12 measurements on for a total of 419 IL12 data points. The random effect in the model was the 118 individual buffalo, and we evaluated the fixed effects including all two way interactions of age, BTB status, animal body condition, the amount of IL12 already in the blood before stimulation (circulating IL12), and the batch number for the assay. We found no significant two-way interactions and so presented the main effects model.

We then further investigated the effect of BTB, asking if those that had acquired BTB changed their IL12 response to the nonspecific mitogen (pokeweed) over the time

course of infection. We did this by evaluating only the animals that acquired BTB during the course of the study with a known time since infection (32 individuals, 134 total samples) and running a generalized linear mixed model (log link, Gaussian distribution, random effect=individual buffalo, dependent effects of: body condition, IL12 batch number, amount of IL12 in the blood before stimulation, and 6 month period of time since infection). We chose a 6-month time period because animals were captured twice a year.

Lastly we investigated whether IL12 response to the specific mitogen changed over time since BTB infection using a subset of animals (n=27). We calculated a proportional change in IL12 production as described above and compared the groups using an ANOVA. These samples were all run in one batch in 2011 in animals with similar body condition scores. We then investigated the difference between the groups, to see which group was driving the pattern, by using Fishers multiple comparisons.

Question 2: How does the hosts ability to mount a lymphocyte response to nonspecific mitogens (pokeweed and LPS) change with BTB Status?

We collected this data on 40 animals in June 2011. Proliferative ability was calculated as a proportion, as noted above. Proportions were then arcsine transformed to induce normality. All plots with lymphocyte proliferation are plotted as the raw ratio, not transformed, but all analyses were done with the transformed data. A GLM (Gaussian distribution, log link) was used to look for associations between the independent variable, transformed lymphocyte proliferation, and the dependent variables - body condition, BTB status, age and all 2-way interactions. Age was not a useful predictor so was eliminated from all models.

Question 3: How does the host's general innate immune capability as measured by bactericidal ability of whole blood change with BTB Status?

A generalized linear model (quasipoisson distribution, log link) was used to determine if the number of colonies killed by whole blood differed by BTB status, body condition, age or any two-way interaction effects. Because the number of colonies grown on the control plates differs by environmental conditions each day we included the average number of colonies on the control plates as an offset term in the GLM (Beechler et al, 2012). This was performed on a subset of 97 individual buffalo from July 2010 until July 2011.

RESULTS

Question 1: How does the hosts ability to mount an IL12 response to a nonspecific mitogen (pokeweed) and a specific mitogen (RVF) change with BTB Status?

Animals with bovine tuberculosis (BTB) animals have increased IL12 production in response to stimulation with pokeweed (GLMM; figure 5.1) after accounting for IL12 batch number, initial amount of RVF circulating and body condition. The increase in IL12 response after infection does not change within the first year of BTB infection (GLMM, figure 5.2). Animals with BTB also have increased IL12 production in response to invitro stimulation with a diseases specific mitogen (RVF) (ANOVA, $F(3,26)=2.372$, $p=0.09$, $R2=0.21$) driven by increased production in the first year of infection that returns to normal by Year 2. (Fishers LSD multiple comparisons, Figure 5.3).

Question 2: How does the hosts ability to mount a lymphocyte response to nonspecific mitogens (pokeweed and LPS) and a disease specific mitogen (RVF) change with BTB Status?

BTB infection status correlated with the ability of lymphocytes to proliferate in response to two non-specific mitogens, LPS and Pokeweed (Figure 5.4). Animals with BTB, have increased lymphocyte proliferation in response to PW and LPS, but this is driven by animals in poor body condition with BTB. The host's ability to respond to a disease specific mitogen (RVF) varied by BTB status, but was unaffected by condition. Animals

with BTB had increased response to RVF stimulation (GLM, estimate=0.82, SE=0.3, p=0.02) even after accounting for condition (estimate=-0.38, SE =0.30, p=0.22). In this subset of animals, all captured in June of 2011 (n=22) the mean time of BTB infection was 1.5 years (range 0-2.5 years).

Question 3: How does the host's general innate immune capability change with BTB Status?

Animals with BTB have decreased bactericidal ability of whole blood compared to those without BTB (figure 5.5), which holds even after accounting for animal body condition and age (table 5.2). The majority of animals in this subset had had BTB for greater than 6 months, only 4 of 40 positive buffalo were infected within the last 6 months. The mean time since since BTB infection was 1 year (range 0-2.5 years). All statistical results were the same whether the 4 recently infected buffalo were included or not.

DISCUSSION

BTB has strong and detectable effects on host immune responses in free-ranging African buffalo that may alter susceptibility to other microparasitic intracellular pathogens, such as Rift Valley fever. We found that BTB was associated with higher IL12 response to invitro stimulation with a nonspecific mitogen, pokeweed, and a disease specific mitogen, RVF, which is detectable for the first year of infection with BTB. The increase in the signaling molecule (IL12) in response to stimulation was also reflected with increased cellular function as measured by the LPA. BTB+ animals showed increased in vitro lymphocyte proliferation in response to pokeweed stimulation, compared to their BTB- counterparts. This provides evidence that when exposed to another pathogen, the host would also be able to mount a stronger IL12 response, perhaps making it less likely that the pathogen is able to infect the animal. The IL12 response and the NK cells they activate are

one of the most important mediators of viral infections (Biron et al, 1999). In addition, IL12 is important in helping immature T cells develop into Th1 cells which are also important in mediating the response to other intracellular infections (Trinchieri, 2003).

We also found that the increased lymphocyte proliferation in response to both LPS (B cell mitogen) and pokeweed (B & T cell mitogen) in BTB + individuals was condition dependent. BTB+ animals in poor condition had increased proliferation, but BTB+ animals in good condition did not. This condition-dependent immune response could indicate that the costs of mounting a strong proliferation response may be higher than the benefit, Alternatively, an animal in better body condition may have a more localized or efficient response to BTB infection that are not seen in assays using circulatory cells. In support of this hypothesis, Thacker et al (2007) found that cows with systemically increased Th1 cytokine mRNA expression had increased pathology associated with BTB infection, and that animals with lower systemic levels had decreased pathology associated with infection. However the pattern was reversed when the local (head and lung) lymph nodes were evaluated, possibly indicating that a high systemic response is not necessarily a more effective response. This could possibly explain why we did not see suppressed IL12 production or lymphocyte proliferation in animals chronically infected with BTB. Perhaps the suppression associated with infection is not strong enough to be detectable in circulating lymphocytes, but a Th0 skew may be detectable in local lymph nodes of the head and neck. This could lead to easier invasion of other pathogens that are transmitted by inhalation or direct contact where the head and neck lymph nodes are the first round of defense.

We hypothesized that in BTB infections more than a year old, we would see a skew to a TH0 immune response (Welsh et al, 2005). A TH0 skew may be represented by

suppressed IL12 response to invitro stimulation with mitogens (RVF and pokeweed) and/or suppressed lymphocyte proliferative ability; but we did not find support for this hypothesis in our study. Thacker et al (2007) also saw an initial upregulation in IL12 production that peaked at 30 days post BTB infection, but did not see any reduction up to 3 months later. Similarly in our study, the animals IL12 response to pokeweed and RVF stayed upregulated through the first year of BTB infection. After a year of infection, the IL12 response to RVF had returned to "normal" but no reduction in response that would represent a Th0 skew was noted in the 3 years post infection. Unfortunately we do not have any data on what happens to the IL12 response to pokeweed more than 1 year after infection, but it is likely it follows the same pattern as RVF.

Although we did not find any evidence of a systemic Th0 skew in animals with BTB, we did see suppressed innate immunocompetence, in BTB + animals as measured by the bactericidal ability of whole blood. Piron et al (2013) suggested that while cytokines were elevated systemically in response to BTB, the receptors and function of APC's (antigen presenting cells) were suppressed, which would decrease the host's ability to respond to an insult from a pathogen, despite the increased circulatory cytokine expression. The majority of BTB infected animals in this subset had been infected for greater than a year indicating that animals with older infections of BTB have a decreased ability to respond to an insult from a pathogen, despite there being no evidence of "suppression" in IL12 production or lymphocyte response. Future work needs to focus on finding the pathways by which innate immune function is suppressed in BTB infected animals to better understand the impacts of suppressed innate immunity.

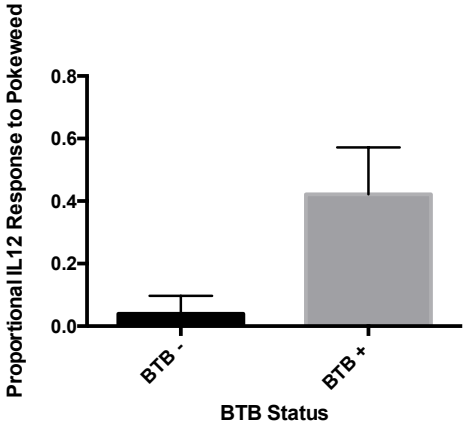
BTB has dynamic effects on the immune system of hosts, which are detectable as population level patterns in free-ranging African buffalo. Although systemic IL12 response and lymphocyte proliferation were increased in animals with BTB, BTB + animals showed decreased overall innate immune function with BTB. This reduced ability to respond to a pathogen with a strong innate response may increase the likelihood that animals with BTB become infected with another pathogen.

Table 5.1: Variables used in this study

Disease & Demographic Variables	Immune Parameters
Age (months)	Invitro IL12 production in response to a nonspecific mitogen (Pokeweed) and a disease specific mitogen (Rift Valley Fever modified live virus strain - Smithburn Strain)
Body Condition (scale of 0-5)	In Vitro Lymphocyte Production in response to two nonspecific mitogens (pokeweed & lipopolysaccharide)
Bovine Tuberculosis Status	Bactericidal Ability of Whole Blood

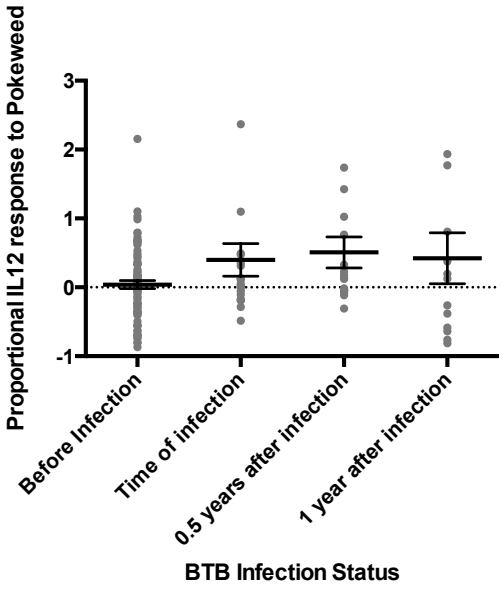
Table 5.2: Animals with BTB have reduced bactericidal ability of whole blood after accounting for age and body condition. The table represents the estimates for each parameter in a model with the independent variable of (μ(# colonies on control plates)- μ(# colonies on the experimental plates). The dependent variables are BTB Status, Body Condition and Age of the animal, with an offset term to account for the average number of colonies on the control plate.

	Estimate	**Standard Error**	**p-value**
BTB Status (Pos)	-0.52	0.24	0.03*
Body Condition	-0.41	0.2	0.04*
Age	0.02	0.04	0.49



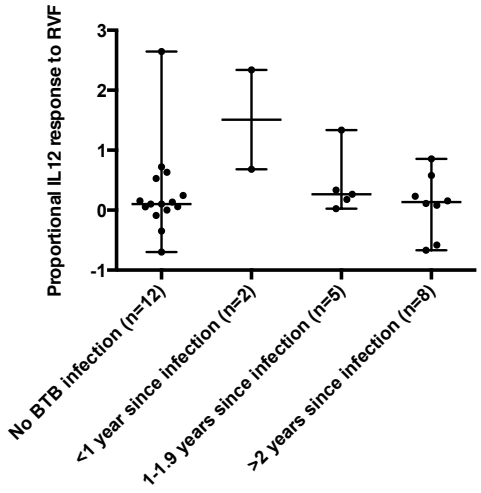
	Estimate	SE	P value
IL12 Batch #	-88.63	15.86	<0.01*
Circulating IL12	-0.28	0.04	<0.01*
Animal Body Condition	224.19	195.72	0.25
	718.48	323.84	0.03*

Figure 5.1: Animals with BTB initially have a stronger IL 12 response to pokeweed stimulation that those animals without BTB (Table contains estimates, SE and p values for the GLMM). All animals with BTB were within 1 year of infection. The figure represents the mean change in IL12 production after stimulation with pokeweed with standard error. The table on the right represents the model parameters for a generalized linear model (Gaussian family, log link) with formula (IL12 Difference~IL12 Plate + IL12 Base circulating level + Animal Body Condition + BTB Status).



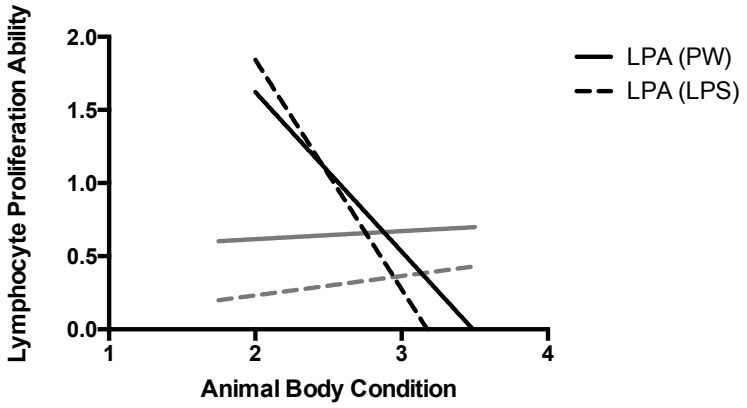
	Estimate	SE	p-value
IL12 Batch #	-118.26	29.07	<0.01*
Circulating IL12	-0.22	0.06	<0.01*
Animal Body Condition	25.98	325.94	0.94
Time (Years) since BTB infection	282.39	221.27	0.20

Figure 5.2: BTB infected animals have upregulated IL12 response to pokeweed but the mean magnitude of the increase does not change in the first year of BTB infection. Lines are at the mean; error bars are standard error of the mean. The table on the right shows the model parameters for a generalized linear model (Gaussian family, log link) with formula (IL12 Difference~IL12 Plate + Circulating IL12 + Animal Body Condition + Years after BTB infection) for only individuals that converted from BTB - to BTB + over the course of the study.



	Mean	SE	p-value
No BTB infection (n=12) vs. <1 year since infection (n=2)	-1.23	0.51	0.02*
No BTB infection (n=12) vs. 1-1.9 years since infection (n=5)	-0.15	0.35	0.68
No BTB infection (n=12) vs. >2 years since infection (n=8)	0.19	0.30	0.54

Figure 5.3: Animals with recent BTB infections have a stronger IL12 response to RVF vaccine than animals that never converted. By year 2 the response has returned to pre-BTB levels.



LPA (PW)	Est	SE	p-value
BTB Status (+)	3.19	1.5	0.05*
Body Condition	0.02	0.36	0.95
BTB (+) * Condition	-1.12	-2.16	0.05*

LPA (LPS)	Est	SE	p-value
BTB Status (+)	2.36	1.00	0.03*
Body Condition	0.17	0.36	0.64
BTB (+) * Condition	-0.73	0.35	0.05*

Figure 5.4: Animals with BTB (solid lines) have increased lymphocyte responsiveness to nonspecific mitogens (LPS and Pokeweed) compared to BTB - (dashed lines), but this varies by condition. Animals with BTB have increased lymphocyte proliferative ability if they are in poor body condition, but if they are in better body condition they have reduced proliferative ability. A body condition score of 3 would be an animal in normal condition.

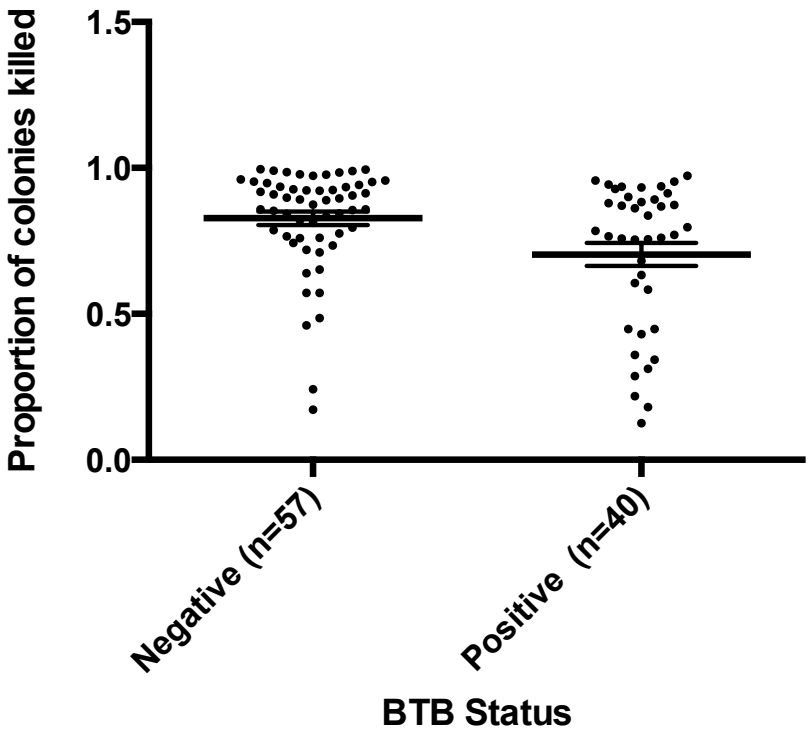


Figure 5.5: Animals with BTB have reduced bactericidal ability of whole blood. Each point is a data point; line is at the mean proportion of colonies killed with SEM error bars. In this subset of animals the majority of the individuals had "chronic" BTB infections with the mean time since infection 1 year (range 0-2.5 years).

6 – Conclusion

While much attention has been given to the direct impact of emerging diseases, such as chytridiomycosis, white nose syndrome, Hendra virus and ebola virus, including how they may affect host populations or spillover into humans and livestock, very little attention has been given to how these emerging diseases alter parasite interactions within a host. Most hosts are infected with multiple parasites that may interact with each other competitively or synergestically, and parasite interactions within a host can drive population level transmission patterns of disease (Ezenwa et al, 2011). Therefore, it is important to consider the impact emerging diseases may have on parasite interactions within a host, and how these individual level impacts can scale up to affect disease transmission patterns of the common diseases within the population.

This dissertation demonstrates that individual African buffalo with the emerging disease BTB have an increased risk of acquiring a native pathogen, Rift Valley fever (Chapter 3). Chapters 4 and 5 explore potential immune mechanisms through which two pathogens may achieve this, illustrating that animals with BTB have altered immune function that may change their susceptibility to other pathogens, such as RVF. Preliminary work suggests that animals with BTB have decreased innate immune capability, which may hinder their ability to fight initial pathogen exposure and increase the likelihood of acquiring RVF. However, more work on the mechanisms by which BTB inhibits immune response should be conducted to elucidate the primary interactions involved. Understanding the exact mechanism behind BTB related immunosuppression may allow a better understanding of

what other native pathogens might be affected by the invasion of BTB into the ecosystem, allowing park managers to make better informed risk assessments and conservation decisions.

The immune-mediated interactions between Rift Valley fever (RVF) and Bovine tuberculosis (BTB) not only alter individual level susceptibility to RVF, but may scale up to affect the population level transmission dynamics of RVF (based on model of RVF in chapter 2, BTB modified model in chapter 3) in buffalo. RVF outbreaks are larger and of a longer duration in the presence of BTB. Future work should investigate whether increases in the size of buffalo outbreaks match to increased risk of RVF transmission to other wildlife and domestic animal populations, or an increase in human risk of acquiring the disease. More sophisticated mathematical modeling also needs to be accomplished to explore whether the role of buffalo in the interepidemic transmission of disease (chapter 1) is affected by the presence of BTB.

To my knowledge this is the first report of an emerging disease altering native parasite infection dynamics in a common host species. This would be important to replicate in other systems, to fully understand the impact of emerging diseases. Our full understanding of how emerging disease alter disease dynamics within a system may be especially important for diseases affecting conservation of at-risk species, such as bat white nose syndrome, Tasmanian devil facial tumor disease, or FIV in lions and other exotic felids. It may also aid One Health initiatives, as scientists seek to limit the transmission of zoonotic diseases and use interdisciplinary approaches to answer key questions about emerging and existing infectious pathogens.

Bibliography

Allen, J.E., and R.M. Maizels. 2011: Diversity and dialogue in immunity to helminths. Nature Reviews in Immunology 11(6):375-88

Allen, A.R., G. Minozzi, E.J. Glass, R.A.Skuce, S.W.J. McDowell, J.A. Woolliams and S.C. Bishop. 2010. Bovine Tuberculosis: the genetic basis of host susceptibility. Proceedings of the Royal Society B. doi: 10.1098/rspb.2010.0830

Aly K., S. Darwish, A. Ramoun, K. Tawfeek, N. Hanh, N. Sousa, J. Sulon, O. Szenci and J.F. Beckers. 2011. Accuracy of transrectal palpation for early pregnancy diagnosis in Egyptian buffaloes. Tropical Animal Health and Production 43:5-7

Ameni, G., H. Miorner, F. Roger, and M. Tibbo. 2000. Comparison between comparative tuberculin and gamma-interferon tests for the diagnosis of bovine tuberculosis in Ethiopia. Tropical Animal Health & Production 32:267–276.

Anderson, E.C., L.W. Rowe, 1998: The prevalence of antibody to the viruses of bovine virus diarrhoea, bovine herpes virus 1, Rift Valley fever, ephemeral fever and bluetongue and to Leptospira sp in free-ranging wildlife in Zimbabwe. Epidemiology and Infection, 121:441-9.

Anderson, R.M. and R.M.May. 1986: The invasions, persistence and spread of infectious diseases within animal and plant communities. Philosophical Transactions of the Royal Society B 314, 533-570

Anyamba, A., J.-P. Chretien, J. Small, C. J. Tucker, P.B. Formenty, J.H. Richardson, S.C. Britch, D.C. Schnabel, R.L. Erickson, and K.J. Linthicum, 2009: Prediction of a Rift Valley fever outbreak. PNAS 2009 106 (3) 955-959.

Archer, B.N., J. Weyer, J. Paweska, D. Nkosi, P. Leman, K. San Tint and L. Blumberg. 2011: Outbreak of Rift Valley fever affecting veterinarians and farmers in South Africa, 2008. South African Medical Journal 101 (4), 263-266.

Ardia D.R., H.K. Parmentier and L.A. Vogel. 2011. The role of constraints and limitation in driving individual variation in immune response. Functional Ecology 25:61-73

Augustine, D.J. 1998. Modelling Chlamydia-Koala interactions: coexistence, population dynamics and conservation implications. J. Appl. Ecol., 35, 261–272.

Balkhy, H.H. and Z.A. Memish, 2003: Rift Valley fever: an uninvited zoonosis in the Arabian Peninsula. International Journal of Antimicrobial Agents 21:153-157.

Barnard, B.J. and M.J. Botha, 1977: An inactivated Rift Valley fever vaccine. Journal of the South African Veterinary Association 48(1), 45-48.

Baqar, S., C.G. Hayes, J.R. Murphy and D.M. Watts, 1993: Vertical transmission of West Nile virus by Culex and Aedes species mosquitoes. American Journal of Tropical Medicine and Hygiene 48(6):757-762.

Becker, N., 1989: Life strategies of mosquitoes as an adaptation to their environment. Bulletin of the Society of Vector Ecology. 14:6-25.

Beechler, BR, R. Bengis, R. Swanepoel, J.T. Paweska, A. Kemp, P.J. van Vuren, J. Joubert, V.O. Ezenwa, and A.E. Jolles. 2012. Rift Valley fever in Kruger National Park: do buffalo play a role in the interepidemic circulation of virus? Transboundary And Emerging Diseases.

Beechler, B.R., H. Broughton, A. Bell, V.O. Ezenwa and A.E. Jolles, 2012: Innate immunity in free-ranging African buffalo (Syncerus caffer): Associations with parasite infestation and white blood cell counts. Physiological and Biochemical Zoology 85(3), 255-264.

Bengis R., R. Grant and V. deVos. 2006. in The Kruger Experience: Ecology and Management of savannah heterogeneity. Editors: Biggs, H. duToit, Johan. Rogers, K. Sinclair A. and B. Walker. Island Press, Washington DC

Betelli E., M. Oukka and V.K. Kuchroo. 2007. TH-17 cells in the circle of immunity and autoimmunity. Nature Reviews Immunology 8:345-350

Biron CA, Nguyen KB, Pien GC, Cousens LP & Salazar-Mather TP. 1999. Natural killer cells in antiviral defense: function and regulation by innate cytokines. Annual Reviews in Immunology 17:189-220

Bordes, F & Morand, S. 2009. Coevolution between multiple helminth infestations and basal immune investment in mammals: cumulative effects of polyparasitism. Parasitology Research 106: 33-37

Bradley JE and J.A. Jackson. 2008. Measuring immune system variation to help understand host-pathogen community dynamics. Parasitology 135:807-823.

Boughton R.K., G. Joop and S.A.O. Armitage. 2011. Outdoor immunology: methodological considerations for ecologists. Functional Ecology 25:81-100

Buehler D.M.., T. Piersma, K. Matson and B.I. Tieleman. 2008. Seasonal Redistribution of Immune Function in a Migrant Shorebird: Annual Cycle Effects Override Adjustments to Thermal Regime. The American Naturalist 172:783-796

Caron A., P.C. Cross and J.T. du Toit. 2003. Ecological implications of bovine tuberculosis in African Buffalo herds. Ecological Applications 13:1338-1345.

Cabaret J., N. Gasnier and P. Jacquiet. 1998. Fecal egg counts are representative of digestive-tract strongyle worm burdens in sheep and goats. Parasite 5:137-142.

Chevalier, V., S. de la Rocque, T. Baldet, L. Vial and F. Roger, 2004: Epidemiological processes involved in the emergence of vector-borne diseases: West Nile fever, Rift Valley fever, Japanese Encephalitis and Crimean Congo haemorrhagic fever. Revues in Science and Technology office of international epizootics 23(2): 537-555.

Chevalier, V., M. Pepin, L. Plee, R. Lancelot, 2010: Rift Valley Fever - a threat for Europe?. EuroSurveillance 15(10);1950-6 .

Chitnis, N., J.Cushing, and J. Hyman, 2006: Bifurcation Analysis of a Mathematical Model for Malaria Transmission. SIAM Journal on Applied Mathematics, 67:1, 24-45.

Chitnis N., J.M. Hyman, C.A. Manore, 2013: Modelling vertical transmission in vector-borne diseases with applications to Rift Valley fever. Journal of Biological Dynamics, Vol. 7, Iss. 1. DOI:10.1080/17513758.2012.733427.

Cremer S. and M. Sixt. 2009. Analogies in the evolution of individual and social immunity. Philosophical Transactions of the Royal Society B 364:129-142

Cross, P.C., D.M. Heisey and J.A. Bowers, 2009: Disease, predation & demography: assessing the impacts of bovine tuberculosis on African buffalo by monitoring at individual and population levels. Journal of Applied Ecology 46, 457-465.

Daubney R., J.R. Hudson, P.C. Garnham, 1931: Enzootic hepatitis or Rift Valley fever: an undescribed virus disease of sheep, cattle and man from East Africa. Journal of Pathology and Bacteriology, 34:545-79.

Davies, F.G. and L. Karstad. 1981. Experimental infection of the African buffalo with the virus of Rift Valley fever. Tropical Animal Health Production 13(4):185-188

Davies, F.G., 1975: Observations on the epidemiology of Rift Valley fever in Kenya. Journal of Hygiene, 75:219-230.

Davies W.A., V.P. Ackerman and D.S. Nelson. 1981. Mechanism for nonspecific immunity of Listeria monocytogenes in rats mediated by platelets and the clotting system. Infection and Immunology 33:477-481

de Garine-Wichatitsky M, Caron A, Gomo C, Foggin C, Dutlow K, Pfukenyi D, et al. 2010. Bovine tuberculosis in buffaloes, southern Africa [letter]. Emerging Infectious Diseases. http://wwwnc.cdc.gov/eid/article/16/5/09-0710.htm

Demas G.E., D.A. Zysling, B.R. Beechler, M.P. Muehlenbein and S.S. French. 2011. Beyond phytohaemagglutinin: assessing vertebrate immune function across ecological contexts. Animal Ecology 80:710-730

DeVos V, RG Bengis, NPJ Kriek, A Michel, DF Keet, JP Raath, HFKA Huchzermeyer. 2001. The epidemiology of tuberculosis in free-ranging African buffalo (Syncerus caffer) in the Kruger National Park South Africa. Onderstepoort Journal of Veterinary Research, 68: 119-130.

Diallo M., .J Thonnon, D. Fontenille, 2007: Vertical transmission of the yellow fever virus by Aedes aeypti (Diptera, Culicidae): dynamics of infection in F1 adult progeny of orally infected females. American Journal of Tropical Medicine and Hygiene 62(1):151-156.

Driscoll EE, Hoffman JI, Green LE, Medley GF, Amos W (2011) A Preliminary Study of Genetic Factors That Influence Susceptibility to Bovine Tuberculosis in the British Cattle Herd. PLoS ONE 6(4): e18806. doi:10.1371/journal.pone.0018806

DuToit J.T. 2003. Large Herbivores and Savanna Heterogeneity in The Kruger Experience: Ecology and Management of savannah heterogeneity. Editors: Biggs, H. duToit, Johan. Rogers, K. Sinclair, A. and Walker B. Island Press, Washington DC

Espach, A., M. Romito, L.H. Nel and G.J. Viljoen. 2002. Development of a diagnostic one-tube RT-PCR for the detection of Rift Valley fever virus. Onderstepoort Journal of Veterinary Research 69(3):247-252

Evans, A., F. Gakuya, J.T. Paweska, M. Rostal, L. Akoolo, P.J. VanVuren, T. Manyibe, J.M. Macharia, T.G. Kziazek, D.R. Feiken, R.F. Breimen, K. Njenga, 2008: Prevalence of antibodies against Rift Valley Fever virus in Kenyan wildlife. Epidemiology and Infection 136:1261-1269.

Ezenwa V.O., L.S. Ekernas. and S. Creel.. 2011 Unraveling complex associations between testosterone and parasite infection in the wild. Functional Ecology, in press. doi: 10.1111/j.1365-2435.2011.01919.x

Ezenwa V.O. and A.E. Jolles. 2011. From host immunity to pathogen invasion: the effects of helminth coinfection on the dynamics of microparasites. Integrative and Comparative Biology 51(4):540-551

Ezenwa V.O., R.S. Etienne, G. Luikart, A. Beja-Pereira and A.E. Jolles. 2010. Hidden Consequences of living in a wormy world: nematode-induced immune suppression facilitates tuberculosis invasion in African Buffalo. The American Naturalist 176(5):613-624

Ezenwa V.O., A.E. Jolles and M. O'Brien. 2009. A reliable body condition scoring technique for estimating condition in African buffalo. African Journal of Ecology 47: 476-481

Ezenwa V.O. and A.E. Jolles. 2008. Horns honestly advertise parasite infection in male and female buffalo. Animal Behavior 75:2013-2021

Fenton A., T. Lamb and A.L. Graham. 2008. Optimality analysis of Th1/Th2 immune responses during microparasite-macroparasite co-infection, with epidemiological feedbacks. Parasitology 135:841–853

Fischer-Tenhagen, C., C. Hamblin, S. Quandt, K. Frolich, 2000: Serosurvey for selected infectious disease agents in free-ranging black and white rhinoceros in Africa. Journal of Wildlife Disease 36:316-323.

Flick R and M Bouloy, 2005: Rift Valley Fever virus. Current Molecular Medicine, 5:827-834.

Forsman AM, L.A. Vogel, S.K. Sakaluk, J.L. Grindstaff and C.F. Thompson. 2008. Immune-challenged house wren broods differ in the relative strengths of their responses among different axes of the immune system. Journal of Evolutionary Biology 21:873-878

Freitak D., C. Wheat, D. Heckel and H. Vogel. 2007. Immune system responses and fitness costs associated with consumption of bacteria in larva of Trichoplussia ni. BMC Biology 5:56

Friedl T.W.P. and R. Edler. 2005. Stress-Dependent trade-off between immunological condition and reproductive performance in the polygynous red bishop (Euplectes Orix). Evolutionary Ecology 19:221-239

Gaff, H.D., D.M. Hartley, and N.P. Leahy, 2007: An epidemiological model of Rift Valley fever. Electronic Journal of Differential Equations, (115):1-12.

Gaff, H., C. Burgess, J. Jackson, T. Niu, Y. Papelis, and D. Hartley. 2011: Mathematical Model to Assess the Relative Effectiveness of Rift Valley Fever Countermeasures. IJALR 2.2 1-18. Web. 27 Sep. 2012.

Gargan, T.P., P.G. Jupp, and R.J. Novak, 1988: Panveld oviposition sites of floodwater Aedes mosquitoes and attempts to detect transovarial transmission of Rift Valley fever virus in South Africa. Medical and Veterinary Entomology 2: 231-236.

Geering, W.A., F.G. Glyn Davies and V. Martín, 2002: Preparation of Rift Valley Fever contingency plans. Food and Agriculture Organization of the United Nations. Emergency Prevention System for Transboundary Animal and Plant Pests and Diseases.

Gogal, R.M., S.A. Ahmed and C.T. Larsen. 1997. Analysis of avian lymphocyte proliferation by a new, simple, nonradioactive assay (lympho-pro). Avian Diseases 41(3):714-725

Graham A.L., A.D. Hayward, K.A. Watt, J.G. Pilkington, J.M. Pemberton and D.H. Nussey. 2011. Fitness Correlates of heritable variation in antibody responsiveness in a wild mammal. Science 330(6004):662-665

Graham AL. 2008. Ecological rules governing helminth-micro parasite coinfection. PNAS 105(2): 566-570

Graham, A.L., I.M. Cattadori, J.O. Lloyd-Smith, M.J. Ferrari and O.N. Bjornstad. 2007: Transmission consequences of coinfection: cytokines writ large? Trends in Parasitology 23: 284–291.

Graham A.L., T.J. Lamb, A.F. Read and J.E. Allen. 2005. Malaria-filaria coinfection in mice makes malarial disease more severe unless filarial infection achieves patency. Journal of Infectious Diseases 191:410-421

Grasman K.A.. 2002. Assessing immunological function in toxicological studies of avian wildlife. Integrative and Comparative Biology 42:34-42

Grenfell B.T. and A.P. Dobson. 1995. Infectious Disease in Natural Populations. Cambridge University, Cambridge

Grobbelaar, A.A., J. Weyer, P.A. Leman, A. Kemp, J.T. Paweska and R. Swanepoel, 2011: Molecular epidemiology of Rift Valley fever virus. Emerging Infectious Diseases, 17, 2270-2276.

Hamblin, C. and R.S. Hedger, 1978:Neutralising antibodies to parainfluenza 3 virus in African wildlife, with special reference to the Cape Buffalo (Syncerus caffer). Journal of Wildlife Diseases 14, 377-388.

Hanski C., M. Naumann, A. Grutzkau, G. Pluschke, B. Friedrich, H. Hahn and E.O. Rieckens. 1991. Humoral and cellular defense against intestinal murine infection with Versinia entercolitica. Infection and Immunology 59:1106-1111

Hasselquist D. (2007) Comparative immunoecology in birds: hypotheses and tests. Journal of Ornithology 148:S571-S582

Hawley D.M. and S. Altizer. 2010. Disease ecology meets ecological immunology: understanding the links between organismal immunity and infection dynamics in natural populations. Functional Ecology 25:48-60

Houston A.I., J.M. McNamara, Z. Barta and K.C. Klasing. 2007. The effect of energy reserves and food availability on optimal immune defense. Proceedings of the Royal Society B 274:2835-2842

Jackson J.A., M. Begon, R. Birtles, S. Paterson, I.M. Friberg, A. Hall, A. Lowe, C. Ralli, A. Turner, M. Zawadzka and J.E. Bradley. 2011. The analysis of immunological profiles in wild animals: a case study on immunodynamics in the field vole, Microtus agrestis. Journal of Molecular Ecology 20(5):893-909

Jiao X, R Lo-Man, N Winter, E Deriaud, B Gicquel, C Leclerc. 2003. The shift of Th1 to Th2 immunodominance associated with the tonicity of mycobacterium bovis Bacilli Chalmette-Guerin Infection does not affect the memory response. Journal of Immunology 170:1392-1398.

Johnson, P. T. J. and J. T. Hoverman (2012). Parasite diversity and coinfection determine pathogen infection success and host fitness. Proceedings of the National Academy of Sciences 109: 9006-9011

Jones KE, Pattel NG, Levy MA, Storeygard A, Balk D, Gittleman JL, & Daszak, P. 2008. Global Trends in Emerging Infectious Diseases. Nature 451:990-994

Jolles A.E., V.O. Ezenwa, R.S. Etienne, W.C. Turner and H. Off. 2008. Interactions between macroparasites and microparasites drive patterns of infection in free ranging African buffalo. Ecology 89:2239-2240

Jolles A.E. 2007. Population biology of African buffalo (Syncerus caffer) at Hhuhluwe-iMfolozi Park, South Africa. African Journal of Ecology 45:398-406

Jolles A.E., D. Cooper and S.A. Levin. 2005. Hidden effects of chronic tuberculosis in African buffalo. Ecology 86(9):2358-2364.

Joly, D.O. and F. Messier. 2005. The effect of bovine tuberculosis and brucellosis on reproduction and survival of wood bison in Wood Buffalo National Park. Journal of Animal Ecology 74: 543-551.

Jupp, P.G., B.M. McIntosh, and D.L. Thompson, 1983: Isolation of Rift valley fever virus from Aedes (neomelaniconinion) circumluteolus and/or luteolateralis during an outbreak in cattle in the coastal regions of Natal, South Africa. South African Journal of Science 79, 377.

Jupp, P.G., 1996. Mosquitoes of Southern Africa. Ekoguild Publishers, Hartebeestpoort South Africa.

Kasari, T.R., D.A. Carr, T.V. Lynn & J.T. Weaver, 2008: Evaluation of pathways for release of Rift Valley fever virus into domestic livestock, ruminant wildlife and human populations in the continental United States. Journal of the American Veterinary Medical Association 232(4): 514-529.

Klasing K.C. 2004. The costs of immunity. Acta Zoologica 50:961–969

Labeaud, A.D., P.C. Cross, W.M. Getz, 2011: Rift Valley Fever virus infection in African buffalo (Synceruscaffer) Herds in Rural South Africa: Evidence of Interepidemic Transmission. American Journal of Tropical Medicine and Hygiene 84: 641-646.

Lee K.A., M. Wikelski,. W.D. Robinson, T.R. Robinson, and K.C. Klasing. 2008. Constitutive immune defences correlate with life-history variables in tropical birds. Journal of Animal Ecology 77:356-363

Lilenbaum, W., E. R. Ribeiro, G. N. Souza, E. C. Moreira, L. S. Fonseca, M. A. S. Ferreira, and J. Schettini. 1999. Evaluation of an ELISA-PPD for the diagnosis of bovine tuberculosis in field trials in Brazil. Research in Veterinary Science 66: 191–195.

Linthicum, K.J., C.L. Bailey, C.J. Tucker, D.R. Angleberger, T. Cannon, T.M. Logan, P.H. Gibbs and J. Nickeson, 1991: Towards real-time prediction of Rift Valley fever epidemics in Africa. Preventive Veterinary Medicine 11:325-334.

Llamazares, O. R. G., C. B. Gutierrez Martin, D. Alvarez Nistal, V. A. de la Puente Redondo, L. Dominguez Rodriguez, and E. F. Rodriguez Ferri. 1999. Field evaluation of the single intradermal cervical tuberculin test and the interferon-gamma assay for detection and eradication of bovine tuberculosis in Spain. Veterinary Microbiology 70: 55–66.

Lochmiller R.L. and C. Deerenberg. 2000. Trade-offs in evolutionary immunology: just what is the cost of immunity? Oikos 88:87-98

Long K.Z. and N. Nanthakumar. 2004. Energetic and nutritional regulation of the adaptive immune response and trade-offs in ecological immunology. American Journal of Human Biology 16(5):499-507

Lutomiah, J.L., C. Mwandawiro, J. Magambo and R.C. Sang, 2007: Infection and vertical transmission of Kamiti river virus in laboratory bred Aedes aegypti mosquitoes. Journal of Insect Science 7:55.

Manore, C.M. and Beechler, B.B. 2013. Inter-epidemic and Between-Season Persistence of Rift Valley fever: Vertical Transmission or Cryptic Cycling?. Transboundary and Emerging Diseases. In Press.

Martin L.B., D.M. Hawley and D.R. Ardia. 2011. An introduction to ecological immunology. Functional Ecology 25:1-4

Martin L.B., Z.M. Weil and R.J. Nelson. 2008. Seasonal changes in vertebrate immune activity: mediation by physiological trade-offs. Philosophical Transactions of the Royal Society B. 363:321-339

Martin L.B., Z.M. Weil and R.J. Nelson 2007. Immune defense and reproductive pace of life in peromyscus mice. Ecology 88:2516-2528

Martin L.B., Z.M. Weil and R.J. Nelson. 2006. Redefining approaches and diversifying directions in ecoimmunology. Integrative and Comparative Biology 46:1030-1039

Martin V, V. Chevalier, P. Ceccato, A. Anyamba, L. DeSimone, J. Lubroth, S. de La Rocque and J. Domenach, 2008: The impact of climate change on the epidemiology and control of Rift Valley fever. Revues in Science and Technology 27(2): 413-426.

Martinez-Carrasco, C., E. Serrano, R.R. de Ybanez, J. Penalver, J.A. Garcia, A. Garcia-Ayala, S. Morand and P. Munoz. 2011. The European eel - the swim bladder-nematode systems provides a new view of the invasion paradox. Parasitology Research 108 (6):1501-1506.

Matson K.D., B.I. Tieleman and K.C. Klasing. 2006. Capture Stress and the Bactericidal Competence of Blood and Plasma in Five Species of Tropical Birds. Physiological and Biochemical Zoology 79:556-564

McCallum, H., M. Jones, C. Hawkins, R. Hamede, S. Lachish, D.L. Sinn, N. Beeton and B. Lazenby. 2009. Transmission dynamics of Tasmanian facial tumor disease may lead to disease-induced extinction. Ecology 90:3379-3392

McIntosh, B.M., 1972: Rift Valley fever: vector studies in the field. Journal of the South African Veterinary Medical Association 43(4), 391-395.

McIntosh, B.M. 1980: Epidemiology of Arthropod-borne Viruses in Southern Africa, DSc Thesis, University of Pretoria.

Michel A.L., D. Cooper, J. Jooste, L.M. deKlerk and A.E. Jolles. 2011: Approaches towards optimizing the gamma interferon assay for diagnosing Mycobacterium bovis infection in African buffalo. Preventative Veterinary Medicine 98:142-151

Michel A.L, M.L. Coetzee and D.F. Keet. 2009: Molecular epidemiology of Mycobacterium bovis isolates from free-ranging wildlife in South African game reserves. Veterinary Microbiology 133: 335-343

Michel Al, RG Bengis, DF Keet, M Hofmeyer, LM deKlerk, PC Cross, AE Jolles, I Cooper, J Whyte, P Buss, J Godfroid, et al. 2006. Wildlife tuberculosis in South African Conservation areas: implications and challenges. Veterinary Microbiology 112:91-100.

Millet S., J. Bennett, K.A. Lee, M. Hau and K.C. Klasing. 2007. Quantifying and comparing constitutive immunity across avian species. Developmental and Comparative Immunology 31:188-201

Mills, M.G.L., H.C. Biggs and I.J.Whyte, 1995. The relationship between rainfall, lion predation and population trends in African herbivores. Wildlife Research 22(1): 75-87

Moret Y. and P. Schmid-Hempel. 2001. Entemology - immune defense in bumblebee offspring. Nature 414:506

Moret Y. and M.T. Siva-Jothy. 2003. Adaptive innate immunity? Responsive-mode prophylaxis in the mealworm beetle, Tenebrio molitar. Proceedings of the Royal Society of London Series B-Biological sciences 270:2475-2480

Munang'andu, H.M., V. Siamudaala, W. Matandiko, A. Nambota, J.B. Muma, A.S. Mweene and M. Munyeme. 2011. Comparative intradermal tuberculin testing of free-ranging African Buffalooes (Syncerus caffer) captured for Ex Situ conservation in the Kafue Basin Ecosystem in Zambia.

Nelson A, 1999. The Cost of Disease Eradication: Smallpox and Bovine Tuberculosis. Annals of the New York Academy of Science, 894:83-91.

Owen-Smith, N.and J. Ogotu, 2003: Rainfall influences on ungulate population. In: DuToit, J.T., K.H. Rogers and H.C. Biggs (eds), The Kruger Experience: Ecology and Management of Savannah Heterogeneity, pp 310-331. Island Press, Washington DC.

Paweska, J.T., F.J. Burt, F. Anthony, S.J. Smith, A.A. Grobbelaar and J.E. Croft ,2003: IgG-sandwich and IgM-capture enzyme-linked immunosorbent assay for the detection of antibody to Rift Valley fever virus in domestic ruminants. Journal of Virological Methods.113, 103-112.

Paweska, J.T, E. Mortimer, P.A. Leman, R. Swanepoel, 2005: An inhibition enzyme-linked immunosorbent assay for the detection of antibody against Rift Valley fever virus in domestic and wild ruminant sera. Onderstepoort Journal of Veterinary Research 70:49-64.

Paweska, J.T., P.J. VanVuren, A. Kemp, P. Buss, R.G. Bengis, F. Gakuya, R.F. Breiman, M.K. Njenga, R. Swanepoel, 2008: Recombinant nucleocapsid – based ELISA for detection of IgG antibody to Rift Valley fever virus in African Buffalo. Veterinary Microbiology 127: 21-28.

Paweska, J.T., P. Jansen van Vuren, A. Kemp, R. Swanepoel, P. Buss, R.G. Bengis, F. Gakuya, R.F. Breiman and M. Kariuki Njenga, 2010: A recombinant nucleocapsid-based indirect ELISA for serodiagnosis of Rift Valley fever in African wildlife. In: Odongo, N.F., M. Garcia and G.J. Vilioen (eds) Proceedings of Sustainable Improvement of Animal Production and Health, pp309-312. FAO, Rome 2010.

Pederson, A.B., and A. Fenton, 2008. Emphasizing the ecology in parasite community ecology. TRENDS in Ecology and Evolution 22(3): 133-139.

Pepin, M., M. Bouloy, B.H. Bird, A. Kemp and J. Paweska, 2010: Rift Valley fever virus (Bunyaviridae: Phlebovirus): an update on pathogenesis, molecular epidemiology, vectors, diagnostics and prevention. Veterinary Research 41(6).

Pirson, C., G.J. Jones, S. Steinbach, G.S. Besra and H.M. Vordermeier, 2012: Differential effects of Mycobacterium bovis - derived polar and apolar lipid fractions on bovine innate immune cells. Veterinary Research 43: 54

Plowright, W. 1982. The effects of rinderpest and rinderpest control on wildlife in Africa. Symposia of the Zoological Society of London 50:1–28

Pollock J.M., J.D. Rogers, M.D. Welsh and J. McNair. 2006. Pathogenesis of bovine tuberculosis: The role of experimental models of infection. Veterinary Microbiology 112:141-150

Prins H.T. 1996. Ecology and Behavior of the African Buffalo: Social Inequality and Decision Making. London: Chapman and Hall.

Raja A. 2004. Immunology of tuberculosis. Indian Journal of Medicine and Research 120:213-232

Rodwell, T.C., I.J. Whyte and W.M. Boyce. 2001. Evaluation of population effects of bovine tuberculosis in free-ranging African buffalo. Journal of Mammalogy 82(1): 231-238.

Rostal, M.K., A.L. Evans, R. Sang, S. Gikundi, L. Wakhule, P. Munyua, J. Macharia, D.R. Feiken, R.F. Breiman, M.K. Njenga and M. Kariuki, 2010: Identification of potential vectors and detection of antibodies against Rift Valley fever virus in livestock during interepizootic periods. American Journal of Veterinary Research 71(5):522-526.

Sadd B.M. and P. Schmid Hempel. 2009. Principles of Ecoimmunology. Evolutionary Applications 2:113-121

Schiller, I., H.M. Vordermeier, W.R. Waters, M. Palmer, T. Thacker, A. Whelan, R. Hardegger, B. Marg-Haufe, A. Raeber and B. Oesch. 2009: Assessment of Mycobacterium tuberculosis OmpATb as a novel antigen for the diagnosis of bovine tuberculosis. Clinical Vaccine Immunology 16(9): 1314-1321

Schulenberg H., J. Kurtz, Y. Moret and M.T. Siva-Jothy. 2009. Introduction. Ecological Immunology. Proceedings of the Royal Society B 364:3-14

Scott, R.M., F.M. Feinsod, I.H. Allam, T.G. Ksiazek, C.J. Peters, B.A. Botros and M.A. Darwish. 1986. Serological tests for detecting Rift Valley fever viral antibodies in sheep from the Nile Delta. Journal of Clinical Microbiology 24(4): 612-614

Seydack, A.H., C.C. Grant, I.P. Smit, W.J. Vermeulen, J. Baard and N. Zambatis. 2011. Large herbivore population performance and climate in a South African semi-arid savanna. Koedoe 54(1): 1-20

Sinclair, A.R.E., 1977: The African buffalo: a study of resource limitation of populations, University of Chicago Press, Chicago.

Singh, K.V., O.A. Osman, I.F. El Cicy and T.I. Baz, 1967:Colostral Transfer of rinderpest neutralizing antibody to offspring of vaccinated dams. Canadian Journal of Comparative Medical Veterinary Science 31, 295-298.

Stear M. J., S.J. Bishop, J.L. Duncan, Q.A. McKellar and M. Murray. 1994. The repeatability of faecal egg counts, peripheral eosinophil counts, and plasma pepsinogen concentrations during deliberate infections with Ostertagia circumcincta. International Journal For Parasitology 25:375-380.

Swanepoel, R., J.K. Struthers, M.J. Erasmus, S.P. Shepherd, G.M. McGillivray, A.J., Shepherd, D.E. Hummitszch, B.J. Erasmus, and B.J.H. Barnard, 1986: Comparative pathogenicity and antigenic cross-reactivity of Rift Valley fever and other African phleboviruses in sheep. Journal of Hygiene, 97, 331-346.

Swanepoel, C. and J. Coetzer, 2006: Rift Valley Fever. Infectious Diseases of Livestock, Oxford UP: Oxford. 1037-1070.

Telfer, S. and K. Bown. 2012. The effects of invasion on parasite dynamics and communities. Functional Ecology 26:1288-1299

Thacker, T.C., M.V. Palmer and W.R. Waters, 2007. Associations between cytokine gene expression and pathology in Mycobacterium bovis infected cattle. Veterinary Immunology and Immunopathology 119: 204-213

Tieleman B.I., J.B. Williams, R.E. Ricklefs and K.C. Klasing. 2005. Constitutive innate immunity is a component of the pace-of-life syndrome in tropical birds. Proceedings of the Royal Society B 272:1715-1720

Thomson, G.R., 1996: Foot and Mouth Disease in the African Buffalo. Proceedings of a Symposium on African Buffalo as Game Ranch Animal, ed. BL Penzhorn, p. 13.

Thorne, E.T. and E.S. Williams. 1988: Disease and endangered species: the black-footed ferret as a recent example. Conservation Biology 2,: 66–74.

Tizard I. 2004. Veterinary Immunology. Saunders, Philadelphia

Trinchieri, G. 2003. Interleukin 12 and the regulation of innate resistance and adaptive immunity. Nature Reviews in Immunology 3(2):133-146

Turell, M.J., D.J. Dohm, C.N. Mores, L. Terracina, D. L. Wallette, Jr., L.J. Hribar, J.E. Pecor, and J. A. Blow, 2008: Potential for North American Mosquitoes to Transmit Rift Valley Fever Virus1. Journal of the American Mosquito Control Association 24 (4), 502-507.

Van der Lugt, J.J., J.A. Coetzer and M.M. Smit. 1996. Distribution of viral antigen in tissues of new-born lambs infected with Rift Valley fever virus. Onderstepoort Journal of Veterinary Research 63(4): 341-347

Venter, F.J., R.J. Scholes and H.C. Eckhardt, 2003: The abiotic template and its associated vegetation pattern. In: DuToit, J.T., K.H. Rogers and H.C. Biggs (eds), The Kruger Experience: Ecology and Management of Savannah Heterogeneity, pp 83-129. Island Press, Washington DC.

Vordermeier, M., A. Gobena, S. Berg, R. Bishop, B.D. Robertson, A. Aseffa, R.G. Hewinson and D.B. Young. 2012. The influence of cattle breed on susceptibility to bovine tuberculosis in Ethiopia. Comparative Immunology and Microbiology and Infectious Disease 35(3):227-232

Vredenburg, V.T., R.A. Knapp, T.S. Tunstall and C.J. Briggs. 2010: Dynamics of an emerging disease drive large-scale amphibian population extinctions. Proceedings of the National Academy of Sciences of the United States of America 107: 9689-9694

Waters, W.R., M.V. Palmer, T.C. Thacker, W.C. Davis, S. Sreevatsan, P. Coussens, K.G. Meade, J.C. Hope and D.M Estes. 2011: Tuberculosis Immunity: Opportunities from Studies with Cattle. Clinical and Developmental Immunology. doi:10.1155/2011/768542

Welsh, M.D., R.T. Cunningham, D.M. Corbett, R.M. Girvin, J. McNair, R.A. Skuce, D.G. Bryson and J.M. Pollock: 2005. Influence of pathological progression on the balance between cellular and humoral immune responses in bovine tuberculosis. Immunology 114: 101-111

Widdison, L.J., J. Schreuder, B. Villarreal-Ramos, C.J. Howard, M. Watson and T.J. Coffey. 2006: Cytokine expression profiles of bovine lymph nodes: effects of Mycobacterium bovis infection and bacille Calmette–Guérin vaccination. Clinical and Experimental Immunology 144:281-289

Wilcoxen T.E., R.K. Boughton and S.J. Schoech. 2010. Selection on innate immunity and body condition in Florida scrub-jays throughout an epidemic. Biology Letters 6:552-554

Wonham, M.J., M.A. Lewis, J. Rencławowicz and P. van den Driessche, 2006: Transmission assumptions generate conflicting predictions in host–vector disease models: a case study in West Nile virus. Ecology Letters 9(6): 706-725.

Wood, P.R. and S.L. Jones. 2001: BOVIGAMTM: an in vitro cellular diagnostic test for bovine tuberculosis. Tuberculosis 81: 147–155.

Woods, C.W., A.M. Karpati, T. Grein, N. McCarthy, P. Gaturuku, E. Muchiri, et al. 2002: An outbreak of Rift Valley fever in northeastern Kenya, 1997–98. Emerging Infectious Diseases.;8(2):138-44.

van Vuren, P.J., C.T. Tiemessen and J.T. Paweska. 2011 Anti-Nucleocapsid Protein Immune Responses Counteract Pathogenic Effects of Rift Valley Fever Virus Infection in Mice. PLoS ONE 6(9): e25027. doi: 10.1371/journal.pone.0025027

Xu, W., D.M. Watts, M.C. Constanzo, X. Tang, L.A. Venegas, F. Jiao, A. Sette, J. Sidney, A.K. Sewell, L. Woolridge, S. Makino, J.C. Morrill, C.J. Peters and J. Kan-Mitchell. 2013. The nucleopcapsid protein of Rift Valley Fever Virus Is a Potent Human CD8+ T cell Antigen and Elicits Memory Responses. PLOS ONE 8(3):e59210 doi:10.137/journal.pone.0059210

Xue, L., H.M. Scott, L.W. Cohnstaedt, C. Scoglio, 2012: A network-based meta-population approach to model Rift Valley fever epidemics. Journal of Theoretical Biology, Volume 306, Pages 129-144, DOI 10.1016/j.jtbi.2012.04.029.

Zeller, H.G., D. Fontenille, M. Traore-Lamizana, Y. Thiongane and J.P. Digoutte, 1997: Enzootic activity of Rift Valley fever virus in Senegal. American Journal of Tropical Medical Hygiene 56(3), 265-272.

Zhao, J.,K.D. Kim, X. Yang, S. Auh, Y.X. Fu and H. Tang. 2008. Hyper innate responses in neonates lead to increased morbidity and mortality after infection. Proceedings of the National Academy of Science USA 105:7528–7533.