AVIAN HAEMOPARASITE PREVALENCE IN KRUGER NATIONAL PARK AND THE SURROUNDING HUMAN SETTLEMENTS

by

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A dissertation submitted to the Faculty of Science, University of the Witwatersrand, Johannesburg, in fulfilment of the requirements for the degree of Master of Science

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30 May 2018

DECLARATION

I declare that this dissertation is my own, unaided work. It is being submitted for the Degree of Master of Science at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at any other University.

(Signature of candidate)

30th day of May 2018

at Johannesburg

Abstract

The drivers and implications of avian haemoparasite infection in wild birds are complicated to understand and predict, especially in areas where infections are endemic and the parasites have co-evolved together with their hosts. I studied the prevalence of avian haemoparasite infections in the Kruger National Park and the impact of haematozoa on host immune response. Six sites were sampled and blood from 685 birds of 87 species was microscopically screened for parasites. *Haemoproteus*, microfilariae, *Trypanosoma*, *Plasmodium*, *Leucocytozoon*, *Aegyptianella* and *Hepatozoon* spp. were detected. Overall prevalence was 27.33 % with 29 cases of mixed infections, which were mostly in association with *Haemoproteus*. Prevalence was similar for all sites and seasons, with no apparent influence of host life history traits on infection. Interestingly, immune status and body condition were better in infected than uninfected individuals. These findings reveal the complex relationship between parasites and their avian hosts in a southern African environment.

In memory of my father

Stephen Pori

1957 – 2013

It is my hope that this achievement has fulfilled part of the dreams you had for me, during the years when you sacrificed to provide me with best the education you could find

ACKNOWLEDGEMENTS

I would like to extend my most heartfelt thanks, gratitude and appreciation to the following people: My Principal Supervisor, Dr Mduduzi Ndlovu, and Co-Supervisor, Prof. Miles Markus, with whom all this work was done. I thank them for affording me this opportunity, their patience, guidance and constructive critical comments.

Secondly, I thank Alfredo Colina, Miranda Thomas, Damaris Chenoweth, Jackson Siminara, Maliki Wardomto, Tapiwa Gumbo and many other undergraduates from the Organisation for Tropical Studies for their help with fieldwork.

Grateful thanks go to SANparks and Bushbuckridge Local Municipality for permission to conduct this project. The staff at SANparks deserve special thanks, in particular Samantha Mabuza for logistical arrangements, as well as Dr Danny Govender and Purvance Shikwambane for assistance and permission to use the laboratory. Thanks also to Judith Botha and Adolf Manganyi for organising my accommodation in the park; it was indeed a privilege living in such wilderness for two years.

Deep appreciation goes to Dr Michael Peirce for taking the time to share his expertise and knowledge as regards the identification of avian parasites. It was through this time that I felt I was able to learn and improve the most in developing my skills in microscopy and parasitology in general.

Also, Donovan Tye, Bernard Coetzee, Keenan Stears and Melissa Schmitt kindly provided useful comments on my thesis. Without the funding from National Research Foundation (NRF) through the Wits Deputy Vice-Chancellor (Research), this work would not have been possible.

Last, but not least, I would like to express my sincere gratitude to Precious and the rest of my family for their undying and fervent support, encouragement and, most importantly, believing in me.

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CHAPTER 1

General Introduction

Disease ecology in natural populations aims at understanding how environmental conditions shape species interactions that drive disease dynamics (Anderson and May 1978, Price *et al.* 1986, Poulin 2010). Recently, the consequences of landscape heterogeneity for ecological processes and the environmental correlates that perpetuate the spread of infectious diseases have become a central theme in disease ecology (Sehgal *et al.* 2011). The environment influences the ecological processes such as the frequency of host-parasite interactions, host community assembly processes, and dispersal processes of host, vector, and parasite populations that collectively influence pathogen emergence and infectious disease spread (Watts 2015).

The implications of landscape heterogeneity for parasitic disease communities are poorly understood (LaPointe *et al*. 2012), although it is thought that anthropogenic impacts such as human and domestic animal population encroachment, agriculture and increased host density in captive populations may contribute to heightened susceptibility to outbreaks involving both pathogenic parasites and other pathogens (Daszak *et al*. 2001). Parasites are an integral and important component of natural communities, not only because they represent a substantial proportion of the species diversity and biomass (Poulin and Morand 2004, Kuris *et al*. 2008) but also because they can directly or indirectly alter the composition of the community by impacting the number of free-living species or their relative abundance (Mouritsen and Poulin 2005, Wood *et al.* 2007).

Birds are useful indicators of biodiversity abundance in an area (Mikusinski *et al*. 2001). Hence their population trends can reflect the functioning of the ecosystem (Shackelford and Reid 1989). Also, avian species have developed a stable mutual association with their habitats (Shackelford and Reid 1989), such that any changes to this relationship ultimately alter the ecology and behaviour of birds, making them an important tool in understanding ecosystem health (Eidson *et al.* 1999). For instance, the expansion of human populations, coupled with climate change, deforestation and urbanisation, leads to irreversible effects on the habitats of wildlife and their pathogens (Sehgal 2015). Given that birds are particularly well adapted to serve as reservoirs of various pathogens, including hosting of disease-causing parasites, avian diversity offers an opportunity to study host-parasite relationships (Causey and Edwards 2008). Despite the widespread distribution of avian haemoparasites, the variables that drive the spatial distribution and diversity of species within a genus are still poorly understood. There are more

than 200 described haemosporidian species, classified by taxonomists in the genera *Plasmodium, Haemoproteus, Leucocytozoon* and *Fallisia*. However, accurate species identification is one of the major obstacles to studying the diversity and distribution of haemosporidian parasites of birds (Martinsen *et al*. 2006). Furthermore, though the morphology of microfilarial parasites can be valuable for identifying its genus, it is difficult to identify the species without examining the adult worm (Bartlett 2008).

The parasites that have been observed in blood smears most commonly are *Haemoproteus* and *Plasmodium,* which are responsible for causing avian malaria (Benedict *et al*. 2009, La Pointe *et al.* 2012), to use the term "malaria" broadly and as it was sometimes used historically (by including *Haemoproteus*).

The prevalence of Haemosporidia in birds seems to have increased in African endemic areas, possibly because of climate change (Roger 2015). These parasites are widely distributed and are transmitted to the host by vectors (Valkiūnas 2005), as explained further two paragraphs below. A number of species of *Plasmodium* have been reported as the causal organisms of avian malaria, a significant form of haemosporidian infection. Although avian haemoparasites are generally of little clinical significance, some infections may be pathogenic in naive hosts (Warner 1968) or when hosts are subjected to stressful environmental conditions (Graham *et al.* 2016). A well-documented example is that of the Hawaiian Islands, where accidental introduction of avian malaria (*Plasmodium relictum*) and one of its vectors took place. This caused mass mortality of birds. Species distributions were also affected and some species became endangered (van Riper *et al.* 1986). Similar high mortality rates after exposure to avian malaria have likewise been recorded for African black-footed penguins *Spheniscus demersus* in South Africa (Daszak *et al*. 2000). Such documented threats to biodiversity warrant acquisition of a deeper understanding of avian haemoparasite prevalence in less studied species and regions.

Haemosporidians (Sporozoa: Haemosporida) are covered here by the description "haemoparasites" (but this latter term also includes microfilariae, *Trypanosoma* etc.). Haemosporidians are an exclusive group of parasites that are transmitted by dipteran insects (Insecta: Diptera) to birds (Valkiūnas 2005). Trypanosomes (Kinetoplastea: *Trypanosoma*) are common dixenous parasites of three different evolutionary groups and several lineages and are also transmitted by insects (Zidkova *et al.* 2012, Svobodová *et al.* 2015). They are parasites of concern for the reason that they cause diseases in both humans and animals (Kirchloff 2001). Although trypanosomes are considered to be less pathogenic in wild birds, they nevertheless have the potential to elicit clinical signs in some instances (Mandel *et al.* 2008).

Microfilariae are an immature parasite form produced by adult female filarioid nematodes living in the avian host. These microfilariae are ingested by a vector when it takes a blood meal and development then takes place in the vector which can, later, inoculate infective larvae into another host (Bartlett 2008). Adult filarial worms occur in various organs in birds and are difficult to detect (Campbell 1995).

The complex heteroxenous life cycle and epidemiology of haemosporidians (Valkiūnas 2005) and that of trypanosomes (Slapeta *et al.* 2015) are similar in that they both infect cellular components of blood and the assessment of their impacts on hosts is extremely difficult to understand (Valkiūnas 2005, Svobodová *et al.* 2015). Environmental change and the expanding human population increase the likelihood of domestic poultry coming into contact with wild birds, which can facilitate disease crossover (Jones *et al.* 2013). Moreover, as infected animal and human populations increase their ranges and meet with naive populations, the consequences of new infections can be devastating (Lindahl and Grace 2015). When this takes place, unstable host-parasite systems are formed, which cause severe epizootics (Valkiūnas 2004). Anthropogenically induced distortions of the balance in natural ecosystems also lead to epizootics among wild birds (Valkiūnas 2005). Haemoparasites in birds in the deforested areas of the African rainforest have exceeded a 30% prevalence rate, with trypanosome infection involving *T. avium* and *T. everetti* being the most commonly detected (Valkiūnas *et al.* 2011).

Furthermore, the determinants and consequences of infection amongst wild birds are challenging to estimate, especially in areas where infections are endemic and the hosts and parasites have evolved together (Graham 2016). Wild birds and their parasites have undergone a long period of co-evolution and mutual adaptation (Valkiūnas 2005). The interaction of various factors (genotype, immunological status, age, food resources, stressors, availability of shelters, etc.) can bring about unforeseen impacts on the ecology of diseases.

Our understanding of various species of Haemosporidia is poor (Valkiūnas 2005). This is because most of the work done on Haemosporidia to date has been focused on human malaria parasites, being of medical importance. Only a few parasites that are used as model organisms in research on avian haemoparasitaemia (family Plasmodiidae) have received proper attention (Valkiūnas 2005). Other groups of haemosporidians, primarily representatives of the families Haemoproteidae, Leucocytozoidae and Garniidae have been studied to a relatively lesser extent. This has slowed down the progress of scientific research in the field of parasitology (Valkiūnas 2005, Svobodová *et al.* 2015). Mosquitoes have been found to be carriers and transmitters of many avian haemosporidians and *T. culicavium* protozoa. The former reproduce in erythrocytes of the infected host (Valkiūnas 2005, Slapeta *et al.* 2015). Blood-sucking

dipteran vectors such as blackflies (Simuliidae), biting midges (Ceratopogonidae) and hippoboscid flies (Hippoboscidae) are known to transmit trypanosomes. When haemosporidan parasites reproduce in their hosts, they lose red blood cells, which can eventually result in anaemia when the parasite load becomes sufficiently high (Atkinson 2005). Human malaria parasites cause similar effects (Valkiūnas 2005, Braga *et al*. 2011).

Few studies have compared the traditional morphological methods used to identify these parasites with modern molecular techniques of identification (e.g. Garamszegi 2010, Krams *et al.* 2012, Okanga *et al*. 2013) and thus the true diversity of the parasites, especially in summer rainfall areas of southern Africa, is unknown (Martinsen *et al*. 2006). It is, however, appropriate to adopt a holistic approach, involving both microscopic and molecular methodology, in order to acquire a better understanding of host-parasite relationships in the wild (Nordling *et al*. 1998).

Although the reasons for the spatial distribution of avian haemoparasites are unclear, global warming has been implicated as extending the breeding ranges and cycles of ectothermic vectors such as mosquitoes (Rogers 2015). Additionally, avian malaria parasites have been reported to affect host competitiveness in naïve host populations (Valkiūnas 2005, Lachish *et al.* 2011) and possibly lead to extinction of bird species (van Riper III *et al.* 1986), because parasitaemia affects host function (Valkiūnas 2005).

The consequences of avian haemoparasite infections for hosts in endemic areas of Sub-Saharan Africa are not well understood (Lachish *et al.* 2011, Njabo *et al.* 2011). The dynamics of the pathology of most avian species of *Plasmodium* and *Haemoproteus* in birds ranges from chronic infections to acute infections that may have devastating effects on host survival and populations (LaPointe *et al*. 2012). On the other hand, there are conflicting results as to whether infections in wild populations where haemoprotozoan transmission is endemic, and hosts have a long evolutionary history with these parasites, actually has any appreciable fitness effects (Korpimaki 1993, Sanz *et al.* 2001, Marzal *et al.* 2005).

Studies based on the polymerase chain reaction (PCR) together with microscopy have shown that double infections are common in host-parasite associations (Marzal *et al*. 2008). However, research concerning the true burden of these mixed infections on hosts has been limited, with the result that the recent results reported are inconclusive (Marzal *et al*. 2008).

Host traits

The role of wild birds as reservoirs for parasitic diseases and their impact on host ecology has been extensively discussed (Loye and Zuk 1991). The practical importance of studying avian haemosporidians is that it provides a unique opportunity to analyse the agents of diseases in

this group of parasites (Haemosporidia), which can threaten the survival of birds. Considering the spatial variations across the world in relation to the occurrence of parasites in birds, it has been suggested that avian migratory behaviour may have evolved as a way of avoiding infections (Møller and Szép 2010, Altizer *et al.* 2011). This hypothesis is supported by Jenkins *et al.* (2012), who found a much higher diversity of *Leucocytozoon* species in the blood of migratory birds than in resident birds. The distribution of parasites globally can be determined by the movement of birds, especially when they introduce novel parasites into a new environment (Altizer *et al.* 2011). For example, it is thought that migration of birds to the Hawaiian Islands introduced problematic avian malaria parasites there (LaPointe *et al.* 2012, Levin *et al.* 2013).

Social nesting behaviour has been known to reduce nest predation (Hamilton 1971), but it is also associated with higher parasite prevalences and load (Brown and Brown 1996, Hoi *et al*. 1998). Parasite load is dependent upon the density of the host population and the way in which a parasite is transmitted (Fenton *et al*. 2002). There is a positive correlation between the transmission of parasites and the density of hosts (density-dependent transmission). Another hypothesis suggests that there is a positive correlation between the transmission of parasites and the frequency of interactions between the host and the parasite's infective stage, i.e. frequency-dependent transmission (Bush *et al*. 1997).

A study by Astudillo *et al.* (2013) noted differences in the probability of occurrence of haemoparasite infections between birds of different foraging guilds. Bird species that foraged in the low-middle vertical stratum were more prone to *Haemoproteus* spp. infection. In comparison, bird species foraging in the middle-upper vertical stratum were at a higher risk for *Plasmodium* spp. infections compared to birds foraging on the ground. *Leucocytozoon* spp. infections were more common in ground vertical stratum foraging birds compared to other foraging heights, whereas the middle-upper vertical stratum was dominated by *Trypanosoma* spp. The prevalence of microfilariae was similar across different foraging guilds. Other studies have found the prevalence of *Haemoproteus* spp. and *Leucocytozoon* spp. to be positively correlated to nest height and foraging stratum because the potential vectors for these parasites, i.e. biting midges (Ceratopogonidae) and black flies (Simuliidae) are more active in understory-canopy than ground strata (Cerny *et al.* 2011, Swanson *et al.* 2012, González *et al.* 2014).

Study design

The threats posed to biodiversity as well as human and wildlife health by avian parasitism (Edison *et al.* 1999) demand that we better understand the factors that drive parasite prevalence.

Epidemiological studies have shown that temporal variation in the prevalence and the severity of infections can be driven by seasonal variations (Cook *et al*. 1990, Randolph *et al*. 2000). Climatic variables are thought to have the greatest impact on the development of parasitic diseases. Most vectors are exothermic and their ecology is strongly influenced by seasonal variations in temperature. Their efficacy is usually higher during summer and suppressed in winter, as temperatures become less favourable (Rogers and Randolph 1988).

The primary interest behind the research in Kruger National Park is to acquire an understanding of the prevalence of avian haemoprotozoa there and in the surrounding human settlements. The findings will contribute towards both the ecological and veterinary monitoring of diseases within the park. The results are also useful for the potential risk mapping of avian malaria that could be transmitted to domestic poultry in surrounding human settlements or *vice versa*. Given that parasitism is driven by environmental and avian intrinsic factors (Sehgal 2015), I therefore hypothesise that both the environment and host life history traits affect avian parasite prevalence. My first objective is to explore how season (wet vs dry), study areas and land use type (inside vs outside the park) affect parasite prevalence. The second objective will examine how avian parasite prevalence differs by host species, and whether it seems to be affected by intrinsic traits such as, foraging height and gregariousness. I assume that haemoparasites in birds at sites in the southern region of the park would be more prevalent, considering a high rainfall gradient from the south to the north and which has a direct impact on potential vector establishment. Thirdly, I also investigate, using selected criteria, the possible impact of parasitism on host body condition and immunity. Each of these three objectives form the basis of my thesis chapters.

Study sites

Data collection was carried out from May 2015 to July 2016 in the Kruger National Park (KNP) and the nearby surrounding human settlements (Figure 1). The KNP lies in the northern part of South Africa, spanning the Limpopo and Mpumalanga provinces (Jupp 1996). The land use around the protected area is dominated by small-scale cropping and communal conservation areas (Ronaldson 2008). The park covers an area of approximately 20 000 km^2 in the two provinces and is located within the Lowveld region (Ronaldson 2008) in low-lying savannas, with elevations from about 250 m to a small section over 800 m above sea level (Mucina and Rutherford 2006).

The climate of the Kruger National Park is subtropical. Summer days are humid and hot with temperatures often soaring to above 38 °C. Winters are mild and generally frost-free.

The rainy season is from September until May, with a gradient of rainfall decreasing from the south (750 mm per annum) to the northern parts of KNP (350 mm per annum). Drought is endemic in the KNP, despite this rainfall gradient (Ronaldson 2008). Habitats comprise wide-open savanna in the south of the park, whereas the north is characterised by Mopani woodlands. The most dominant trees include Apple leaf, Tamboti, Baobab and Leadwood, to mention but a few (Mucina and Rutherford 2006). This variation in microhabitats allows the KNP to support a wide diversity of approximately 520 bird species, half of which are resident, 107 have a limited distribution range, 94 are seasonal**,** 109 are cryptic while 18 are purely nocturnal (Barnes and Behrens 2014)

Surveys have shown that *Aedes* and *Culex* spp. mosquitoes are common in the KNP (Jupp 1996), but little is known about the distribution and densities of non-mosquito potential vectors in the region that are capable of transmitting avian haemoparasites (Becker *et al.* 2010)

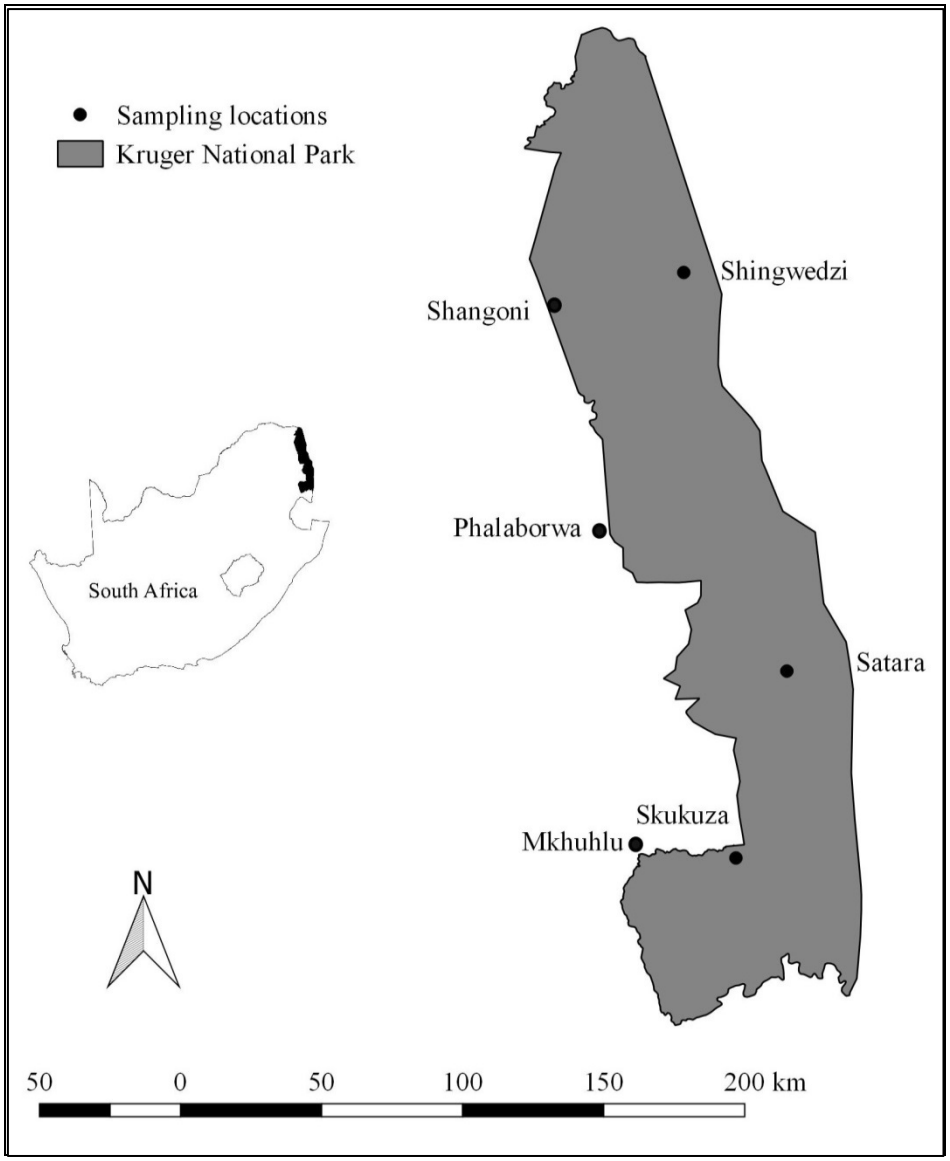

Figure 1.1 Geographical locations of the study sites inside and outside the Kruger National Park

The primary focus was to get a representative sample of birds that have different host traits and occupy the different sites found in the study areas. Birds were trapped at five sites in the Kruger National Park, namely: Skukuza (24º 99′ S, 31º 60′ E), Satara (24º 39′ S, 31º 77′ E), Shangoni (23º 45′ S, 30º 97′ E) and Shingwedzi (23º 11′ S, 31º 43′ E). Skukuza lies in the southern area, while Shingwedzi and Shangoni are in the northern region of the park. Satara is located in the central part. Birds in two adjacent settlements immediately outside the park were also sampled in an attempt to determine whether different land use types and human disturbance can influence the prevalence of avian parasites. Phalaborwa (23º 94′ S, 31º 14′ E) was the outside

settlement in the north, while Mkhuhlu (24º 59´ S, 31º 14´ E) was an adjacent settlement in the southern area of the park (see Figure 1.1).

Thesis outline

Each chapter is structured and written as an individual paper to ease the passage of future publication. This has resulted in the repetition of some literature citations and parts of the methodology explanations. The second chapter attempted to assess the influences of the environment and bird life-history traits on avian haemoparasite prevalence. The objective was to determine the identity and prevalence of haemoparasites at all the study sites and, further, to try to examine the effects of landscape heterogeneity on parasite communities. To the extent that it was feasible, I analysed how different environments might influence the prevalence of avian haemoparasites. Seasonality was considered, to assess how it may generate annual and geographic variations in the timing and severity of epidemics. Differences in parasite prevalence between sites (inside vs outside the park and the north-south vegetation contrast) were also investigated. In essence, I tested the latitude and rainfall gradient effects on prevalence. Intrinsic life-history traits such as social grouping and solidarity of birds were assessed in regard to how they might affect infection by blood parasites. I also explored whether foraging behaviour could be related to haemoparasite prevalence.

Chapter 3 focused on the consequences of parasite infection for avian immunity and body condition. In this chapter, I evaluated the impact of haemoparasites on the immunity of birds. This was done by assessing how the parasites influenced differential white blood cell count profiles, as a measure of integrated immune function. Generally, an elevated total number of leucocytes indicates an inflammatory process in response to both microbiological and macroparasite infections (Dein 1986). I then looked at how infection affected the body condition of parasitised birds. Furthermore, the impacts of double or multiple haemoparasite infections on the host were also considered.

In the final chapter, I summarise my findings in relation to the objectives outlined in chapters two and three. A complete study synthesis is done, to reflect upon the extent to which the environment and life history traits of birds might influence their susceptibility to haemoparasite infections. In conclusion, I suggest applications of my findings to management strategies in disease ecology and propose research directions for future studies.

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CHAPTER 2

Influence of the environment and life history traits on avian haemoparasite prevalence

Abstract

Prevailing environmental change events are likely to affect the distribution and prevalence of parasitic diseases. The prevalence of haematozoan infections in birds caught in and around the Kruger National Park was determined in order to test the hypothesis that environmental and life history traits influence parasitaemia. Microscopy was used to screen blood smears prepared from captured birds (685 individuals of 87 species). The most commonly occurring avian haemoparasite was *Haemoproteus* (prevalence: 18.57 %) and it was mainly detected in starlings (Family Sturnidae). Other parasites identified were microfilariae (4.99 %), *Trypanosoma* (4.34 %), *Plasmodium* (4 %), *Leucocytozoon* (1.03 %), *Aegyptianella* (0.42 %) and *Hepatozoon* (0.05 %) spp. Greater Blue-eared Starlings (n = 5) and House Sparrows (n = 5) had the highest diversity of parasites. There were 29 cases of double infections, which were mostly in association with *Haemoproteus* (n = 27), the leading combination having been *Haemoproteus* together with *Plasmodium* (n = 13). Overall, haemoparasite prevalence was similar for all sites and seasons. However, the highest parasite diversity was recorded in the southern regions of the park, and there was no detectable influence of host life history traits on infection. Findings suggest that the timing and occurrence of parasitaemia in the park is not necessarily mediated by site or season, and overall haemoparasite infection persisted everywhere, all year round. These findings represent new knowledge on avian haemoparasite prevalence in a subtropical protected area of South Africa.

Introduction

Climate change is affecting the spread of parasitic diseases worldwide, with serious implications for human health and biodiversity conservation (Gavrilles 2013). The transmission of parasites between infected and non-infected populations is important, given the effects of climate change on wildlife distribution. It is predicted that global warming will change the spatio-temporal distribution of wildlife, parasites and vectors, which can lead to altered infection risks (Dobson and Foufopoulos 2001, McMichael *et al.* 2003) and new disease outbreaks in historically isolated populations (Hobbelen *et al*. 2012). Understanding how wildlife parasitic diseases are distributed and function can help to predict, mediate and shape climate change response, guide conservation practice, and protect livestock (including poultry) from acquiring infections from wildlife.

Birds are a highly diverse group of vertebrates and both they and their parasites have a worldwide distribution. This makes birds a perfect model for developing an understanding of the drivers and effects of parasitism in the wild (Valkiūnas 2005). Although avian haemoparasites are ubiquitous, considerable variation exists in both prevalence and parasitaemia at different spatio-temporal scales (Atkinson and Van Riper III 1991, Valkiūnas 2005). The protozoa in the blood are classified in the order Haemosporida, an exclusive group of unicellular sporozoan (apicomplexan) organisms that infect birds, amongst other vertebrates, through transmission by insect vectors belonging to the order Diptera (Valkiūnas 2005). The geographic distribution of haemoparasites as revealed by their prevalence is thought to be determined by the environment and seasonal meteorological variations (Møller and Nielsen 2007). These factors influence the spatio-temporal changes in vector abundance and density (Bensch and Akesson 2003, Møller and Nielsen 2007, Dunn *et al*. 2011). Since insect vectors are ectothermic, it is to be expected that environmental temperature directly affects vector traits and ecology (Gallana 2013).

Epidemiological studies have also demonstrated the importance of seasonal weather fluctuations in affecting geographical variation in the timing and severity of disease outbreaks, involving latitudinal and altitudinal zonation effects on the emergence and existence of parasitic infections (Cook *et al*. 1990, Randolph *et al*. 2000). Although the environment and social interactions between avian species are considered to influence the distribution of haematozoa, climate is expected to be the most decisive contributor to frequency of infection. Seasonal variations in temperature (Rogers and Randolph 1988), elevation gradient and proximity to water bodies (Valkiūnas 2005, Dunn *et al.* 2011) determine the abundance of

vectors such as mosquitoes, ticks and other arthropods. Populations hereof may fail to become established; or their vectorial efficacy might become reduced when they are exposed environmentally beyond their ideal conditions for persistence (Rogers and Randolph 1988). Understanding the spatio-temporal correlations of disease prevalence in wild populations can help us to appreciate how environmental attributes drive the interactions amongst the species that influence epidemics of diseases (Anderson and May 1978, Price *et al.* 1986).

Generally, the conditions that affect avian haemoparasitaemia remain uncertain. It has nonetheless been suggested that alteration of the environment through anthropogenic changes and global warming increase the geographical distribution (range) of vectors and result in modification of their breeding cycles and habitats (Rogers 2015). Environmental change and expanding human populations have also been implicated in increasing the risk of domestic poultry coming into contact with wild birds, thereby enabling disease crossover to potentially take place (Jones *et al.* 2013). It has been shown that human-related environmental disturbances such as agriculture, the introduction of wild animals into captivity and the intrusion of human and livestock populations may cause host–parasite systems to become unstable (Valkiūnas 2005). This eventually increases the possibility that a given parasitic disease will be problematic (Daszak *et al*. 2000). Furthermore, severe epizootics are likely to have disastrous impacts on naive hosts (Valkiūnas 2005). There have, however, been conflicting statements by Valkiūnas (2005), who concluded that there is a reduced probability of haemosporidian infection in areas with a higher anthropogenic load, due to the elimination of vectors or reduction in their populations caused by the use of insecticides, pesticides, and pollution of the environment.

Although avian parasites are globally distributed, the factors that affect the spatial variations in the prevalence of various parasite lineages amongst their respective hosts are poorly understood (Svobodova *et al.* 2007, Szollosi *et al*. 2011). Additionally, the causes and impacts of infection in natural bird populations are complex to determine and predict, particularly in areas of southern Africa where some organisms are endemic and have co-evolved with their hosts (Knowles 2014). The development of infection in hosts can be greatly influenced by intrinsic (physiological and immune status, age) and extrinsic (forage quality and abundance, habitat quality) parameters affecting the host (Valkiūnas 2005).

Most species of Haemosporidia are thought to be relatively host-specific and restricted to bird species of the same family (Ricklefs *et al*. 2005, Hellgren *et al*. 2008, Marzal *et al.* 2011). However, host specificity at the family level is not necessarily strict (Jaramillo *et al.* 2017). Parasite transmission is thought to be related to the density of the host population

(density-dependent transmission) or with the frequency of host exposure to infective parasites. Social behaviour may increase exposure and can also be associated with higher parasite prevalence and load (Brown and Brown 1996, Hoi *et al*. 1998). These findings are explained by the "amplification effect" hypothesis which predicts that a diverse host population has many more individuals that are susceptible to infection. The hypothesis also takes account of the fact that contact between infected and susceptible hosts is increased in a diverse host situation, which often leads to higher haemoparasite prevalences (Blomberg *et al.* 2003). Mixed-species foraging flocks have been found to be more susceptible to *Haemoproteus* spp. and *Leucocytozoon* spp. infections compared to solitary species (Astudillo *et al.* 2013). However, in contrast to the amplification effect hypothesis, the "dilution effect" hypothesis predicts divergent outcomes for the interactions between host diversity and parasite prevalence. The theory suggests that an increased diversity of hosts will decrease the number of susceptible hosts and also reduce the chances of contact between the susceptible and infected hosts, thereby lowering the prevalence of parasitic infections (Gregory 1990).

The study by Astudillo *et al.* (2013) noted differences in the probability of haemoparasite infections between birds of different foraging guilds. Bird species that foraged in the low-middle vertical stratum were more prone to *Haemoproteus* spp. infection. In comparison, bird species foraging in the middle-upper vertical stratum were at a higher risk of contracting *Plasmodium* spp. infections compared to the ground-foraging birds. *Leucocytozoon* spp. infections were more common in ground vertical stratum foraging birds compared to other foraging heights, while the middle-upper vertical stratum was dominated by *Trypanosoma* spp. The prevalence of microfilariae was similar across different foraging guilds. Other studies have found the prevalence of *Haemoproteus* spp. and *Leucocytozoon* spp. to be positively correlated with nest height and foraging stratum because the potential vectors for these parasites, i.e. biting midges (Ceratopogonidae) and black flies (Simuliidae), are more active in understory-canopy than ground strata (Cerny *et al.* 2011, Swanson *et al.* 2012, González *et al.* 2014).

Our understanding of the different groups of haemoparasites is limited. For example, despite the global distribution of avian trypanosomes, studies on this group of parasites have not been prioritised whilst they can be useful as models for host parasite interactions (Zidkova *et al.* 2012). In addition, parasitic infections, the ecology of vectors, and the exact timing and location of acquisition of infection within the life-history of hosts are also poorly documented (Valkiūnas 2005, Svobodova *et al.* 2007, Zidkova *et al.* 2012). Furthermore, most research in this field is biased towards human malaria, being of medical importance, as well as to a limited

number of other species in the family Plasmodiidae, including malarial parasites used for modelling avian haemoparasitaemia (Valkiūnas 2005). Parasites from the other groups of haemosporidians such as the families Haemoproteidae, Leucocytozoidae, and Garniidae have rarely been studied, and that hinders the progress of scientific research in parasitology (Valkiūnas 2005).

The focus of this study was to determine the prevalence of avian haemoparasites around the Kruger National Park so as to further understand the influence on infection prevalence of the environment and life history traits of the host. On the assumption that parasitism is driven by environmental and avian intrinsic factors (Sehgal 2015), I therefore hypothesised that both the environment and host life history traits affect avian haemoparasite prevalence. The first objective was to explore how study locations, season (wet vs dry), and land use type (inside vs outside the park) affect haemoparasite prevalence. The second objective was to consider how avian haemoparasite prevalence differed amongst host species, and whether it was affected by intrinsic traits such as foraging height and gregariousness. These research findings will contribute towards planning both the ecological and veterinary monitoring of avian parasitic diseases within the park.

Materials and methods

Study site

Birds were sampled at five sites in the Kruger National Park, namely: Skukuza (24º 99′ S, 31º 60′ E), Satara (24º 39′ S, 31º 77′ E), Shangoni (23º 45′ S, 30º 97′ E) and Shingwedzi (23º 11′ S, 31º 43′ E). Skukuza lies in the southern area of the park, whereas Shingwedzi and Shangoni are in the northern part. Satara is located in the central region. Blood from birds in two adjacent settlements immediately outside the park was also sampled, in order to determine whether different land use types and human disturbances can influence the prevalence of avian parasites. Phalaborwa (23º 94′ S, 31º 14′ E) was the outside settlement in the north, and Mkhuhlu (24º 59′ S, 31º 14′ E) was the chosen settlement adjacent to the southern part of the park. Full attributes of the study sites are described in detail in Chapter 1.

Fieldwork

So as to obtain a representative sample for the whole region, the primary focus was to get blood from as many bird species as possible that have different life history traits and occupy diverse habitats in different localities. The assumption was that birds foraging near water bodies have a higher haematozoan prevalence rate since they would spend a significant part of their day

closer to where vectors breed, thereby inadvertently increasing their chances of becoming infected. Birds that forage in tree canopies were expected to have lower prevalence of infection since the risk of transmission decreases with vertical distance (See host traits in Chapter 1 for a full review). Sampling sites were located in areas where there was a source of attraction for birds such as the waste disposal sites, water points and residential gardens. Birds were caught in 2016 using mist-nets and walk-in traps during the dry (i.e. April – September) and wet (i.e. October – March) seasons at 2 sampling sites per location. Sampling effort was even across all sites with 4 mist-nets used at each location. All sites were sampled the same number of times except for Shangoni and Phalaborwa where logistical challenges could not permit an equal sampling chance. Walk-in traps were baited with maize and placed near the water's edge. Trapping of birds was carried out in the morning (06h00 – 09h00) and late afternoon (16h00 – 19h00) when temperatures were low, so as to minimise stress on the part of captured birds. Mist-nets and walk-in traps were constantly monitored and checked for caught birds.

Screening for haemoparasites

Captured birds were individually marked with a SAFRING band, and standard morphometric measurements were recorded, i.e. mass, moult status, tarsus length, and head and culmen lengths. Blood samples were obtained by venepuncture of the right wing using a sterile 25G needle, with blood drawn into a 75 µl micro-haematocrit capillary tube. Two blood smear slides were prepared from each bird sampled. The slides were air-dried in the field, and then fixed with absolute ethyl alcohol to preserve the integrity of the cells and increase their rigidity before the slides were transported to the Skukuza Scientific Services Laboratory. Blood smears were stained with 10 % Giemsa solution prior to screening. Coverslips were not used. One blood smear from each bird was searched for avian haemoparasites (mainly *Haemoproteus* and *Plasmodium* spp.) under a compound microscope using the battlement technique, while the other slide was catalogued with SANParks scientific services. An area of the blood film approximately one third from the end of the slide was selected for examination, beginning at low magnification, i.e. x100, followed by x400 and then x1000 under immersion oil. Each slide was examined by moving two fields along the edge, two fields up and then two down, observing and recording any parasites present. The total number of fields covered on each slide at x400 magnification was 20. Photographs of all parasites and any abnormalities were captured using analySIS getIT software (Version 5.1). Detected parasites were identified to genus level and photographs of organisms (Appendix 1) were also sent to an avian parasitologist expert, Dr Michael Peirce (UK), for further confirmation of identification.

Data analysis

Firstly, the overall prevalence of avian haemoparasite infection (regardless of site and seasonal variation) in and around the Kruger National Park was established. This was then followed by calculating the prevalence of individual parasites at each site, while taking into account seasonal variations. Prevalence amongst different family groups, foraging guilds and social status was also determined. A Krustal-Wallis test for independent samples was used to ascertain if there was any significant variation in infection prevalence amongst sites, ignoring seasonal variations. To address the second question as to whether there were any seasonal variations in infection, I used a Mann-Whitney U test. A Wilcoxon signed ranks test was also used on data for only four sites from which there were samples for both the wet and dry seasons. Data from Shangoni and Phalaborwa were excluded from this analysis as there was only information for the dry season, due to logistical constraints. A Krustal-Wallis test was also used to test for the significance of infection as a function of foraging guild.

Birds were classed into five most dominant family groups (group sample $n \geq 50$) and comparative prevalence was determined for each group. It was deemed that sample of less than 50 would not be adequate for an accurate statistical analysis. Infection prevalence was also compared between solitary and gregarious birds using a Wilcoxon signed ranks test. All analyses were tested at the 5 % level of significance using the statistical package IBM SPSS 23 (IBM Corp. 2015).

Results

A total of 685 birds of 87 species were captured and smears of blood from them were examined (Appendix 3). One hundred and ninety-four birds were found to be infected by at least one form of haemoparasite. In addition to microfilariae, six genera of haemoparasites were detected by microscopy, namely: *Haemoproteus*, *Plasmodium*, *Leucocytozoon*, *Trypanosoma*, *Aegyptianella* and *Hepatozoon* spp. (Appendix 1). The overall prevalence of haematozoa for all sites was 28.3 %. *Haemoproteus* (18.57 %) was the parasite that occurred most commonly. Prevalences for other parasites identified were: microfilariae (4.99 %), *Trypanosoma* (4.34 %), *Plasmodium* (4 %), *Leucocytozoon* (1.03 %), *Aegyptianella* (0.42 %) and *Hepatozoon* (0.05 %) spp. (Figure 1).

Greater Blue-eared Starlings (n = 5) and House Sparrows (n = 5) harboured the greatest diversity of parasites (Table 2.1). Behaviourally, ground-foraging birds and solitary birds had the highest parasite diversity (Table 2.2 and Table 2.4). There were 29 birds with double

infections (4.2 %). The combinations included *Haemoproteus* + *Plasmodium* (n = 13), *Haemoproteus* + *Leucocytozoon* (n = 1), *Haemoproteus* + microfilariae (n = 3), *Haemoproteus* + *Trypanosoma* (n = 7) and *Haemoproteus* + *Aegyptianella* (n = 1) spp. The other combinations were *Trypanosoma* + *Plasmodium* (n = 1) and *Trypanosoma* + *Aegyptianella* (n = 1) spp. Double infections mostly occurred in the Greater Blue-eared Starling *L. chalybaeus* (n = 8), the Yellow-fronted Canary *S. mozambicus* (n = 5) and the Southern Grey-headed Sparrow *P. diffusus* (n = 3). *Haemoproteus* (14.58 %), microfilariae (6.94 %) and *Leucocytozoon* (2.78) spp. were the most commonly detected parasites during the wet season, whereas *Haemoproteus* (22.55 %) and *Plasmodium* spp. infections (3.51 %) were more prevalent in the dry season. Low occurrence was recorded for *Hepatozoon* in the dry season (0.19 %) and *Aegyptianella* spp. in the wet (1.39 %) and in the dry season (0.37 %). Infections with *Trypanosoma* spp. were similar in the wet (1.39 %) and dry (3.14 %) seasons (Figure 2.1).

Despite equal sampling efforts at all sites and during different seasons, there is insufficient data to determine how infections in individual species varied between sites and season. Phalaborwa, one of the two sites located outside the park, could only be sampled once, hence it was not possible to do an inside vs outside prevalence comparison. Shangoni, a site located inside the park, also became inaccessible to researchers after my single visit, partly due to a change of Section Rangers. Furthermore the abundance of some bird species was dissimilar between sites and seasonally (See Appendix 2 for a full inventory of birds captured), making it difficult to capture the same species during both seasons and at all sites. The overall prevalence of haemoparasites was highest at Satara (35 %, n = 37) and Skukuza (32.65 %, n = 343), while birds from Shingwedzi (21.10 %, n = 109) had the lowest infection rates. A higher diversity of parasites was recorded in birds caught in Mkhuhlu and Skukuza (southern sites), whereas birds from Shangoni and Shingwedzi (northern sites) had the lowest parasite diversity (Figure 2.1). *Haemoproteus* spp. prevalence was highest in Skukuza (29.74 %) and Satara (29.73 %). The prevalence of microfilariae increased gradually from the southern to the northern sites (Figure 2.1).

For birds with a sample size of ≥ 20, the highest prevalence recorded was in Greater Blue-eared Starlings *Lamprotornis chalybaeus* (infection prevalence = 35 %, n = 170) while the lowest prevalence rate was recorded for Dark-capped Bulbuls *Pycnonotus tricolor* (7.41 %, n = 27) and Fork-tailed Drongos *Dicrurus adsimilis* (10 %, n = 20). The prevalence of infection

was highest in the family Sturnidae (35 %, n = 188), by at least one form of haemoparasite.

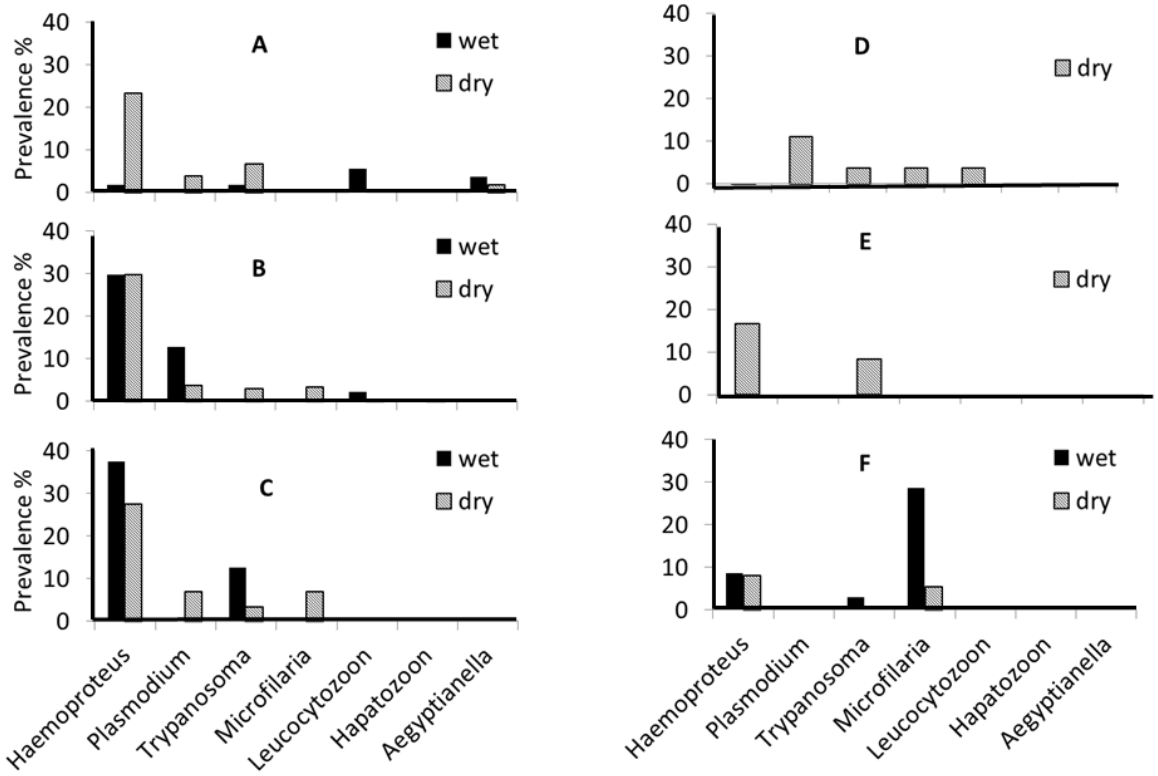

Figure 2.1 Prevalence and diversity of haemoparasites at Mkhuhlu (A), Skukuza (B), Satara (C), Phalarowa (D), Shangoni (E) and Shingwedzi (F).

Table 2.1 Birds with the highest infection diversity and their life-history traits. Haemoparasite presence labels: H = *Haemoproteus*, P = *Plasmodium*, L = *Leucocytozoon*, T = *Trypanosoma*, M = Microfilariae and A = *Aegyptianella*. Values in parentheses represent the number of birds infected by the corresponding parasite.

Common name	Species	Foraging guild	Social association	Sample size	Haemoparasite presence
Greater Blue-eared Starling	*Lamprotornis chalybaeus*	Ground	Gregarious	170	H (55), T (2), P (4), L (1), M (4)
House Sparrow	*Passer domesticus*	Ground	Gregarious	50	H (3), P (1), M (1), A (2), T (1)
Red-billed Hornbill	*Tockus erythrorhynchus*	Ground	Solitary	35	H (1), M (5), T (2), M (5)
Southern Grey-headed Sparrow	*Passer diffusus*	Ground	Solitary	14	H (9), P (1), M (1), T(2)
Southern Yellow-billed Hornbill	*Tockus leucomelas*	Ground	Solitary	19	H (2), P (1), T (2), M (2)
Yellow-fronted Canary	*Serinus mozambicus*	Ground	Solitary	22	H (11), P (3), L (3), T (6), A (1)
Total				310	

Haemoparasite infection was similar at all sites (*Haemoproteus*: $H_5 = 7.527$, $p = 0.184$; *Plasmodium*: $H_5 = 5.462$, $p = 0.362$; *Trypanosoma*: $H_5 = 5.735$, $p = 0.333$; Microfilariae: $H_5 = 5.959$, $p = 0.310$; *Leucocytozoon*: $H_5 = 6.006$, $p = 0.306$; *Hepatozoon*: $H_5 = 4.000$, $p = 0.549$; *Aegyptianella*: $H_5 = 8.889$, $p = 0.114$). There were no seasonal variations in infection prevalence, at all sites combined, for *Haemoproteus* ($U = 9.00$, $p = 0.522$), *Plasmodium* ($U = 9.00$, $p = 0.495$), *Trypanosoma* ($U = 8.50$, $p = 0.454$), microfilariae ($U = 9.00$, $p = 0.495$), *Leucocytozoon* ($U = 9.00$, $p = 0.471$), *Hepatozoon* ($U = 9.00$, $p = 0.471$) and *Aegyptianella* ($U = 10.50$, $p = 0.648$).

Ground foraging birds (e.g. Greater Blue-eared Starling *L. chalybaeus*, African Mourning Dove *S. decipiens* and House Sparrow *P. domesticus* as well as tree canopy stratum foraging birds (e.g. Lesser Masked Weaver *P. intermedius*, Dark-capped bulbul *P. tricolor* and Green Wood-hoopoe *P. purpureus*) had the highest haemoparasite infection rates. The lowest prevalence was recorded in aerial foraging birds (e.g. European Swallow *H. rustica* and Wire-tailed Swallow *H. smithii*) and aquatic birds (e.g. African Jacana *A. africanus* and White-faced Duck *D. viduata*) (Table 2.2).

Table 2.2 Haemoparasite prevalence as a function of foraging guild.

Foraging guild	Sample size	Prevalence %
Aquatic	12	17
Ground	496	32
Canopy	97	25
Aerial	80	14

Overall, haemoparasite prevalence was higher in ground foraging birds and lower in aerial foragers (Table 2.2). However, individual pathogen prevalence did not differ significantly amongst foraging guilds (*Haemoproteus*: $H_3 = 4.554$, $p = 0.208$; *Plasmodium*: $H_3 = 4.918$, $p = 0.178$; *Trypanosoma*: $H_3 = 2.601$, $p = 0.457$; Microfilariae: $H_3 = 0.473$, $p = 0.925$; *Leucocytozoon*: $H_3 = 4.156$, $p = 0.245$; *Hepatozoon*: $H_3 = 3.000$, $p = 0.392$; *Aegyptianella*: $H_3 = 3.000$, $p = 0.392$).

Haemoproteus spp. was the most prevalent pathogen in all avian families. The highest prevalence of this organism was recorded in Sturnidae (32.45 %), while the lowest prevalence was in Bucerotidae (5.36 %). *Plasmodium* spp. was generally rare to absent in all the bird families. Microfilariae occurred most frequently in Bucerotidae (12.50 %) and Columbidae (11.48 %), whilst their lowest prevalence was in Passeridae (1.60 %), with none detected in Hirundinidae (0 %). *Hepatozoon* was only found in Columbidae (1.64 %), *Leucocytozoon* only in Sturnidae (0.53 %) and *Aegyptianella* only in the family Passeridae (Table 2.3).

Table 2.3 Prevalence of parasites according to Aves families. Only bird families from which more than 50 individuals were examined are included here.

Parasite	Prevalence (%)				
	Sturnidae	Passeridae	Columbidae	Bucerotidae	Hirundinidae
Haemoproteus	32.45	20	11.48	5.36	9.80
Plasmodium	2.66	1.54	0	1.79	1.96
Leucocytozoon	0.53	0	0	0	0
Microfilariae	2.13	1.06	11.48	12.50	0
Trypanosoma	1.60	1.60	0	7.14	1.96
Hepatozoon	0	0	1.64	0	0
Aegyptianella	0	3.08	0	0	0

Gregarious birds (n = 328) had the highest *Haemoproteus* and *Aegyptianella* prevalences, while solitary birds (n = 357) had the highest infection prevalences of microfilariae, *Plasmodium, Trypanosoma* and *Leucocytozoon*. *Hepatozoon* was only detected in one solitary bird, a Spectacled Weaver *P. ocularis* (Table 2.4).

Table 2.4 Haemoparasite prevalence as a function of social association.

	Prevalence (%)	
Parasite	Gregarious	Solitary
Haemoproteus	22.5	16.16
Plasmodium	3.44	3.84
Trypanosoma	1.25	4.38
Leucocytozoon	0.31	1.37
Microfilariae	2.19	20.00
Aegyptianella	0.63	0.55
Hepatozoon	0	0.27

Infection prevalence between the two groups (gregarious vs solitary birds) was similar for each parasite genus (*Haemoproteus*: $W_s = 0.447$, $p = 0.655$; *Plasmodium*: $W_s = 0.447$, $p = 0.655$; *Trypanosoma*: $W_s = 0.447$, $p = 0.665$; Microfilariae: $W_s = 1.342$, $p = 0.180$). All statisctical details are appended in Appendix 3.

Discussion

The diversity of avian haemoparasites in the Kruger National Park was high. In addition to microfilariae, six genera were encountered. This diversity further supports the notion that avian parasites are endemic in the subtropical Lowveld regions of southern Africa (Okanga *et al*. 2013). H*aemoproteus*, which is the cause of a usually relatively benign form of avian malaria (reference to a *Haemoproteus* infection as "malaria" being a concept that is mainly historical), was by far the most common organism detected and it was mainly found in starlings (family Sturnidae). The prevalence of the often more pathogenic avian plasmodial parasites appeared to be suppressed in the region all year round. Microfilariae were the second most common parasite present after *Haemoproteus* spp., but their overall prevalence was low. Cases of double infection were mostly in association with *Haemoproteus* spp. However, very few instances of this were detected (4.2 %).

The similarity in haemoparasite prevalence at the different sites is evidence of the widespread abundance of avian haematozoa in the Kruger National Park in different habitats that range from wide-open savanna thornveld in the southern parts of the park to the broad-leaved Mopani woodland that dominates in the northern parts. These research results are contrary to those of Poulin (2007), Paaijmans and Thomas (2011), Dubiec *et al*. (2018) and Turcotte *et al*. (2018), who found that avian haemoparasite infections differed between localities and was influenced by variations in landscape, vegetation structure and geographic location. Those authors demonstrated some important roles of landscape and environmental processes (e.g. abiotic conditions and species interactions) in driving disease dynamics. Considering that sampling during the present investigation was carried out in different landscapes within the park, the results suggest that the vectors responsible for the transmission of these parasites are ubiquitous in the Kruger National Park.

Although other studies have postulated that the chances of birds acquiring haematozoan infections are reduced in areas with a higher anthropogenic load (Rieter 2001, Valkiūnas 2005), due to the influence of insecticides, environmental pollution on vector ecology, etc., my

observations suggest that anthropogenic disturbances might actually be instrumental in increasing haemoparasite prevalence in birds (Figure 1). Anthropogenic land-use changes such as deforestation, habitat fragmentation, dam construction, urbanisation (Morse 1995, Daszak *et al.* 2000), and changes in habitat quality (Mostowy and Engelstaedter 2011) have been implicated as key drivers of disease emergence. This causes some novel interactions among vectors, hosts and parasites (Patz *et al.* 2004), which can alter the exposure of birds to infections and redefine their niche in the transmission and epidemiology of parasitic infections (Mostowy and Engelstaedter 2011). In recent years, Mkhuhlu has been marked by rapid population growth (Bushbuckridge Local Municipality 2010), possibly resulting in the formation of microhabitats suitable for vectors, which could prolong their breeding cycles and thereby increase the vector populations (Becker *et al.* 2010). This, in turn, is likely to lead to elevated parasite transmission and prevalence (Marquardt *et al*. 2000). Over a long period of time, such changes might in theory facilitate the evolution of novel emergent infections in these bird populations, concomitantly being accompanied by a greater diversity of parasite taxa.

As human populations continue to grow in this region, the demand for land will increase towards areas surrounding the park. This is likely to enhance the potential for spread of diseases such as avian malaria *sensu lato* (Haemosporidia of the genera *Plasmodium* and *Haemoproteus*) from wild birds to domestic poultry or *vice versa* (Taylor *et al.* 2001, Jones *et al.* 2008). Any such outbreaks may cause high mortality of domestic poultry and result in serious financial losses for the local poultry industry (Lebea 2014).

Haemoparasite infections are thought to be influenced by seasonal weather variations, considering the direct bearing that seasonality has in determining the abundance and population density of vectors throughout the year (Githeko *et al.* 2000, Bensch and Akesson 2003, Rogers and Randolph 2006, Møller and Nielsen 2007, Dunn *et al*. 2011 and Mordecai *et al.* 2017). However, my findings suggest that this hypothesis is not supported in the Kruger National Park area setting. The most plausible explanation is that although temperature is an important variable for predicting the prevalence, distribution and diversity of haemoparasites (Pérez-Rodríguez *et al.* 2013**)** because of its influence on vector ecology (Gallana 2013), the recent shift in seasons and high temperatures that were experienced in the Lowveld region in the past year (Scientific Services 2017) are likely to have contributed to an increased haemoparasite prevalence rate in the dry season. Temperatures were unusually high during the winter season of 2016 (mean = 20 ºC and maximum = 29.2 ºC) (Scientific Services 2017), which could have extended the vector breeding period into the dry season. An increase in mean annual temperatures causes a shift in wildlife ranges, dynamics of diseases and vector ecology, which

can lead to altered, novel risks (Dobson and Foufopoulos 2001, McMichael *et al.* 2003). In chronically infected birds, some haemosporidian parasites become dormant in an unknown form and are not seen microscopically in peripheral blood. For reasons that are unclear, asexual replication is then at some point triggered, leading to a recurrence characterised by elevated parasitaemia levels (Martin *et al.* 2010, Murdock *et al.* 2012). In the northern hemisphere, this was given the name "spring relapse" many years ago, when coincidence of parasitaemic recurrence with the onset of the spring breeding season of birds was detected. Furthermore, given that little is currently known about avian haemosporidian vectors in southern Africa (Hellard *et al.* 2016), it is possible that there are vectors which are "homeothermic" in that they function independently of potential temperature constraints and are active throughout the year.

Contrary to my findings, foraging in wetlands was not a predictor for haemoparasite prevalence, as was the case in studies in Costa Rica (Mendenhall *et al.* 2013) and Latvia (Krama *et al*. 2015); even for *Haemoproteus*, *Plasmodium* and *Leucocytozoon*. Certainly, the vectors of the last-mentioned two genera are water-dependent. The assumption is that birds foraging near water bodies have a higher haematozoan prevalence rate since they would spend a significant part of their day closer to where vectors breed, thereby inadvertently increasing their chances of becoming infected. By contrast, birds that forage in tree canopies are expected to have a lower infection prevalence (See chapter 1, host traits) since the risk of transmission decreases with vertical distance (elevation). Birds feeding on the ground had the highest incidence of infection, whereas birds foraging in tree canopies had higher infection prevalence than those foraging in wetlands. Aerial foragers had the least risk of infection. However, cognisance should be taken of the communal roosting habit of the European Swallow *H. rustica* in reed beds at water bodies (in the present study, some individuals were found to be infected with haemoparasites). This avian social phenomenon occurs when the species is overwintering in southern Africa, making the aerial foraging criterion of questionable significance in practice as far as *H. rustica* is concerned. Another example is the aerial-foraging European Bee-eater *M. apiaster*. This species roosts in trees at night, where it could be bitten by vectors (unlike when it is foraging aerially). The haemoparasite prevalence findings raise questions concerning the paradox of 'vector ecology' (habitat, epidemiology and pathogenesis), which is still poorly understood in this region, even though parasites utilise haematophagous vectors with specific habitat preferences (Martens *et al*. 1999, Reiter 2001). Tsomafo (2013) found that anthropogenic influences on the environment can establish microhabitats in which vectors can successfully breed. It is also interesting to note that prevalence was parasite-specific in a comparison between gregarious and solitary birds (Table 2.4). Such a finding does not fully

conform to the amplification hypothesis (Blomberg *et al.* 2003), which predicts a higher prevalence in more social birds. I therefore suggest that avian social trait (gregarious vs solitary) as a prevalence predictor might be highly dependent on parasite genus; which affects bird family groups differently.

In conclusion, these findings challenge current paradigms on avian parasitology which predict clear variations in haemoparasite prevalence according to environmental conditions (location, season and land use) and social traits. Worldwide, the situation probably varies, however. The generally low levels of parasite prevalence (although diverse) may have hindered the application of conventional analysis to elucidate any apparent patterns in the prevalence. Nevertheless, this study suggests that a bird's family group and guild may generally still be a good predictor for prevalence. Future work should pay attention to nesting height, because infection of a bird as a nestling might be important. Also, adult birds that are immobile at the nest would no doubt be particularly susceptible to vector bites.

Of course, there is obviously a detection limitation when using a microscope to screen avian blood samples for haemosporidian infections. Firstly, parasites in chronically infected birds with a low parasitemia of below 40 infected red blood cells per microlitre of blood, which is equivalent to one parasite per 10,000 red blood cells (Bruce and Day 2002), can be difficult to find by microscopy (Jarvi *et al.* 2003, Waldenström *et al.* 2004). The overall infection prevalence of 27.3 % is a conservative estimation, hence the need for a molecular screening technique (Valkiūnas *et al.* 2008) to supplement the current microscopy-based findings. A study by Tsomafo (2013) found a 23 % overall prevalence of haemosporidian blood parasites using microscopy, whereas prevalence determined by Polymerase Chain Reaction diagnostics was much higher at 44 % in birds sampled in the central region of Ghana. Unfortunately, the cost of molecular analysis still remains unaffordable to most researchers working in developing countries, and therefore microscopy is the most practical alternative. Lastly, little is known about avian haemoparasite vectors in southern Africa (Hellard *et al.* 2016). Future studies should thus also focus on resolving questions relating to the identity, distribution and ecology of avian haemoparasite vectors in the region.

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CHAPTER 3

Consequences of blood parasite infection for avian immunity and body condition

Abstract

Understanding the host immune response to haemoparasite infections is important in elucidating the true burden of these parasites on birds in endemic areas of southern Africa. Using blood smears from 685 individuals of 87 species captured in and adjacent to the Kruger National Park, I examined the impact of haemoparasites on body condition and heterophil to lymphocyte ratio (H:L) as a measure of the immune response. Parasites recorded were microfilariae and the genera *Haemoproteus*, *Trypanosoma*, *Plasmodium*, *Leucocytozoon*, *Aegyptianella* and *Hepazotoon*. The presence of infection, including double infections, did not affect body condition except for birds parasitised by *Haemoproteus* in the wet season. Their body condition was better than that of birds in which infection was not diagnosed. Furthermore, the heterophil to lympocyte ratio was significantly lower for birds that had *Haemoproteus*, *Leucocytozoon* and *Trypanosoma* spp. infections, compared to birds in which infection was not detected. Birds harbouring filarial worms and plasmodial parasites had a similar H:L ratio to uninfected individuals. Double infections did not have any effect on the H:L ratio. Overall, the results suggest that haemoparasite infection lowered the heterophil to lymphocyte ratio, which is thought to be positively correlated to stress levels. Haemoparasite infection in these birds therefore appears to be of little significance for the immune status of the avian host and may even be interpreted as being beneficial. Of course, some inapparent confounding factors could be involved such as the age and sex of the host. To further evaluate the findings, I recommend that other techniques which measure specific host responses triggered by haemoparasite infections should also be used, as well as full blood cell counts. It is, however, possible that because haemoparasite infections in the Kruger National Park are endemic, hosts have co-evolved with parasites such that there are now, in general, no significant deleterious physiological consequences for infected birds.

Introduction

Knowledge concerning the ecology, evolution and association of parasites with their hosts in natural and endemic populations is important in assessing host-parasite relationships and their implications (Lachish *et al*. 2011). Southern Africa has a highly diverse geographic landscape which supports more than 700 avian species (Newman 2002). The widespread abundance of birds in this region therefore offers a unique opportunity to study their complex interactions with parasites (Lachish *et al*. 2011) and the related ecology, which is still poorly understood (Valkiūnas 2005). Avian haemoparasites are endemic to tropical terrestrial regions and are thought to have mutually evolved with their hosts over many years of their existence (Korpimaki *et al.* 1993, Sanz *et al.* 2001, Marzal *et al.* 2005, Bensch *et al.* 2007). As a result, they are thought to be of little pathogenic importance to their hosts (Kallio *et al*. 2007), but rather cause chronic infections (Bennett *et al.* 1993), whereby haemoparasites remain dormant inside the host cells as parasitaemia stabilises. Generally, the extent to which parasites become pathogenic is dependent on environmental variables that the host is exposed to (Kallio *et al.* 2007, Woodhams *et al.* 2008). Stressful conditions for the host such as extreme temperatures (Wegner *et al.* 2003), breeding (Weatherhead and Bennett 1991), or migration (Atkinson and LaPointe, 2009), lower the host's immunity. If immunity is compromised during the chronic phase of haemosporidiosis, this can result in asexual replication and expansion of the dormant infection via penetration of parasites into new red blood cells, thereby significantly elevating parasitaemia levels (Valkiūnas 2005, Martin *et al.* 2010, Mydlarz *et al.* 2010, Murdock *et al.* 2012).

Although avian haemoparasites are normally of little clinical significance, *Plasmodium* spp. have frequently been shown to be pathogenic, causing considerable host mortality and having serious implications for the diversity and distribution of avian communities (Hudson *et al.* 1998, Daszak *et al.* 2000, LaPointe *et al*. 2012, Dunn *et al.* 2013). A well-documented example is the introduction of *Plasmodium relictum* into the Hawaiian islands, where birds in naïve populations subsequently became infected. This parasite caused massive outbreaks of disease that resulted in high mortality and disturbance of bird distributions (van Riper *et al.* 1986, Van Riper 1991, Valkiūnas 2005). *P. relictum* was partly responsible for the extinction of several endemic Hawaiian bird species (Valkiūnas 2005). Outbreaks of *P. relictum* malaria have from time to time been reported in birds in captivity (Belo *et al.* 2009, Chagas *et al.* 2017), including among penguin species such as the Magellanic penguin *Spheniscus magellanicus* in

United States of America (Fix *et al.* 1988) and the African penguin (*Spheniscus demersus*) in South Africa (Daszak *et al*. 2000).

Although symptoms of haemoparasite infection in birds are not always apparent, these organisms can nevertheless affect avian body condition (Valkiūnas *et al.* 2006). Studies have found a negative association between body condition and haematozoan infection in wild birds (Dawson and Bortolotti 2000, Merino *et al*. 2000). Furthermore, high *Leucocytozoon*-associated mortality has been recorded in domestic poultry, resulting in a reduction in farming profits (Morii 1992). In Asia, *Plasmodium gallinaceum* has been reported to cause significant morbidity and high mortality in infected birds (Rao *et al.* 1951). Peirce (1984) found *Leucocytozoon marchouxi* to be pathogenic in an Emerald-spotted Wood Dove *Turtur chalcospilos*, sampled in Zambia. Marked parasitaemia of the same parasite species (*L. marchouxi*) had been noted in a nestling Laughing Dove *Streptopelia senegalensis* that was examined in Pretoria, South Africa, but an obvious pathogenic effect was not apparent (Oosthuizen and Markus 1968). Also in columbids in Pretoria, *Haemoproteus* was seen to be pathogenic in a feral domestic pigeon (Markus and Oosthuizen 1972) and in exotic caged doves (Earlé *et al*. 1993).

The impacts of avian haemoparasites on birds are generally extremely complex to determine in natural systems (Bennett *et al.* 1988) due to the diversity of haematozoan species affecting bird populations (Bensch *et al*. 2004). However, it has been suggested that elevated parasitaemia levels cause the host to compete with the parasite for energy (Sheldon and Verhulst 1996, Valkiūnas 2005), which could be a significant factor in selection and control of avian population dynamics (Daszak *et al.* 2000, Albon *et al.* 2002). Certainly, there is a fitness cost associated with mounting an immune response to *Plasmodium relictum* (see Delhaye *et al*. 2018), which could be disadvantageous for the individual bird. Differential white blood cell counts are considered to be a reliable means of reflecting a host's immune defence status (Kilgas *et al*. 2006, Salvante 2006) and are further used to elucidate host-parasite interactions. Leukocyte counts tend to be elevated by haemoparasite infections (Salvante 2006). The weakening of the host's immunity by parasitic infections decreases the number of lymphocytes and can leave the host susceptible to secondary infections (Pedersen and Fenton 2007). Studies by Fix *et al.* (1988) and Davis *et al*. (2008) have shown lymphocytes to be associated with protection from infectious diseases and their numbers are increased during the initial parasitic infections. Therefore, the heterophil to lymphocyte (H:L) ratio is commonly used in disease ecology studies as a stress indicator in respect of infectious diseases and it is positively correlated with the severity of the infection in birds (Davis *et al.* 2008).

In contrast, however, some studies provide conflicting results as to whether haemoparasite infections have considerable effects on their hosts (Sanz *et al.* 2001, Marzal *et al.* 2005, Valkiūnas 2005, Bensch *et al.* 2007). The impact of avian haemoparasites on wild birds is difficult to predict, especially in areas with endemic infections where the host and parasites have co-evolved. This is exacerbated by the fact that when birds co-evolve with their haemoparasites, they have the ability to sustain parasitaemia below a level at which an infection impacts negatively on the host (Atkinson and Van Riper 1991). Though microfilarial infections may impact host fitness in some instances, most cases have been found to be generally non-pathogenic (Campbell 1995). Bennett *et al.* (1993) and Hunter *et al.* (1997) have considered *Leucocytozoon* species to be non-pathogenic in wild populations. Moreover, *Haemoproteus* and *Trypanosoma* spp. are also thought to be generally non-pathogenic (Baker 1976, Fallis and Desser 1977). Nevertheless, despite much research, the effects of blood parasites on host fitness in the wild are still poorly understood (Bensch *et al.* 2007, Kulma *et al.* 2014). Studies on the pathogenicity of haematozoa are rare and results remain unclear (Marzal *et al*. 2008, LaPointe *et al*. 2012). Given that haemoparasites can in theory exert strong selective forces on their hosts, it is important to understand host-parasite interactions in wild bird populations. In the present study, I assessed the impact of parasitism on body mass and lymphocyte ratios. The hypothesis tested is that avian haemosporidian infections cause appreciable effects on avian body condition and for immunity (i.e. considered via heterophil to lymphocyte ratios).

Materials and methods

Study site

Birds were sampled from six sites in and around the Kruger National Park, namely: Skukuza (24º 99′ S, 31º 60′ E), Satara (24º 39′ S, 31º 77′ E), Shangoni (23º 45′ S, 30º 97′ E), Shingwedzi (23º 11′ S, 31º 43′ E), Phalaborwa (23º 94′ S, 31º 14′ E) and Mkhuhlu (24º 59′ S, 31º 14′ E). Skukuza and Mkhuhlu lie in the southern areas, while Shingwedzi, Shangoni and Phalaborwa are situated in the north. Satara is located centrally. Detailed attributes of the study sites are described in chapter 1.

Fieldwork

Study sites were located in areas where there was a source of attraction for birds such as the waste disposal sites, watering points and residential gardens. Birds were caught using mist-nets and walk-in traps (Appendix 2) in 2016 during the dry (i.e. April–September) and wet (i.e.

October–March) seasons. Walk-in traps were baited with maize seeds and placed near the water's edge. Trapping of birds was carried out in the early morning and late afternoon when temperatures were low, to minimise stress to birds. Mist-nets and walk-in traps were constantly monitored and checked for trapped birds.

Haemoparasite screening

Captured birds were individually marked with a SAFRING band and standard morphometric measurements were recorded (tarsus, head and culmen lengths; body mass; state of moult). Blood samples were obtained by venepuncture of the right wing using a sterile 25G needle, with blood drawn into a 75 µl micro-haematocrit capillary tube. Two blood smear slides were prepared from each bird sampled. The slides were air-dried in the field and fixed with absolute ethyl alchohol to preserve the integrity of the cells and increase their rigidity before the slides were transported to the Skukuza Scientific Services Laboratory. Blood smears were stained with 10 % Giemsa solution prior to screening. Coverslips were not used. One blood smear from each bird was then searched microscopically for avian haemoparasites under a compound microscope using the battlement technique, while the other slide was catalogued with SANParks scientific services.

Differential White Blood Cell Count (DWBCC)

To partially evaluate the immune response, white blood cell counts (WBC) were performed on stained smears, using a compound microscope at x1000 magnification. All types of leucocytes (heterophils, eosinophils, lymphocytes, monocytes and basophils) were identified and classified according to their morphologic and staining characteristics while counting with the aid of an electronic differential cell counter (Model: DBC-8E), until a total of 100 cells was reached. This is because avian blood consists of a percentage of each type of leucocyte and the count determines any deviation from a healthy range. Examination of WBC's was done on the edge of the oval-shaped end of the smear where blood cells were present as a single layer and slightly separated, thus facilitating examination.

Body condition

Body Condition Index (BCI) for each bird was calculated from the morphometric measurements (mass and structural lengths) using the following altered formula from Ndlovu *et al*. (2010):

$$BCI = \frac{\sqrt[3]{body\_mass}}{\sqrt[2]{tarsus * culmen}} * 100$$

This equation was also used to calculate the lowest possible BCI, average BCI and highest possible BCI for each bird species, based on reported values in "Robert's Birds of Southern Africa", 7th edition (Hockey *et al*. 2005). Where multiple values were obtained, the values from the largest sample size were used. Morphometrics for the few dimorphic species sampled were averaged to represent values for that particular species. For the lower limit BCI, I used the lowest body mass and highest culmen and tarsus lengths in the equation. In order to calculate upper limit BCI, the highest body mass and lowest culmen and tarsus lengths were used. The BCI % was derived using the following equation:

$$\text{BCI \%} = \frac{BCI - RMinBCI}{RMaxBCI - RMinBCI} * 100$$

Where BCI = calculated body condition; RMin = Roberts lower limit; and RmaxBCI = Roberts upper limit.

Data analysis

A two-way analysis of variance (ANOVA) was used to assess the impact of infection on the body condition of birds. To test for the immune response to haemoparasite infection, I first calculated the heterophil to lymphocyte ratio (H:L) using heterophil and lymphocyte counts and log-transformed the raw data so that it could achieve normality. I then used a two-way ANOVA for the analysis. I used an ANOVA to assess the influence of infection on body condition and H:L ratio in regard to avian family groups. Since infection differed amongst families (see chapter 2), i.e not all families were infected by all the types of parasites recorded, it was difficult to analyse the influence of an individual parasite genus on body condition and H:L ratio in avian families. I then only considered infections by at least 1 genus and *Haemoproteus* infections, which were common and present in all the family groups. I also took into account mixed infections by *Haemoproteus*. All statistics were tested at the 5 % level of significance and all analyses were carried out using the package IBM SPSS 23 (IBM Corp. 2015).

Results

A total of 685 birds of 87 species were captured and smears of blood from them were examined (Appendix 3 has a complete species list). One hundred and ninety-four birds were found to be infected by at least one form of haemoparasite. In addition to microfilariae, six genera of haemoparasites were detected by microscopy, namely: *Haemoproteus*, *Plasmodium*, *Leucocytozoon*, *Trypanosoma*, *Aegyptianella* and *Hepatozoon* spp. (Appendix 1). The overall prevalence of haematozoa for all sites was 28.3%. Prevalence results are given in chapter 2. There was a seasonal variation in mean body condition of birds, regardless of infection status ($F_{1.673} = 12.055$, $p = 0.001$) ie higher in the dry season than the wet season. Body condition was similar between parasitised birds and those in which infection was not detected ($F_{1,673} = 1.2756$, $p = 0.259$). The exception was for birds harbouring *Haemoproteus*. The body condition of these individuals was significantly better than that of the unifected birds in the wet season ($F_{1.138} = 4.400$, $p = 0.037$). Infection with other parasites did not affect body condition (Table 3.1). The details are: *Plasmodium* ($F_{1,673} = 1.579$, $p = 2.094$), *Leucocytozoon* ($F_{1,673} = 0.000$, $p = 0.953$), microfilariae ($F_{1,673} = .001$, $p = 9.814$), *Trypanosoma* ($F_{1,673} = 1.2756$, $p = 0.259$), and double infections ($F_{1,670} = 2.669$, $p = 0.103$).

Table 3.1 Mean body condition index (%) and sample sizes for birds infected with different haemoparasites. "Double infection" indicates birds in which more than one type of parasite was found. Only double infection combinations involving *Haemoproteus* and *Trypanosoma* spp. are included.

Infection detected	Mean Body Condition Index (%)			
	Sample size	Dry Season	Sample size	Wet Season
No parasites	382	47.57 ± 0.78	103	40.42 ± 1.51
Haemoproteus	122	48.34 ± 1.38	21	48.01 ± 3.33
Microfilariae	16	49.55 ± 3.82	10	43.24 ± 4.84
Plasmodium	20	49.02 ± 3.41	5	51.98 ± 6.83
Trypanosoma	17	44.48 ± 3.87	2	44.95 ± 16.18
Leucocytozoon	2	40.05 ± 10.80	4	33.63 ± 7.64
Double infection			5	52.38 ± 6.70

Haemoproteus + Double infection	23	47.54 ± 3.13		
Trypanosoma +	2	41.18 ± 10.75	–	–

The heterophil to lymphocyte ratio (H:L) was significantly lower for infected birds than birds in which infection was not detected ($F_{1.522} = 25.893$, $p < 001$), and for both seasons (Table 3.2). The H:L ratio was significantly higher in the wet season than the dry season for both infected and uninfected birds ($F_{1.522} = 57.722$, $p < 001$). Similarily, the H:L ratio was significantly lower for *Haemoproteus* ($F_{1.522} = 8.7768$, $p = 0.001$), *Leucocytozoon* ($F_{1.522} = 8.8377$, $p = 0.003$), and *Trypanosoma* ($F_{1.522} = 4.2644$, $p = 0.039$) infections. Results were not significant for filarial helminths ($F_{1.522} = 0.3108$, $p = 0.860$), *Plasmodium* ($F_{1.522} = 1.2364$, $p = 0.227$), and double infections ($F_{1.519} = 3.3599$, $p = 0.067$). The sample size was insufficient to calculate the impact of *Aegyptianella* and *Hepatozoon* parasites on body condition and H:L ratio.

Table 3.2 Mean heterophil to lymphocyte (H:L) ratios in birds infected with haemoparasites. "Double infection" indicates birds in which more than one parasite genus was detected. Only double infection combinations involving *Trypanosoma* and *Haemoproteus* spp. are included.

Infection detected	Sample size	Mean H:L ratio
No parasites	368	29.28 ± 1.67
Haemoproteus	119	11.79 ± 3.79
Microfilariae	17	22.99 ± 5.92
Plasmodium	19	10.97 ± 11.98
Trypanosoma	18	6.24 ± 8.72
Leucocytozoon	5	1.22 ± 1.05
Haemoproteus + Double infection	22	6.92 ± 1.87
Trypanosoma +	2	2.35 ± 2.95

Despite equal sampling efforts in this study, there was insufficient data to determine the effect of infections within different seasons on body condition and H:L ratios among all family groups in which parasites were found. The abundance of family groups varied greatly and I only considered families with n ≥ 50. These were Sturnidae (n = 188), Passeridae (n = 65), Columbidae (n = 61), Bucerotidae (n = 56) and Hirundinidae (n = 51) (Table 3.3).

Table 3.3 Overall body condition (index) amongst family groups, as associated with haemoparasite infection.

Parasite	Body Condition Index (%)									
	Sturnidae		Passeridae		Columbidae		Bucerotidae		Hirundinidae	
	In	Un	In	Un	In	Un	In	Un	In	Un
H	50.63	51.32	49.5	36.13	34.68	50.9	41.84	43.22	40.16	42.19
P	46.89	51.32	26.67	36.13	–	50.9	49.95	43.22	39.28	42.19
L	40.55	51.32	–	36.13	–	50.9	–	43.22	–	42.19
M	51.67	51.32	63.29	36.13	46.31	50.9	37.7	43.22	–	42.19
T	55.17	51.32	45.22	36.13	–	50.9	43.84	43.22	38.46	42.19
Hp	–	51.32	–	36.13	–	50.9	–	43.22	–	42.19
A	0	51.32	23.07	36.13	–	50.9	–	43.22	–	42.19
DI *H*+	47.01	51.32	48.60	36.13	47.43	50.9	47.01	43.22	47.43	42.19
DI *T*+	–	–	32.42	36.13	–	–	49.94	43.22	–	–

Parasite column symbols: "*H*" represents *Haemoproteus*, "*P*" represents *Plasmodium*, "*L*" represents *Leucocytozoon*, "*T*" represents *Trypanosoma*, "M" represents Microfilariae, "*A*" represents *Aegyptianella*, "*Hp*" represents *Hepatozoon*, "In" represents infected birds, "Un" represents uninfected birds (i.e. no infection detected), "DI *H*+" represents Double Infection with *Haemoproteus* plus another parasite, and "DI *T*+" represents Double Infection with *Trypanosoma* and another parasite. The "–" in Body condition columns means that no infections in birds were detected.

Overall avian family body conditions were significantly different, regardless of infection detection status ($F_{4.404} = 12.23$, $p < 001$). Infection by at least one form of parasite ($F_{1.408} = 0.07$, $p = 0.795$) and *Haemoproteus* infections ($F_{1.408} = 0.026$, $p = 0.872$) were correlated with a similar body condition to where infection was not detected. There was also correspondence

between family and infection by at least one form of parasite on the one hand ($F_{4.408} = 5.01$, $p = 0.001$) and between family and *Haemoproteus* infection on the other ($F_{4.408} = 6.175$, $p < 001$). Body condition of family groups was not associated with double infections ($F_{1.416} = 0.290$, $p = 0.590$). The H:L ratio for the five avian family groups (Table 3.4) was significantly different, regardlesss of diagnosed infection status ($F_{4.307} = 4.743$, $p = 0.001$). H:L ratios for both birds having at least one form of parasite ($F_{1.307} = 9.068$, $p = 0.003$) and those infected by *Haemoproteus* ($F_{1.307} = 5.350$, $p = 0.021$) were significantly different from ratios for birds in which infection was not detected. Double infections did not affect the H:L ratio ($F_{1.416} = 1.457$, $p = 0.228$).

Table 3.4 Heterophil to lymphocyte ratio amongst family groups, as associated with haemoparasite infection.

Parasite	Heterophil / Lymphocyte ratios									
	Sturnidae		Passeridae		Columbidae		Bucerotidae		Hirundinidae	
	In	Un	In	Un	In	Un	In	Un	In	Un
H	5.37	12.51	4.81	10.38	18.89	32.83	15.27	20.75	22.82	39.79
P	11.16	12.51	0.13	10.38	–	32.83	1.03	20.75	17.2	39.79
L	–	12.51	–	10.38	–	32.83	–	20.75	–	39.79
M	12.39	12.51	24.13	10.38	29.66	32.83	24.88	20.75	–	39.79
T	5.08	12.51	4.09	10.38	–	32.83	8.42	20.75	1.41	39.79
Hp	–	12.51	–	10.38	0.9	32.83	–	20.75	–	39.79
A	–	51.32	2.77	10.38	–	32.83	–	20.75	–	39.79
DI *H*+	6.92	12.51	6.92	10.38	6.92	32.83	6.92	20.75	6.92	39.79
DI *T*+	–	12.51	3.67	10.38	–	32.83	1.03	20.75	–	–

Parasite column symbols: "*H*" represents *Haemoproteus*, "*P*" represents *Plasmodium*, "*L*" represents *Leucocytozoon*, "*T*" represents *Trypanosoma*, "M" represents Microfilariae, "*A*" represents *Aegyptianella*, "*Hp*" represents *Hepatozoon*, "In" represents infected birds, "Un" represents uninfected birds (i.e. no infection detected), "DI *H*+" represents Double Infection with *Haemoproteus* plus another parasite, and "DI *T*+" represents Double Infection with *Trypanosoma* and another parasite. The "–" in Body condition columns means that no infections in birds were detected.

Discussion

There are uncertainties concerning the threats posed by avian haemoparasites to their hosts in natural populations (Sanz *et al.* 2001, Marzal *et al.* 2005). It is therefore crucial to assess infection implications and associations, particularly in protected areas such as the Kruger National Park, which is a hub for conservation in South Africa. The park is an important bird and general biodiversity area and it presents an interesting context in which to try and understand the influence of avian parasites on host fitness in wild bird populations.

Results suggest that overall birds infected by haemoparasites are not subjected to any fitness costs on their body condition, with the exception of *Trypanosoma* alone and double infections by *Trypanosoma*, which appeared to have a slight negative effect on the body condition of the host. As for the double infections, this possible influence may, in fact, have been attributable to a very small sample size (n = 2). Conversely, infection seemed to reduce "stress" on an individual, as indicated by a lower heterophil to lymphocyte ratio. These findings suggest that haemoparasites are endemic in the greater Lowveld region and hence avian hosts may have co-evolved with parasites such that infections do not result in any significant deleterious effects as regards host body condition. Interestingly, the body condition percentage was better for infected birds than uninfected ones in both the wet and dry seasons with an exception of *Leucocytozoon*. The presence of *Haemoproteus*, a haemosporidian that normally appears to be non-pathogenic (Valkiūnas 2005), presented birds with the best recorded body condition. One is tempted to conclude that this indicates that birds have become immunogenetically adapted to *Haemoproteus* infections and perhaps even derive a benefit therefrom. Globally, *Haemoproteus* is the most abundant avian haemosporidian organism, with a long evolutionary host-parasite history, such that *Haemoproteus* has been described as generally non-pathogenic (Baker 1976, Fallis and Desser 1977), as already stated above. However, although the results were not statistically significant, the body condition of birds grouped at the family level was not as good in respect of infected individuals when compared to uninfected ones. Due to low and inconsistent infection detection amongst family groups, it was difficult to come up with a definitive conclusion regarding how the different avian families' body condition correlated with haematozoan infections.

Although *Plasmodium* parasites have been reported to be the most virulent haemosporidian because of their complex life cycle which involves asexual reproduction within the host's erythrocytes, thereby triggering more serious effects on the birds (Valkiūnas 2005), my findings suggest that this may not necessarily be the case in endemic populations, or at least for the birds sampled in this study. *Plasmodium* spp. infections have been reported

to be most virulent in nestlings, especially during their first few days after hatching, before they attain immunity (Gabaldon and Ulloa 1980), at which time infected chicks can often be seen dead under trees (Valkiūnas 2005). Perhaps the most parsimonious explanation of my results is that it is the intensively infected birds that suffer severe body condition costs (Valkiūnas 2005) and the majority of such individuals are likely to be less mobile, hence under-sampled. Such a phenomenon is likely to bias our understanding of the ecology of parasites during the acute stages of parasitaemia in the wild. Therefore, I may have failed to determine the impact of the acute infection on body condition, which is a common challenge that several other researchers have faced when attempting to analyse the relationships between wild birds and their parasites (Bensch *et al*. 2007, Marzal *et al*. 2008). To further complicate these dynamics, the duration of the acute phase of haemoparasite infection is short, which makes it statistically unlikely that wild birds will normally be captured at the time of peak tissue schizogony or high parasitaemia (Atkinson and Samuel 2010). Valkiūnas (2005) noted a reduction in the mobility of infected birds within a couple of days under captive experimental conditions, a situation which is likely to be similar in natural ecosystems. Moreover, it is thought that during the acute stage of infection, birds may lose their competitive ability (to defend themselves and escape from predators) and therefore have a greater than usual chance of being predated on (Valkiūnas 2005, Møller and Nielsen 2007).

Parasitised birds are likely to have become infected initially as nestlings (and survived the primary acute infection). Two South African examples of infections in nestlings were illustrated by Oosthuizen and Markus (1968). With the acquisition of immunity, birds then become carriers of chronic infections (Valkiūnas 2005). However, we cannot discount the possibility that the results in the present study are attributable to combining the impacts of several species of parasites from a single genus, in which case this could have obscured the possibility of any variations in virulence and impacts on host fitness of different parasite species within a particular haematozoan genus (see below). The implications of avian haemosporidian infection in the wild is quite complex, which is perhaps a reason for contradictory findings in this type of research; in addition to which various unknown confounding factors could be involved, such as intestinal coccidiosis or helminthiasis (both being extremely common in wild birds). There are various parasite species within a given haemosporidian genus, and these species can have different virulence characteristics and effects on hosts (Lachish *et al.* 2011). Studies revealed that the survival rate and body condition of birds infected by *P. circumflexum* were much poorer than of those infected by *P. relictum*

(Lachish *et al.* 2011). Likewise, Palinauskas *et al.* (2011) demonstrated variations in the development, virulence and ecology of different *Plasmodium* lineages on avian health.

Elevated H:L ratios are reported to be linked to parasitic infections (Davis *et al.* 2008) through the triggering of stress hormones (Hõrak *et al.* 1998), but my results suggest the opposite if only haematozoa (i.e. excluding the possibility of unknown factors as well) are taken into consideration. The H:L ratio of infected birds was lower compared to that of uninfected individuals. Similar trends were observed in avian family groups, except for (1) Sturnidae infected with microfilariae and *Trypanosoma*, (2) Passeridae infected with filarial worms, and (3) Bucerotidae infected with *Plasmodium*; all of which birds had a slightly higher H:L ratio than uninfected individuals. This could be an erroneous impression caused by a small sample size. Generally, the H:L ratio findings correspond to the body condition results in the sense that infected birds have become carriers such that the parasitaemia levels are probably too low to have a pronounced effect on host immunity. On the other hand, very few researchers have attempted to quantify how stress actually affects H:L ratios of different species and sexes of birds. My analysis did not account for possible disparities attributable to host sex differences and species, which are likely to be related to different responses to parasitic infections (Jain 1986). Granthon and Williams (2017) found that infected female *Vireo* spp. had a higher H:L ratio than infected males and this was attributed to greater breeding demands experienced by female birds. There are also inconsistencies concerning what to regard as the normal parameter of the H:L ratio for a healthy bird (Werner 2007). In addition, differentiating between a H:L ratio that has become elevated as a result of an immune response to parasitic infection from that of a H:L ratio raised by other stressful activity such as breeding and migration, is very complicated. This paradox makes it extremely difficult to come up with a consensus on how to interpret leucocyte profiles and, further, to determine an individual's capacity and potential to fight off parasitic infections. Nonetheless, Figuerola *et al.* (1999) concluded that higher H:L ratios were associated with a better fitness condition in Cirl Buntings *Emberiza cirlus* sampled in Spain.

At face value, my findings suggest that haemoparasite infections in birds in the Kruger National Park are of little significance in regard to immunity and the body condition of the host. In some instances, the infection appears to be beneficial, as suggested by improved immunity and body condition, which is not what one would expect intuitively. It could be a spurious correlation and perhaps H:L ratios cannot be used exclusively to determine the influence of parasitic infections on host immunity. There is a need to concurrently employ other techniques together with H:L ratios, e.g. those that involve determining the specific cytokine

profiles that are elicited by haematozoan infections. Though this may seem to be a desirable approach towards tackling the disparities evident in avian immune response studies, most of the laboratory equipment and consumables required for such analyses are usually expensive and often unaffordable to researchers based in developing countries where avian haemoparasites are common.

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CHAPTER 4

Synthesis

There has been growing interest in the study of avian diseases following a rise in recent years of the emergence and spread of zoonotic infections. The expansion of human populations, coupled with climate change, deforestation and urbanisation leads to irreversible effects on the habitats of wildlife and their pathogens (Sehgal 2015). Given that birds are particularly well adapted to serve as reservoirs of both pathogenic and non-pathogenic organisms, avian diversity offers an opportunity to study host-parasite relationships (Causey and Edwards 2008). Insight into the determinants, pathogenesis and implications of avian diseases in the environment is undoubtedly an invaluable addition to knowledge of the complex field of disease ecology. The primary aim of this study was therefore to acquire an understanding of the prevalence of avian haemoprotozoa in Kruger National Park and the surrounding human settlements and to consider their implications for avian ecology.

My first objective was to explore how season (wet vs dry) and location affects parasite prevalence (Chapter 2). This involved assessing how prevalence trends in the wet season differed from those in the dry season, with the assumption that infections would be much more prevalent in the wet season when the climate becomes more favourable for vectors. However, total prevalence was constant during both seasons; yet there were individual parasite type differences in terms of seasonal variation. *Haemoproteus* infection prevalence was much higher in the wet season, whereas *Plasmodium* was mostly dominant in the dry season. *Trypanosoma* and microfilarial prevalences were also much higher in the dry season. There was clearly no common seasonal trend for all haemoparasites combined. These findings show that birds are carriers of infections and suggest that prevalence persists beyond the wet season when vectors are most active (Gage *et al.* 2008). An analysis of the prevalence of infection according to sampling sites showed no site differences. I had assumed that the prevalence of haemoparasites infection in birds at sites located in the southern region of the park would be the highest, considering a high rainfall gradient from the south to the north which has a direct impact on potential vector establishment (Scientific Services 2017). The ability of birds to fly over long distances is likely to have collapsed environmental site differences as a determinant of infection, allowing birds to spread haemoparasites over a wide area, i.e. throughout the Lowveld region. Lastly, I investigated the impact of parasitism on host body condition and immunity (Chapter 3). Findings were that infected birds did not suffer any body condition losses, or costs that are reflected by heterophil to lymphocyte ratios. The prevalence and

diversity of avian haematozoa were relatively high (six genera plus filarial worms) in the Kruger National Park, compared to other studies done in West Africa. For example, Olayemi *et al.* (2014) found only four kinds of blood parasite in Nigeria. Prevalence of avian haemoparasites detected by microscopy in previous studies at various parts of the African savana showed prevalence rates of 11.5% in Senegal, 13% in Cameroon and 19·1% across sub-Saharan Africa, while east Africa recorded the highest prevalence of 37% (Bennett and Herman 1976, Bennett *et al.*1978, Kirkpatrick and Smith 1988, Bennett *et al.*1992).

Infections in the present study appeared to be of little consequence for the host's body condition, implying that these infections are endemic in the Lowveld region of South Africa. It remains unclear why such infections did not have any pronounced effects on the health of the birds sampled. The most parsimonious explanation is that this South African community of birds has become immunogenetically adapted to these parasites, which are thought to be endemic to the region, as stated above. Hence the effects (should there be any) of infections are now less overt than they might otherwise have been (Bensch *et al.* 2004). Despite the high prevalence and diversity of parasites (Chapter 2), their effects on the physical wellbeing of these study birds, appears to be of no significance, for now. However, the environmental and climate change events that are currently taking place might well come to affect the distribution, spread and prevalence of parasitic diseases of vertebrates, with possible implications for biodiversity, health and conservation in the future (Gavrilles 2013). Studies elsewhere (Valkiūnas 2005, Jones *et al.* 2013) show that such environmental changes can alter the ecology of the host, parasite or vector, leading to new unstable relationships which could trigger outbreaks of diseases. This may lead to redefining the evolution of parasites and emergence of virulent strains. Such novel epidemics might threaten the immune competence of naive birds. Anthropogenic pressures in areas in close proximity to the Kruger National Park have created structural diversity in the environment and its habitat for vectors which, in turn, could in theory support a greater diversity of parasites. A risk then lies in there being a spill-over of parasites from birds outside the park to those inside the park or *vice versa*. Furthermore, it is possible that the proximity of wild birds to domestic birds in these settlements could leave potentially susceptible poultry exposed to novel infections.

Limitations

A major constraint as regards being able to draw definitive conclusions from the results of this work was the limited sample sizes for considering the influence of different genera of parasites on various species and family groups of birds at a particular site and season. This information

is essential for assessing host specificity amongst parasites. Nevertheless, there was a reasonable suggestion that bird family group is for whatever reason(s) a strong determinant of prevalence; and starlings were the most infected group.

Microscopy has been the traditional method used to screen avian blood for parasites (Valkiūnas, 2005). The technique is based on using morphological features exhibited by parasites on blood smear slides for identification (Makler *et al.* 1998). However, there are challenges associated with the use of microscopy. Firstly, chronic infections with low parasitaemia can be difficult to detect in birds by microscopy (Jarvi *et al.* 2003, Waldenström *et al.* 2004). Furthermore, it is possible for the same parasite species to exhibit different morphology, depending on the host in which it is present. *Plasmodium* species were a challenge when screening, given that the genus can only be positively identified from its mature stages; and immature forms are difficult to observe. Another challenge faced was the failure of the ocular method to differentiate between several species within a genus. This is likely to have obscured the reality of any variations in virulence among different species of parasites within the same genus and, in turn, their effects on host fitness. Nevertheless, it is likely that my findings are a conservative estimate of the true burden of parasitaemia in the birds sampled. Few studies have compared the traditional morphological methods used to identify haemoparasites with modern molecular techniques of identification (e.g. Okanga *et al*. 2013) and thus the true diversity of these parasites, especially in summer rainfall areas of southern Africa, remains unknown (Martinsen *et al*. 2006). I recommend the use of molecular screening techniques, i.e. involving DNA amplification by application of the Polymerase Chain Reaction (PCR), in future studies to complement microscopy findings and to distinguish between species within a genus, thereby facilitating elucidation of the impacts of infection on hosts.

Attempts to quantify the immune response to haemoparasite infections in wild birds through the use of leucocyte profiles proved to be quite complicated in terms of analysis. The technique measures stress levels in the host but may fail to differentiate stress levels caused by inflammation, parasitic infection or any other stressful activity, such as handling of trapped birds (Davis *et al*. 2008). This makes heterophil/lymphocyte ratio counts unsuitable to use exclusively. They are more useful when complemented by other approaches and information, such as (1) assessing the concentration of haptoglobin, which is triggered during the immune response to parasites; and (2) Packed Cell Volume (PCV) data, which gives the percentage of erythrocytes circulating in the blood (Matson *et al.* 2006).

Despite the challenges faced in this study, it is, to my knowledge, the first time that the prevalence of avian haemoparasites has been investigated in the Kruger National Park. The

findings reflect the complex relationship between parasites and their hosts in southern African environments. The work has set a foundation upon which future research incorporating more advanced screening protocols and technologies can build, thereby improving the scientific quality of the parasitological research involved. I conclude that this study supports the argument that avian haemoparasite infections have co-evolved with their hosts in endemic areas of South Africa. However, despite the fact that infections may have minimal pathogenic influence, it is important to continue monitoring them, considering the current changes in climate and the environment that are taking place and which may result in alteration of the dynamics of the ecology of wildlife diseases in the future.

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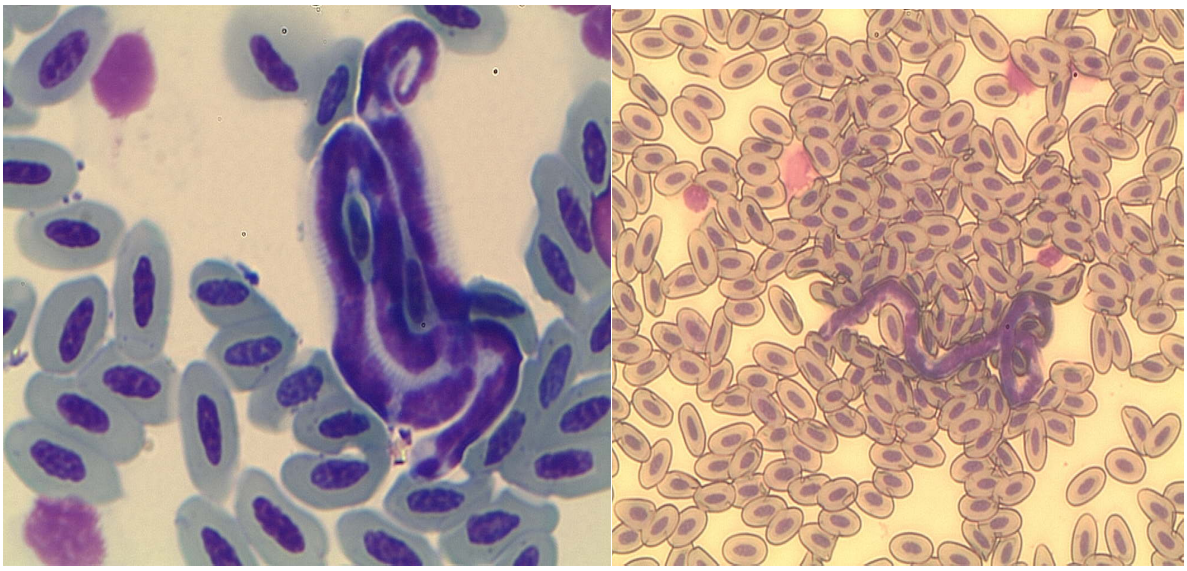
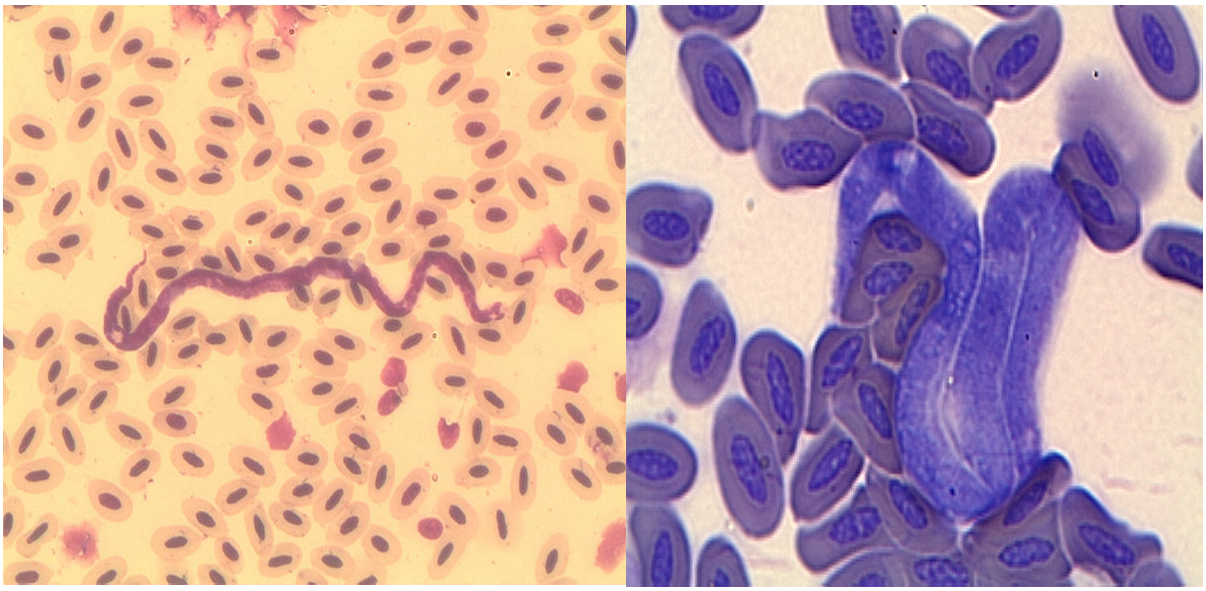
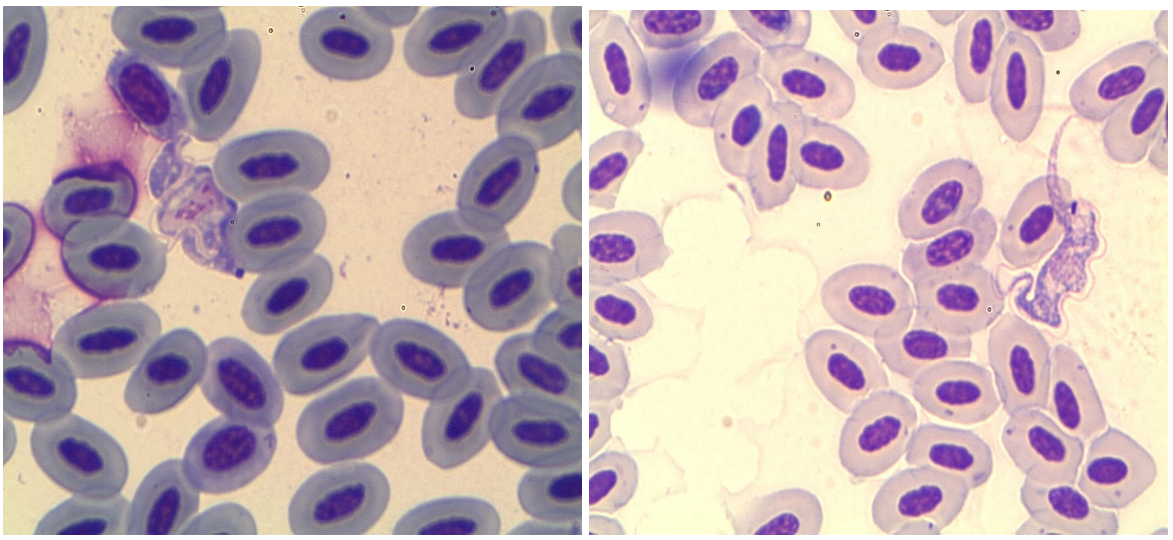
Appendix 1



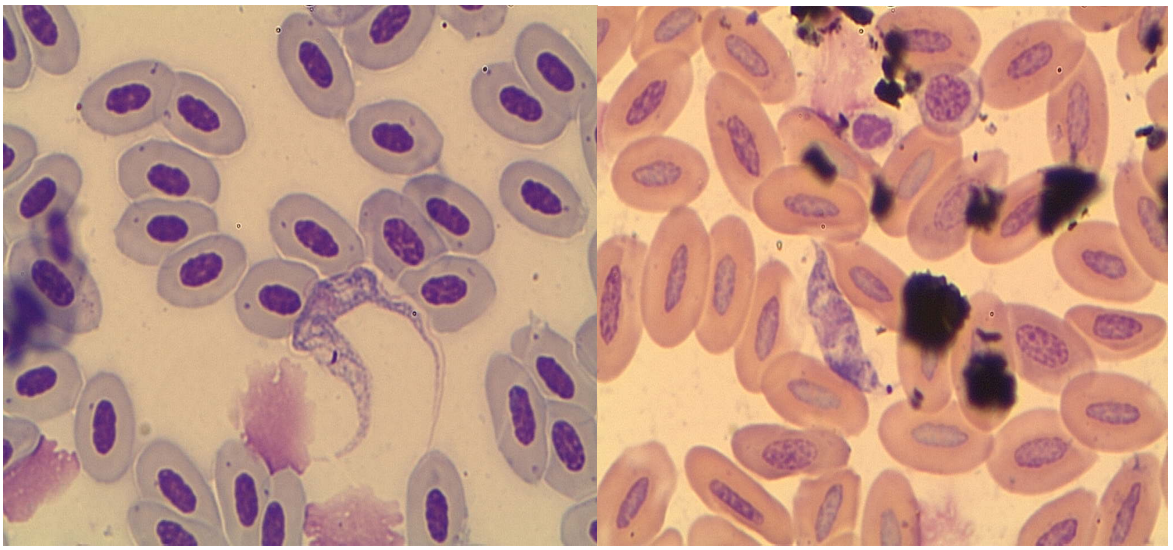
Microfilariae in an African Mourning Dove from Shingwedzi.



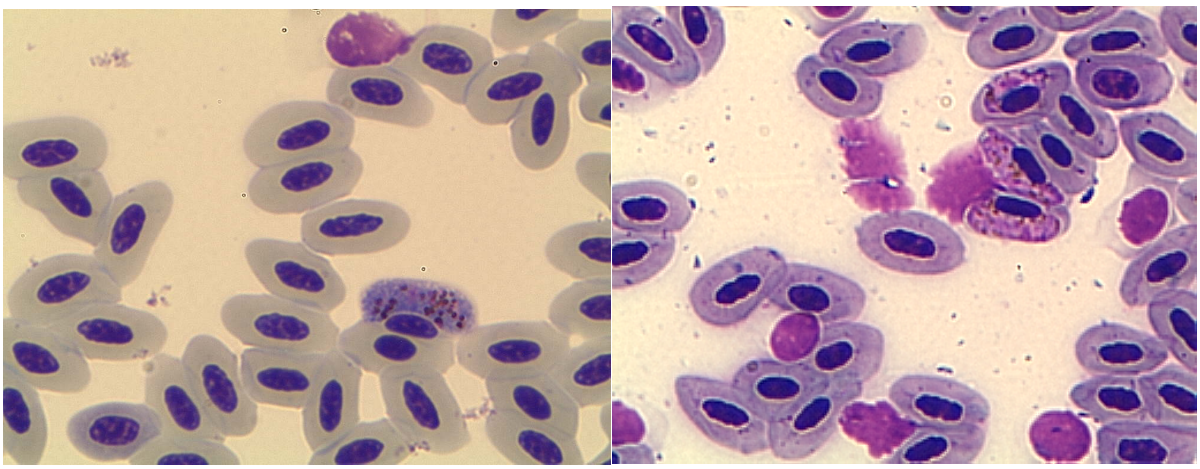
Microfilariae in an African Mourning Dove from Shingwedzi (Left) and a Blacksmith Lapwing from Skukuza (Right).



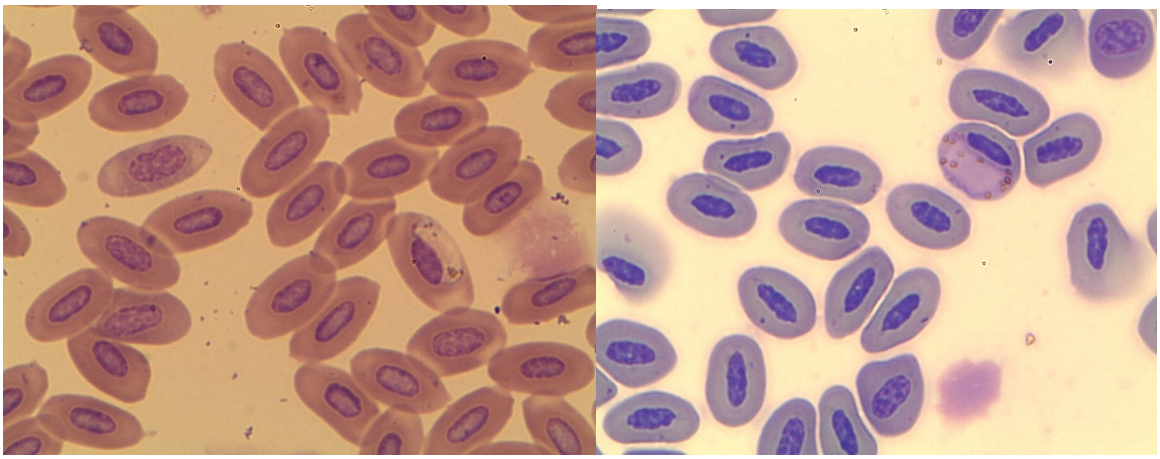
Blood smears from a Red-billed Hornbill from Shingwedzi (Left) and a Yellow-fronted Canary from Skukuza (Right) infected by *Trypanosoma* spp.



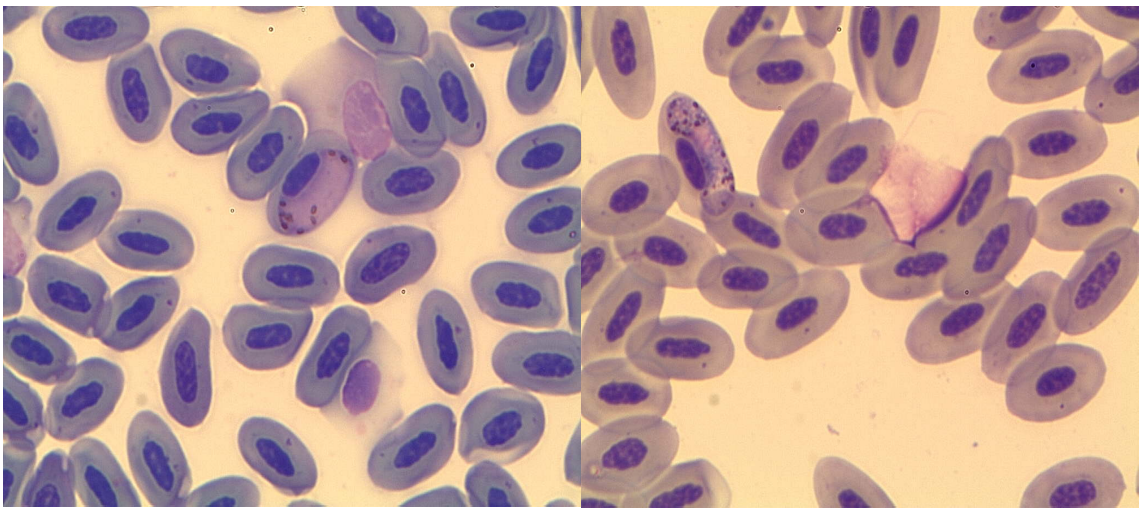
Blood smears from a Yellow-fronted Canary from Skukuza (Left) infected by *Trypanosoma* sp. and a Red-billed Hornbill from Phalaborwa (Right) infected by *Trypanosoma everetti.*



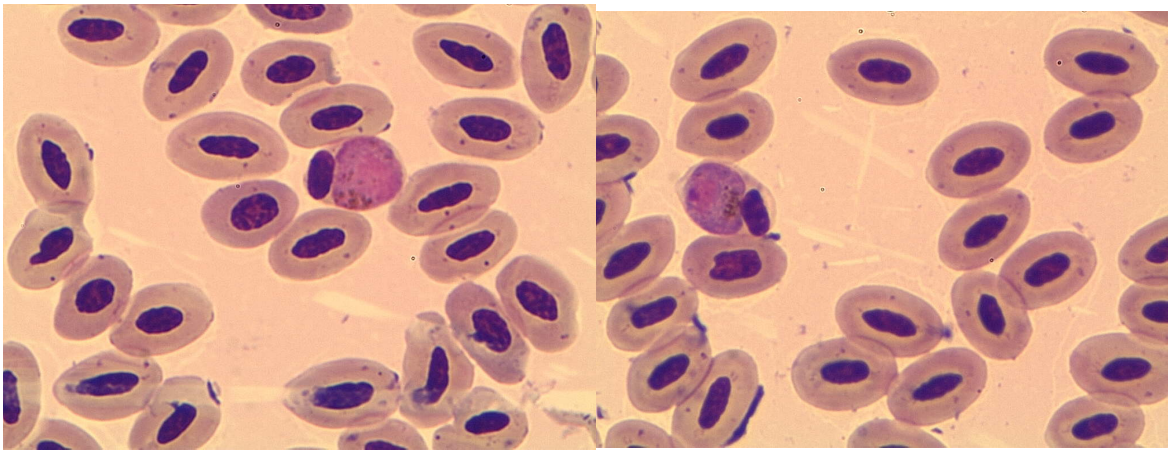
Haemoproteus spp. infection in an African Mourning Dove from Shingwedzi (Left) and Dark-capped Bulbul from Skukuza (Right).



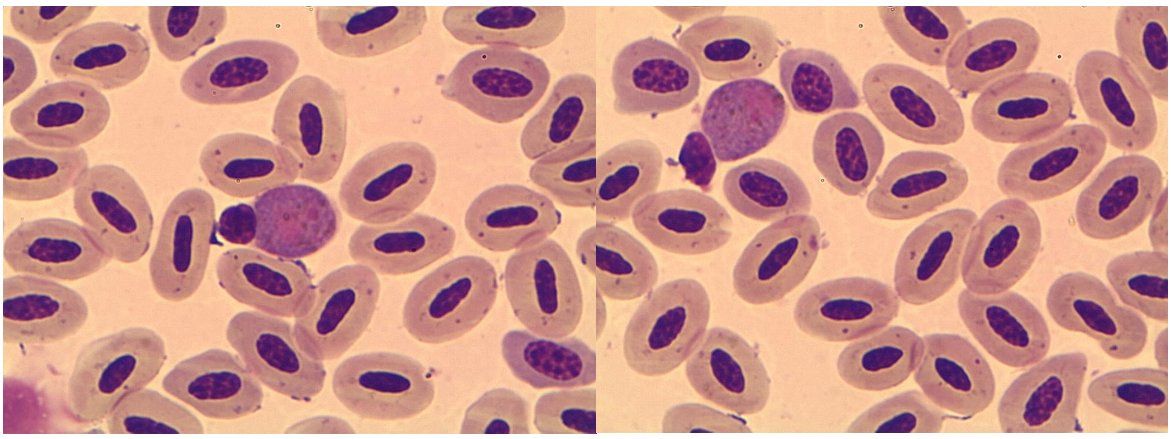
Immature *Haemoproteus* sp. gametocyte in a Red-headed Weaver from Phalaborwa (Left) and *Haemoproteus pastoris* in a Greater Blue-eared Starling from Skukuza (Right).



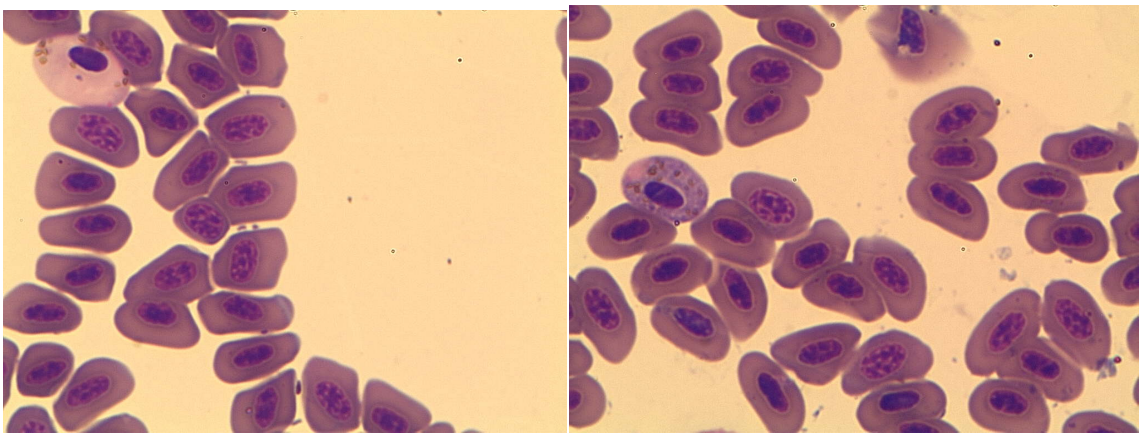
Haemoproteus sp. infection in a Yellow-fronted Canary from Skukuza (Left) and *Haemoproteus burhini* in a Water Thick-knee from Skukuza (Right).



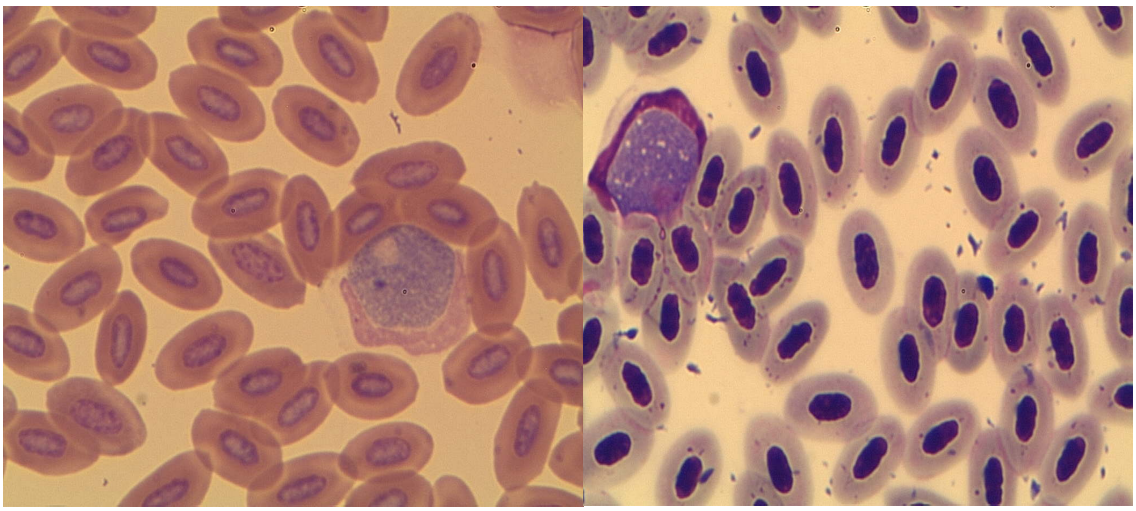
Plasmodium relictum displacing the nuclei of erythrocytes of a Southern Yellow-billed Hornbill at Skukuza.



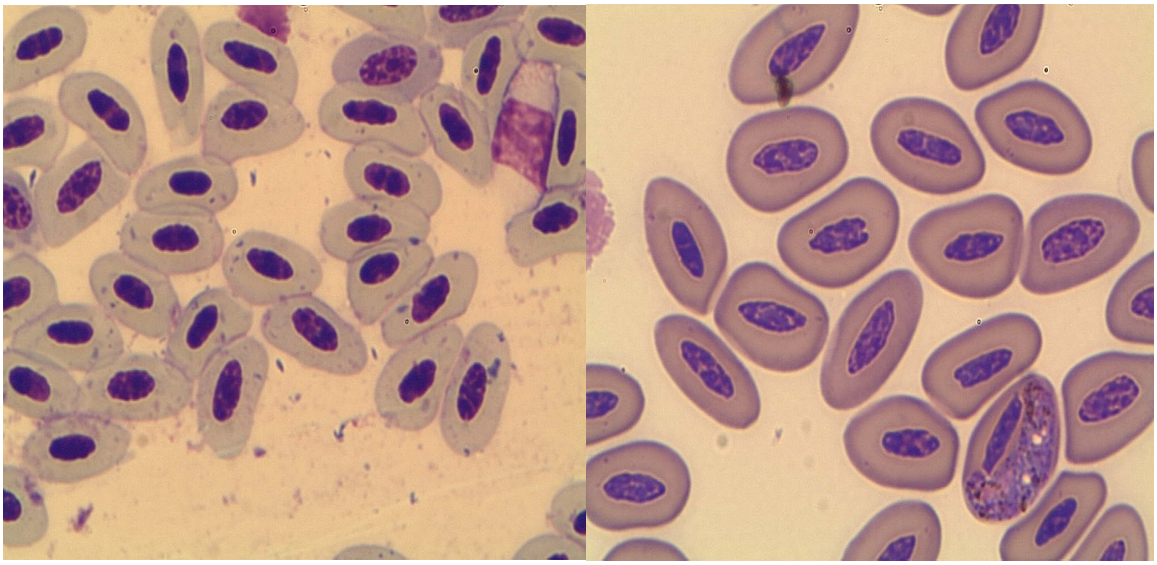
Plasmodium relictum "evicts" the nuclei and occupies erythrocytes of a Southern Yellow-billed Hornbill from Skukuza.



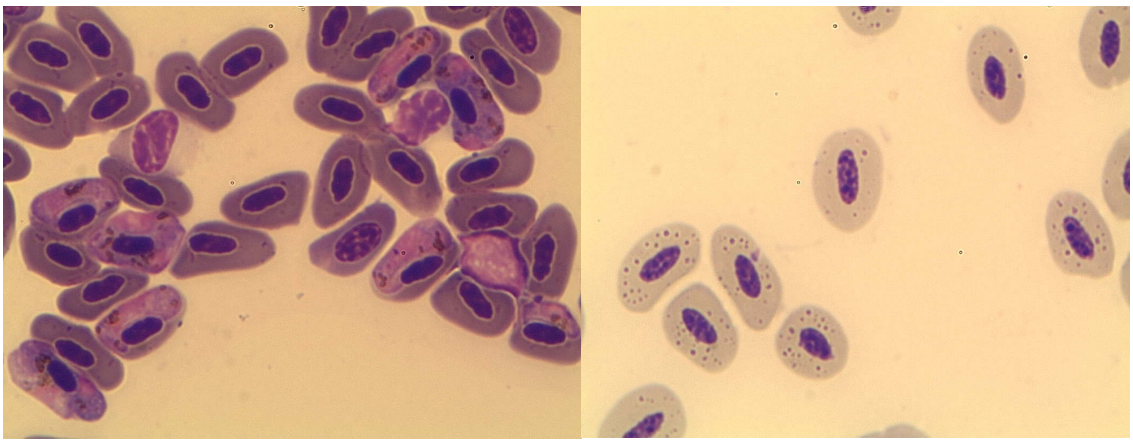
Suspected *Haemoproteus magnus* in a Yellow-fronted Canary from Mkhuhlu.



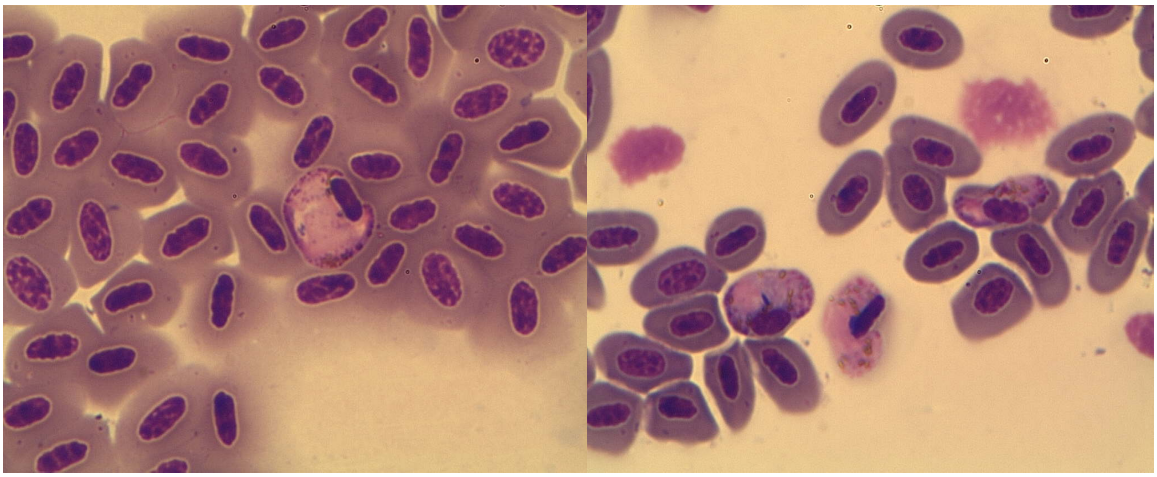
Leucocytozoon bouffardi infection in a Red-headed Weaver from Phalaborwa (Left) and *Leucocytozoon dubreuili* in a Yellow-fronted Canary from Skukuza (Right).



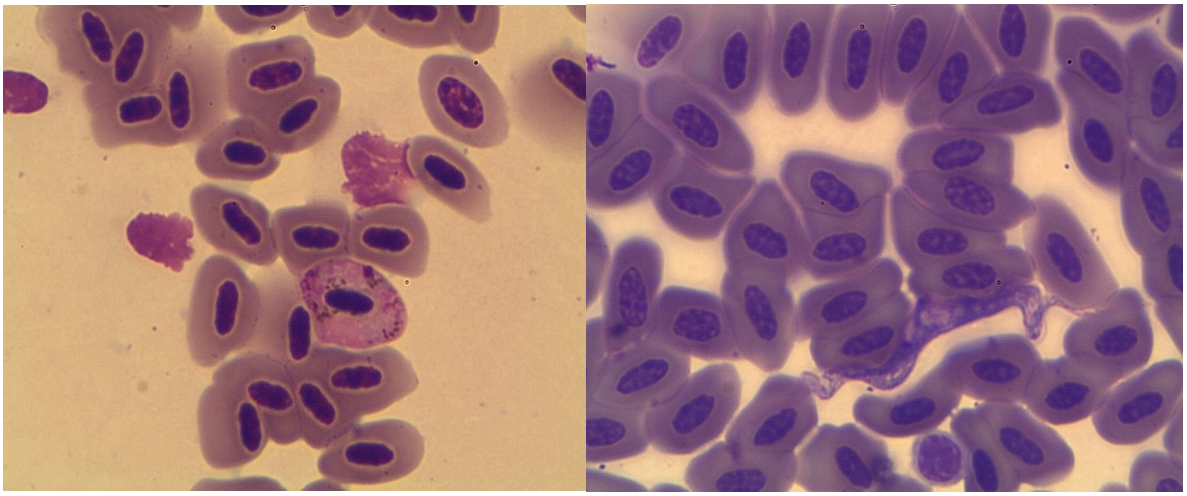
Hepatozoon sp. infection in a Speckled Weaver from Skukuza (Left) and *Haemoproteus* sp. infection in a Marabou Stork in Skukuza (Right).



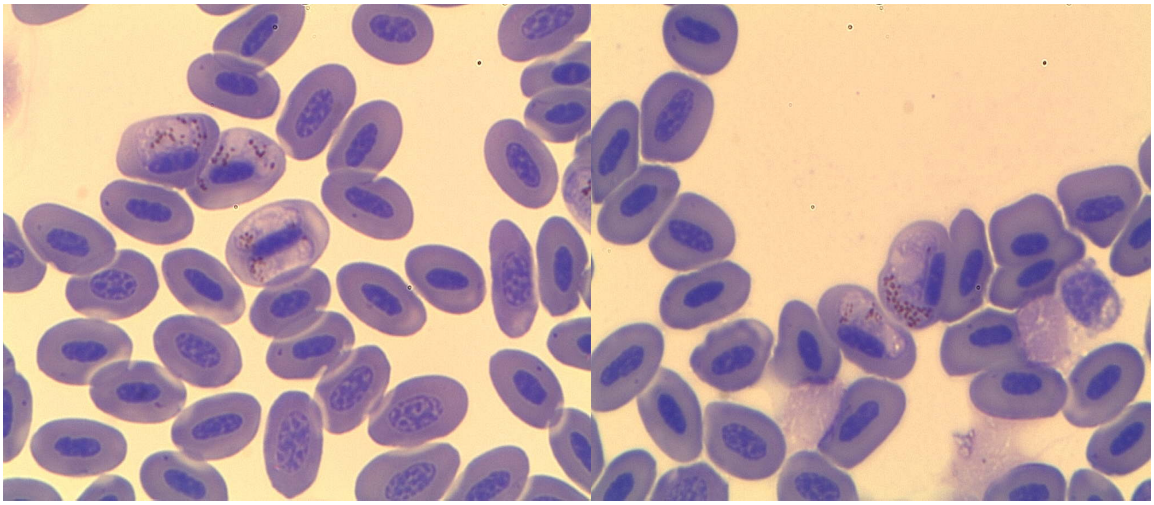
High level of parasitaemia of *Haemoproteus prognei* in a European Swallow (Left) and *Aegyptianella* sp. infection in a Yellow-fronted Canary from Mkhuhlu (Right).



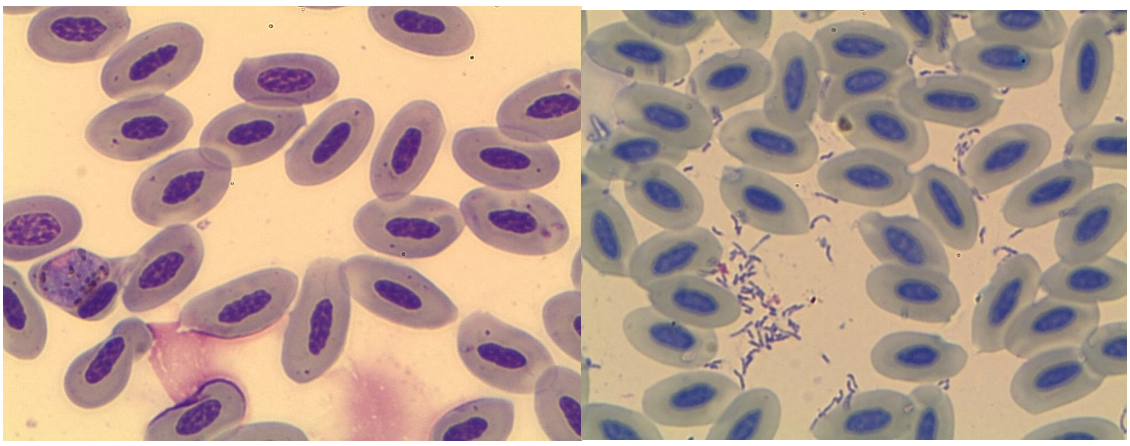
Haemoproteus sp. infection in a Yellow-fronted Canary from Mkhuhlu.



H*aemoproteus* sp. infection in a Greater Blue-eared Starling from Skukuza (Left) and Microfilaria in a Laughing Dove from Phalaborwa (Right).



Two immature gametocytes of *Haemoproteus orizivorae* in the same cell, resulting from high or rising parasitaemia (Left), and a distorted mature gametocyte of *Haemoproteus orizivorae* in which the pigment granules have been "pushed" to one pole. The host was a Blue Waxbill from Shingwedzi (Right).



Plasmodium sp. (Left) and an unidentified bacterial infection (Right) in a Water Thick-knee from Skukuza.

Appendix 2.

Table 1. Total number of birds caught in the wet and dry season at all the sites, their foraging guild and social status, and the associated haemoparasite present. Haemoparasite labels key: "H" represents *Haemoproteus*, "P" represents *Plasmodium*, "L" represents *Leucocytozoon*, "T" represents *Trypanosoma*, "M" represents Microfilaria(e), "A" represents *Aegyptianella*, "Hp" represents *Hepatozoon* and "–" represents uninfected birds. The number in parentheses represents the number of birds infected with that particular parasite out of those from which blood was examined.

Common name	Species	Foraging Guild	Social Status	Number Caught	Haemoparasite Presence
African Grey Hornbill	*Tockus nasutus*	Ground	Solitary	2	–
African Hoopoe	*Upupa africana*	Ground	Solitary	5	–
African Jacana	*Actophilornis africanus*	Aquatic	Solitary	8	M (1)
African Mourning Dove	*Streptopelia decipiens*	Ground	Solitary	31	H (7), M (6)
African Paradise Flycatcher	*Terpsiphone viridis*	Aerial	Solitary	4	H (1)
African Pied Wagtail	*Motacilla aguimp*	Ground	Solitary	7	P (1)
Arrow–marked Babbler	*Turdoides jardineii*	Ground	Gregarious	6	H (2), P (3)
Ashy Flycatcher	*Muscicapa caerulescens*	Aerial	Solitary	4	T (1), M (1)
Bearded Scrub–Robin	*Cercotrichas quadrivirgata*	Ground	Solitary	3	P (1)
Black-backed Puffback	*Dryoscopus cubla*	Canopy	Solitary	2	–
Black Crake	*Amaurornis flavirostris*	Aquatic	Solitary	1	H (1)
Black–collared Barbet	*Lybius torquatus*	Canopy	Solitary	4	H (1), M (1)
Blacksmith Lapwing	*Vanellus senegallus*	Ground	Solitary	2	–
Blue Waxbill	*Uraeginthus angolensis*	Ground	Gregarious	1	H (1), T (1)

Bearded Scrub–Robin	*Cercotrichas quadrivirgata*	Ground	Solitary	1	H (1)
Bronze Mannikin	*Lonchura cucullata*	Ground	Gregarious	3	–
Brown–crowned Tchagra	*Tchagra australis*	Ground	Solitary	3	–
Brown–hooded Kingfisher	*Halcyon albiventris*	Ground	Solitary	4	H (1)
Brubru	*Nilaus afer*	Canopy	Solitary	1	–
Burchell's Starling	*Lamprotornis australis*	Ground	Solitary	1	–
Bushveld Pipit	*Anthus caffer*	Ground	Solitary	1	–
Cape Starling	*Lamprotornis nitens*	Ground	Gregarious	10	H (4), P (1)
Cape Wagtail	*Motacilla capensis*	Ground	Gregarious	2	–
Cape Weaver	*Ploceus capensis*	Ground	Solitary	7	H (4), P (1), A (1)
Cape White–eye	*Zosterops pallidus*	Canopy	Solitary	1	–
Cardinal Woodpecker	*Dendropicos fuscescens*	Canopy	Solitary	1	–
Chinspot Batis	*Batis molitor*	Canopy	Solitary	1	–
Common Myna	*Acridotheres tristis*	Ground	Gregarious	2	H (1), P (1)
Crested Barbet	*Trachyphonus vaillantii*	Ground	Solitary	3	–
Crested Francolin	*Peliperdix sephaena*	Ground	Solitary	9	L (1), P (1)
Dark–capped Bulbul	*Pycnonotus tricolor*	Canopy	Solitary	27	H (1), P (1)
Egyptian Goose	*Alopochen aegyptiacus*	Ground	Gregarious	3	–
Emerald–spotted Wood–dove	*Turtur chalcospilos*	Ground	Solitary	2	–
European Bee–eater	*Merops apiaster*	Aerial	Gregarious	1	–
European Swallow	*Hirundo rustica*	Aerial	Gregarious	29	H (3), T (1)
Fiery–necked Nightjar	*Caprimulgus pectoralis*	Canopy	Solitary	1	H (1)
Fork–tailed Drongo	*Dicrurus adsimilis*	Aerial	Solitary	20	H (2)
Golden–breasted Bunting	*Emberiza flaviventris*	Ground	Solitary	2	–
Greater Blue–eared Starling	*Lamprotornis chalybaeus*	Ground	Gregarious	170	H (55), T (2), P (4), L (1), M (4)
Green Wood–hoopoe	*Phoeniculus purpureus*	Canopy	Gregarious	5	M (1)

Grey Go–Away Bird	*Corythaixoides concolor*	Canopy	Gregarious	1	–
Grey–backed Camaroptera	*Camaroptera brachyura*	Ground	Solitary	1	–
Hadeda Ibis	*Bostrychia hagedash*	Ground	Solitary	2	H (1)
House Sparrow	*Passer domesticus*	Ground	Gregarious	50	H (3), P (1), M(1), A (2), T (1)
Laughing Dove	*Streptopelia senegalensis*	Ground	Solitary	28	H (1), M (2), T (1)
Lesser Masked Weaver	*Ploceus intermedius*	Canopy	Solitary	19	H (3)
Lesser Striped Swallow	*Hirundo abyssinica*	Aerial	Solitary	2	H (1), P (2)
Little Bee–eater	*Merops pusillus*	Canopy	Solitary	4	–
Little Rush Warbler	*Bradypterus baboecala*	Ground	Solitary	2	H (1)
Long–billed Crombec	*Sylvietta rufescens*	Canopy	Solitary	3	T (1)
Malachite Kingfisher	*Alcedo cristata*	Aquatic	Solitary	1	–
Marabou Stork	*Leptoptilos crumeniferus*	Ground	Gregarious	4	H (2), M (1), P (1)
Melba Finch	*Pytilia melba*	Ground	Solitary	2	H (1), M (1)
Orange–breasted Bush–Shrike	*Telophorus sulfureopectus*	Canopy	Solitary	1	H (1)
Red–backed Shrike	*Lanius collurio*	Ground	Solitary	1	H (1), P (1)
Red–billed Buffalo–Weaver	*Bubalornis niger*	Ground	Solitary	1	–
Red–billed Hornbill	*Tockus erythrorhynchus*	Ground	Solitary	35	H (1), M (5), T (2), M (5)
Red–billed Spurfowl	*Pternistis adspersus*	Ground	Solitary	1	–
Red–capped Robin–Chat	*Cossypha natalensis*	Ground	Solitary	4	H (1), L (1)
Red–headed Weaver	*Anaplectes rubriceps*	Canopy	Solitary	1	–
Sand Martin	*Riparia riparia*	Aerial	Gregarious	1	–
Sombre Greenbul	*Andropadus importunus*	Canopy	Solitary	2	–
Southern Black Flycatcher	*Melaenornis pammelaina*	Canopy	Solitary	1	–
Southern Black Tit	*Parus niger*	Canopy	Solitary	3	–

Southern Grey–headed Sparrow	*Passer diffusus*	Ground	Solitary	14	H (9), P (1), M (1), T (2)
Southern Masked Weaver	*Ploceus velatus*	Ground	Gregarious	5	H (1)
Southern Yellow–billed Hornbill	*Tockus leucomelas*	Ground	Solitary	19	H (2), P (1), T (2), M (2)
Speckled Mousebird	*Colius striatus*	Canopy	Gregarious	3	–
Spectacled Weaver	*Ploceus ocularis*	Canopy	Solitary	3	T (1), Hp (1)
Terrestrial Bulbul	*Phyllastrephus terrestris*	Ground	Gregarious	2	–
Thick–billed Weaver	*Amblyospiza albifrons*	Canopy	Gregarious	1	–
Village Weaver	*Ploceus cucullatus*	Ground	Gregarious	10	H (1)
Violet–backed Starling	*Cinnyricinclus leucogaster*	Canopy	Solitary	4	–
Water Thick–knee	*Burhinus vermiculatus*	Ground	Solitary	2	H (2), P (1)
Wattled Starling	*Creatophora cinerea*	Ground	Gregarious	1	–
White–bellied Sunbird	*Cinnyris talatala*	Canopy	Solitary	2	H (1)
White–browed Robin–Chat	*Cossypha heuglini*	Ground	Solitary	4	–
White–browed Scrub–Robin	*Cercotrichas leucophrys*	Ground	Solitary	2	–
White–crested Helmet–Shrike	*Prionops plumatus*	Canopy	Gregarious	5	–
White–faced Duck	*Dendrocygna viduata*	Aquatic	Gregarious	2	–
White–fronted Bee–eater	*Merops bullockoides*	Ground	Gregarious	3	–
Wire–tailed Swallow	*Hirundo smithii*	Aerial	Solitary	19	H (1)
Yellow–fronted Canary	*Serinus mozambicus*	Ground	Solitary	22	H (11), P (3), L (3), T (6), A (1)
Yellow–throated Petronia	*Petronia superciliaris*	Ground	Solitary	1	–
Yellow–bellied Greenbul	*Chlorocichla flaviventris*	Canopy	Solitary	1	H (1)
TOTAL				685	

Appendix 3

Table 1. Kruskal–Wallis test statistics for distribution of parasites across sites.

Parasite	H	df	P
Haemoproteus	7.53	5	0.18
Plasmodium	5.45	5	0.36
Trypanosoma	5.73	5	0.33
Microfilariae	5.96	5	0.31
Leucocytozoon	6.01	5	0.31
Hepatozoon	4.01	5	0.55
Aegyptianella	8.88	5	0.11

Table 2. Mann Whitney U test values for distribution of haemoparasites between seasons.

Parasite	U	df	P
Haemoproteus	9	5	0.61
Plasmodium	9	5	0.61
Trypanosoma	8.5	5	0.48
Microfilariae	9	5	0.61
Leucocytozoon	9	5	0.61
Hepatozoon	10	5	0.76
Aegyptianella	10.5	5	0.76