International Journal for Parasitology: Parasites and Wildlife 21 (2023) 47–54

Contents lists available at ScienceDirect

International Journal for Parasitology: Parasites and Wildlife

journal homepage: www.elsevier.com/locate/ijppaw

Epidemiology of *Anaplasma marginale* and *Anaplasma centrale* infections in African buffalo (*Syncerus caffer*) from Kruger National Park, South Africa☆

Danielle Sisson [a,*], Brianna Beechler [b], Abdul Jabbar [a], Anna Jolles [b,c], Jasmin Hufschmid [a]

[a] Department of Veterinary Biosciences, Melbourne Veterinary School, The University of Melbourne, Werribee, Victoria, 3030, Australia
[b] Carlson College of Veterinary Medicine, Oregon State University, Magruder Hall, 700 SW 30th St, Corvallis, OR, 97331, USA
[c] Department of Integrative Biology, Oregon State University, Cordley Hall, 3029, 2701 SW Campus Way, Corvallis, OR, 97331, USA

1. Introduction

The epidemiology of wildlife disease is a growing field of research. One reason for this is that it provides unique opportunities for the study of the spread of infections in unmanaged settings (Eda et al., 2005). In domesticated animals, disease dynamics are usually altered due to anthropogenic interventions in animal movement, behaviour and disease progression (Siembieda et al., 2011). Wildlife systems, however, can provide us with a deeper understanding of factors affecting prevalence, transmission dynamics, and the effects of concurrent infections with two or more pathogens (Fynn et al., 2016). Another reason is that many livestock diseases have wildlife reservoirs (Buddle et al., 2018; González-Barrio and Ruiz-Fons, 2019). Therefore, management strategies aiming to avoid or, at least, reduce infection and losses at the wildlife-livestock interface, rely on a solid understanding of disease dynamics within the wildlife hosts (Berentsen et al., 2013), which is, however, often missing. For instance, anaplasmosis, heartwater (caused by *Ehrlichia* sp.), theileriosis and babesiosis are known to cause 18% of reported cattle mortalities in South Africa (De Waal, 2000). Nonetheless, although transmission between livestock and wildlife is known to occur for all of these diseases, their epidemiology in wildlife hosts is poorly understood.

Anaplasma species (Rickettsiales: *Anaplasmataceae*) are gram-negative, obligate intracellular bacteria which parasitise blood cells (Aubry and Geale, 2011; Brown, 2012). They have a near global distribution and affect a wide range of host species, though ruminants appear to be particularly susceptible to anaplasmosis. The pathogenicity of *Anaplasma* infection varies with the species (host and pathogen), host age and tissue tropism, with the outcome ranging from subclinical infections, mild illness and abortion to death (Aubry and Geale, 2011; Potgieter and Stoltz, 2004). Infection under the age of six months doesn't usually result in significant clinical disease (Aubry and Geale 2011), but animals have been shown to remain chronically infected, albeit with very low numbers of erythrocytes affected, for the rest of their lives (Grau et al., 2013; Potgieter and Van Rensburg, 1987). Of the total cattle population in South Africa, 99% are predicted to be at risk of developing anaplasmosis, as most cattle farming occurs in areas where the tick vector is endemic (De Waal, 2000). Anaplasmosis is considered one of the most important tick-borne diseases in sub-Saharan Africa, and is estimated to be responsible for 3% of all cattle mortalities in South Africa (De Waal, 2000; Eygelaar et al., 2015). *Anaplasma marginale* and *A. centrale* can also be transmitted by blood-contaminated fomites and biting insects (Kocan et al., 2010). Transmission through fomites is less likely in wildlife species as they do not receive same level of husbandry as domesticated livestock and so are less at risk. Insects remain a possibility, however there is strong overlap of tick species able to transmit the infection, and the presence of the infection, indicate that the tick-borne means of transmission remains strong (Hove et al., 2018; Makgabo et al., 2023).

While there are efforts to minimise the effects of anaplasmosis in cattle through vaccination, management strategies also need to consider interactions of livestock with wildlife that can host these infections. African buffalo (*Syncerus caffer*) are thought to be the main reservoir for anaplasmosis in southern Africa (Debeila, 2012). The three main species of *Anaplasma* found in this host are *A. marginale*, *A. centrale* and *A. omatjenne* (Debeila, 2012; Henrichs et al., 2016). There has also been one instance of infection with *A. phagocytophilum* (normally found in dogs, or wild ruminants in other areas of the world, and occasionally infecting humans), which raises concerns about the expanding zoonotic potential for this parasite (Henrichs, 2014). *Anaplasma marginale* and *A. centrale* infections in wildlife, including African buffalo, appear to be largely subclinical (Henrichs et al., 2016). Little is yet known about *A. omatjenne*.

Previous studies have investigated co-infections with different

☆ Note: Supplementary data associated with this article.

* Corresponding author.
E-mail addresses: danielle.sisson@my.jcu.edu.au (D. Sisson), jabbara@unimelb.edu.au (B. Beechler), huj@unimelb.edu.au (A. Jabbar), brianna.beechler@oregonstate.edu (A. Jolles), jollesa@science.oregonstate.edu (J. Hufschmid).

https://doi.org/10.1016/j.ijppaw.2023.04.005
Received 25 December 2022; Received in revised form 3 April 2023; Accepted 12 April 2023
Available online 14 April 2023

pathogens in African buffalo, and have reported on their role in driving seasonal pathogen dynamics and disease outcomes (Beechler et al., 2019; Combrink et al., 2020; Ezenwa, 2016; Ezenwa et al., 2019; Fagbo et al., 2014; Glidden et al., 2020, 2021; Gorsich et al., 2014, 2018; Henrichs et al., 2016; Sabey et al., 2021). Tissue tropism appears to play a significant role in the impacts of co-infection in this host species. It has been shown, for example, that co-infection with two or more haemoparasite species can impact each species' ability to persist within African buffalo, whereas co-infection with parasites or pathogens occupying different host tissues (e.g. gastrointestinal helminths and protozoans), did not alter patterns of haemoparasitic infections (Henrichs et al., 2016). There remain, however, some important limitations to our current understanding of haemoparasite dynamics in this species. Previous studies focused on presence or absence of infection, but did not consider the intensity of infection. They exclusively sampled adult, female buffalo, with limited consideration of seasonality, and had limitations when examining co-infections between *A. marginale* and *A. centrale*, such as a lack of temporal relationships (i.e. which infection comes first).

The impacts of infection are, of course, dependent on a range of host, environmental and pathogen factors, and it seems likely that the same is true for co-infections. Further work on co-infection interactions of *Anaplasma* spp. in African buffalo, addressing these additional factors, will also allow better comparison with host effects of anaplasmosis in cattle and help to identify risk factors that contribute to transmission at the livestock/wildlife interface. This study therefore investigated whether external (season), internal (sex and age) and temporal factors affect co-infection patterns with different *Anaplasma* spp.

2. Materials and methods

2.1. Study site and animal characteristics

The study population was a herd of African buffalo used in previous studies, resident at Kruger National Park (KNP), South Africa (Couch et al., 2017; Glidden et al., 2018; Sisson et al., 2017). Sampling of individually marked buffalo occurred from February 2014 until July 2016. Animals were sampled every two-to-three months from a 900 ha double-fenced predator-free enclosure near Satara rest camp (S 23°23′52″, E 31°46′40; Supplementary Table 1; Couch et al., 2017; Sisson et al., 2017), resulting in a total of 12 capture events over the study period. The actual number of animals sampled in each capture event varied, ranging from 49 to 70 buffalo at any given time in the study, with 103 individuals (67 females and 36 males) sampled at least once (Supplementary Table 1). At their first sampling, 38% of the buffalo were calves (under one year old), 28% sub-adult (1–5.5 years old), 26% adult (>5.5–15 years old) and 8% geriatric animals (over 15 years old) (Jolles and Ezenwa, 2015). Supplementary feed, including pellets and Lucerne hay, was occasionally supplied to counteract the restriction in grazing grounds that resulted from being fenced in, and a permanent man-made water trough was available year-round.

We used samples from buffalo calves to assess the order of first infection with *A. marginale* or *A. centrale* (Combrink et al., 2020). Only calves that had been sampled within the first six months of their life and were born within the enclosure were included in analyses. This cut-off was chosen because in cattle, calves are protected against *Anaplasma* spp. by maternal antibodies until weaning (i.e. 4-6 months of age) (Jolles et al., 2021; Potgieter and Stoltz, 2004).

2.2. Sampling and measurements

Blood samples were collected via jugular venepuncture in vacutainer tubes coated with CA EDTA (whole blood), resulting in a total of 747 samples over the study period. Age in subadults was estimated from the emergence of permanent incisors, while age of adults was estimated from the wear on incisors (Jolles, 2007).

2.3. DNA extraction and conventional PCR

DNA was extracted from the blood samples using a DNeasy Blood and Tissue Kit (Qiagen, USA) as per the manufacturer's instructions. A conventional PCR was run using the major surface protein one beta (*msp1β*) gene for *A. marginale* and the heat-shock protein *groEL* for *A. centrale*, to identify which samples were positive for single or mixed infections with these pathogens (Sisson et al., 2017). An established protocol was used to amplify the *msp1β* gene of *A. marginale* using a nested PCR (Molad et al., 2006) while new primers were designed to amplify the *groEL* gene of *A. centrale* (Sisson et al., 2017).

2.4. Quantitative (q)PCR

An established multiplex qPCR protocol using TaqMan probes (Decaro et al., 2008) was employed to quantify *A. marginale* and *A. centrale*, targeting *msp1β* and *groEL* genes, respectively, in only those samples which tested positive using conventional PCR. For the multiplex qPCR using TaqMan probes, control plasmids were constructed for *A. marginale* and *A. centrale* by cloning a fragment of the *msp1b* (Carelli et al., 2007) and *groEL genes,* respectively. These plasmids were diluted to produce a 10-fold standard curve for the quantification ranging 10^{10}-10° copies μl^{-1} for both targets in triplicate. The qPCR was performed on a Rotor-Gene Q real time machine (Qiagen, USA). The assays were run in duplicate as a multiplex with the *msp1β* gene assay on the green channel (FAM probe) and *groEL* gene assay on the yellow channel (HEX probe). Bacterial infection intensities were determined by comparing the Ct values of samples to the standard curves for each assay. The *msp1β* is a double copy gene and so the Ct values for this assay were halved before quantification.

For the construction of an *A. marginale* standard curve, a plasmid that contained a 729-base pair (bp) fragment of the *msp1b* gene was used (Carelli et al., 2007), whereas for *A. centrale*, a 488 bp fragment of the *groEL* gene was amplified using the primers groEL-ACF and groEL-ACR (Decaro et al., 2008). These PCR fragments were 1.5% agarose gel-purified using the QIAquick Gel Extraction Kit, (Qiagen, USA) and cloned into the pGEM®-T Easy Vector System (Promega, USA) as per manufacturers' instructions. Plasmid DNA was purified from transformed cells using Wizard Plus Midiprep (Promega) and quantified by spectrophotometrical analysis at 260 nm, and then subjected to bi-directional, automated sequencing using the same primers used in PCR. The quality of the sequences was assessed using the program Geneious Pro 2.0.10 (Kearse et al., 2012).

The multiplex qPCR reaction for the simultaneous detection and quantification of *A. marginale* and *A. centrale* DNA was performed on the Rotor-Gene Q real time machine (Qiagen, USA) using TaqMan probes. For *A. marginale,* the primers AM-For/AM-Rev targeting a 95 bp product within the *msp1β* gene and a probe (AM-Pb) with 6-Carboxyfluorescein (6-FAM) and Black Hole Quencher 1 (BHQ1) dye were used. For *A. centrale*, the primers AC-For/AC-Rev to amplify a 77 bp region of the *groEL* gene and a probe (AC-Pb) with Hexachlorofluorescein (HEX) and BHQ1 dye were used (Decaro et al., 2008). QIAgility (Qiagen) was used to load samples into a 100-well ring. Briefly, the reaction mixture included 12.5 µl of Promega GoTaq probe qPCR Master Mix (Promega, USA), primers at a concentration of 600 nmol/l (*A. marginale*) or 900 nmol/l (*A. centrale*), and each probe at a concentration of 200 nmol/l, and 10 µl of template (1:10 dilution) or plasmid DNA (for the standard curve). The reaction consisted of an initial reaction period at 95 °C for 10 min, followed by 40 cycles of 95 °C denaturation for 1 min, and 1 min of 60 °C of annealing/extension. Samples were run in duplicates and each plasmid DNA was run in triplicate. A no-template control was included in each assay.

Quality control was applied to qPCR results, which meant that some underperforming results had to be discarded, reducing the availability of quantitative results. A 10-fold standard curve was created to assess sensitivities and dynamic ranges using standard DNA ranging from 10^{10}-

10°. A coefficient of variation (CV) was assessed between and within assays by multiple measurements of the absolute copy numbers obtained from standard DNA samples of high, intermediate and low concentrations within the same runs and between runs. Cycle threshold (CT) values were determined automatically with the Rotor-Gene Q Series Software Version 2.3.1 (Build 49) (Qiagen). The intensity of *Anaplasma* spp. from the qPCRs were only included as a positive reading if they exceeded a variable cut-off, specific to each run, which was set to be the higher reading out of either the non-template controls or the lowest standard in the standard curve (Supplementary Table 2). If a qPCR result did not meet the cut-off criteria, it was classified as negative. Infection intensities were also corrected in relation to conventional PCR readings. If there was an insufficient qPCR reading and a positive conventional PCR result, this entry was converted to 'NA' (i.e. data not used); however, if there was an insufficient qPCR reading and a negative conventional PCR result, this entry was converted to a '0' for a negative infection intensities (Supplementary Table 2).

The *msp1β* gene is a double copy gene, and so, if qPCR was working at maximum efficiency, the quantitative result of *A. marginale* infection intensity would be double what is actually present (de la Fuente et al., 2005; Kocan et al., 2003; Tamekuni et al., 2009). To combat this, the reported *A. marginale* infection intensity results were calculated by dividing the original qPCR results by two. However, due to normal error issues with molecular methods, the likelihood of qPCR working at maximum efficiency for every qPCR run is unlikely, and so the quantitative results between *A. marginale* and *A. centrale* cannot be directly compared, and *A. marginale* likely has a slightly higher infection intensity than what is indicated here due to potential for imperfect replications in qPCR. While the infection intensities between the two species are not directly compared, the results can be used in separate statistical analyses to understand what the patterns are in each infection. Also, when comparing infection intensity involved in co-infections, or in concurrent and asynchronous time frames (time-lagged), if infection intensity data was not available for both points in that comparison, the models would not use it, further minimising the available infection intensity data for comparison. Conventional PCR did not have the same data losses, and so was able to be used instead of the qPCR results in those cases, to provide some opportunity to look at co-infection and asynchronous time frame interactions.

2.5. Statistical analyses

Statistical analyses were performed to investigate the associations between a number of predictors (age, sex, season, concurrent or previous *A. marginale* or *A. centrale* co-infections) and dependent variables (presence/absence of detectable infection with *A. marginale* or *A. centrale*, or infection intensity). Separate models were built to predict presence/absence or intensity of infection. In order to investigate temporal relationships, models were built that included infection status in the capture event immediately previous to the current one as a predictor. However, data for subsequent capture events only existed for a limited number of animals, restricting the power of these models to determine significant associations of current infection status with other predictors such as sex, age or season. Therefore, separate models were built to include either all concurrent predictors (age, sex, season, concurrent infection with other *Anaplasma* species) (concurrent models), or previous infection status (previous infection with the same or the other *Anaplasma* species) with concurrent host and environmental predictors (age, sex, season, concurrent infection with other *Anaplasma* species) (time-lagged models).

Anaplasma infection intensity was log-transformed, centred and re-scaled. Generalised linear mixed models (GLMM) were used to investigate relationships between *Anaplasma* infection and age, sex, season, concurrent co-infection or previous infection or co-infection. A binomial error structure and a logit link were used where the outcome was presence/absence of infection, whereas a Gaussian error structure and log link were used when the outcome was infection intensity. An individual identification number (Animal ID) was included as a random effect due to repeat captures of individuals.

Independent variables of season, animal traits (age, sex) and infection or co-infection with *A. marginale* or *A. centrale* were added into the model as main effects if they were significant at the $p < 0.05$ level after univariable screening. Multivariable model selection was performed to minimise the Akaike Information Criterion (AIC) for fitness response models (Akaike et al., 1973). Individual terms were dropped if this improved the AIC by two or more points, and the final model was selected if no more terms were to be dropped that met this criterion. All statistically non-significant interaction terms were removed regardless of AIC. Where *Anaplasma* spp. infection was the outcome, predictor variables were selected primarily based on likely biological significance. Season was coded as a binary variable (wet season = November to April, dry season = May to October). Continuous variables (age) were centred by subtracting the mean (to avoid multicollinearity issues when these variables initially tested as interaction terms in the models) and rescaled by dividing by two standard deviations (to assist with coefficient interpretation by having variables on the same scale). A chi-square test was used to assess the significance associated with the order of infection in calves.

All statistical analyses were performed using RStudio (R Core Team, 2016), with packages lme4 (Bates et al., 2015), lmerTest (Kuznetsova et al., 2015) and nlme (Pinheiro et al., 2017). The level of statistical significance was $p < 0.05$.

3. Results

Of the 747 samples tested, 129 (17.3%) were positive for *A. marginale*, 98 (13.1%) for *A. centrale*, and 113 (15.1%) for a mixed infection with both *Anaplasma* spp. Overall prevalence of infection has previously been described in Sisson et al. (2017), where of the 103 animals that were included in the study, 76 (73.8%) and 63 (61.2%) were infected with *A. marginale* and *A. centrale*, respectively, at some stage throughout the study period. In terms of infection intensities, 220/747 samples (29.4%) fulfilled our criteria for a valid *A. marginale* infection intensity reading and 181/747 for *A. centrale* (24.2%) (Table 1). Infection intensity of *A. centrale* was far more variable than that of *A. marginale*.

3.1. High incidence of co-infections with A. marginale and A. centrale

Anaplasma marginale and *A. centrale* were positively correlated for co-infection, i.e. infection with *A. marginale* meant a concurrent infection with *A. centrale* was more likely, and vice versa ($p < 0.001$ for both) (Table 2; Supplementary Table 3). However, the intensity of infection with one species did not affect probability of infection with the other (p = 0.509 and p = 0.906 for *A. marginale* and *A. centrale* as dependent variables, respectively) (Table 2; Supplementary Table 4).

Moreover, infection with one *Anaplasma* species also meant they were more likely to test positive to that species in the subsequent capture event, as demonstrated by our time-lagged models (Table 3;

Table 1
Mean infection intensities (copies/reaction) of *A. marginale* and *A. centrale* in a herd of African buffalo (*Syncerus caffer*) from Kruger National Park, South Africa. *Anaplasma marginale* and *A. centrale* loads cannot be directly compared due to a double copy gene (*msp1β*) being used for *A. marginale*, and a single copy gene (*groEl*) being used for *A. centrale*. n -sample size; SD – standard deviation.

	A. marginale	A. centrale
n	220	181
Mean	2405	2784
SD	8330	17,211
Range	0–28,298	0–83,428

Table 2
Mixed effects logistic regression (concurrent) model summary predicting the presence of infection, and the infection intensity, of *Anaplasma marginale* and/or *A. centrale* in a herd of African buffalo (*Caffer syncerus*) from Kruger National Park, South Africa, based on co-infection status (statistical values and sample sizes shown in Supplementary Tables 3 and 4). Significant p-values (<0.05) are shown with direction of significance (↓/↑), non-significant p-values are expressed with ∅.

	PRESENCE		INFECTION INTENSITY	
	A. marginale	A. centrale	A. marginale	A. centrale
Age	↓	∅	↓	↓
Sex	Males ↑	∅	∅	Males ↑
Season	Dry ↑	∅	∅	Dry ↑
Co-infection[a]	↑	∅	∅	∅

[a] Co-infection refers to a concurrent infection with the other *Anaplasma* species. Where infection intensity is the outcome, the predictor is co-infection intensity.

Table 3
Mixed effects logistic regression (time-lagged) model summary predicting the presence of infection, and infection intensity, of *Anaplasma marginale* and/or *A. centrale* in a herd of African buffalo (*Syncerus caffer*) from Kruger National Park, South Africa, based on previous infection and co-infection status (statistical values and sample sizes shown in Supplementary Tables 5 and 6). Significant p-values (<0.05) are shown with direction of significance (↓/↑), non-significant p-values are expressed with ∅.

	PRESENCE		INFECTION INTENSITY	
	A. marginale	A. centrale	A. marginale	A. centrale
Age	↑	∅	∅	∅
Sex	∅	∅	∅	∅
Season	∅	∅	∅	∅
Co-infection[a]	↑	↑	∅	∅
Previous infection[b]	↑	↑	∅	↑
Previous co-infection[c]	∅	∅	∅	∅

[a] Co-infection refers to a concurrent infection with the other species.
[b] Previous infection refers to presence of the same species in the previous capture.
[c] Previous co-infection refers to presence of the other species in the previous capture. Where infection intensity is the outcome, the predictor is infection or co-infection intensity.

Supplementary Tables 5 and 6). However, infection with one species did not appear to affect the probability of infection with the other species in the subsequent capture event.

3.2. *Anaplasma* infections peak in the dry season

Anaplasma marginale infections were more likely to occur in the dry season ($p = <0.001$) (Table 2; Supplementary Table 3), and there was a non-significant trend towards higher *A. centrale* infection prevalence in the dry season (Fig. 1). Infection intensity with *A. centrale* was greater during the dry season, ($p = 0.013$) but there was no statistically significant relationship between seasonality and *A. marginale* infection intensity (Table 2; Fig. 2; Supplementary Table 4).

3.3. *A. marginale* infection presence peaks earlier than *A. centrale*

Fifteen calves who were under the age of 6 months at the first capture were analysed for the order of infection. Fourteen of them were first positive for *A. marginale,* while the remaining calf was first coinfected with *A. marginale* and *A. centrale*. Five calves were infected with *A. marginale* on their first capture after birth, with a mean age of 3.4 months of age (SD = 0.202, Chi-square test: $p < 0.001$, $X^2 = 13.133$). By the time the calves turned six months of age, the overall prevalence of infection with *A. centrale* was 40% and 100% for *A. marginale*. The average age of initial *A. marginale* infection was eight months (SD = ± 6 months), whereas that for *A. centrale* was 17 months (SD = ± 14 months).

Younger animals were more likely to test positive for the presence of *A. marginale* ($p = <0.001$) (Table 2; Fig. 1; Supplementary Table 3). Infection intensities of both *A. marginale* and *A. centrale* decreased significantly with increasing age ($p = 0.004$ and $p = 0.043$, respectively) (Table 2; Fig. 3a; Supplementary Table 4). So while *A. marginale* infection peaks earlier than *A. centrale*, both decline in adult and geriatric buffalo.

3.4. *Anaplasma marginale* infection more likely in male buffalo

Males tended to be more likely to be infected with both *A. marginale* and *A. centrale* (Fig. 3), but based on the full concurrent infection model, this was only statistically significant for the *A. marginale* ($p = <0.001$) (Table 2; Supplementary Table 3; Fig. 3b). The intensity of infection with *A. centrale* was higher in males ($p = 0.004$) (Table 2; Supplementary Table 4; Fig. 3c).

4. Discussion

This study confirms that African buffalo are commonly infected with single or co-infections of *A. marginale* and *A. centrale*. A higher number of infections occurred in male animals, the dry season and younger animals. Utilising longitudinal data on individually marked hosts, it was also possible to demonstrate effects of concurrent infections with other *Anaplasma* spp. Nonetheless, limited sample sizes for some data points, such as intensity of infection or subsequent capture data, meant that the statistical power for some models (e.g. the model including previous infection status) was limited. It should also be noted that results came from one herd of buffalo, and results could be strengthened by a larger sample size to allow the infection intensity models more data for statistical power, and also to reassure the epidemiological findings are reproducible in other non-related herds of buffalo.

Infection with one *Anaplasma* species was positively associated with concurrent infection intensity of, and likelihood of infection with, the other. Co-infections with these parasite species were previously reported by Henrichs et al. (2016) in African buffalo from South Africa, and Shkap et al. (2008) in cattle in the USA, using an *A. centrale* strain from South Africa. The reasons for this are unclear. It could be the result of co-exposure, where ticks are infected with both *Anaplasma* species. While prior research has not indicated any significant relationship between tick burden and *Anaplasma* infection (Henrichs et al., 2016), there is currently little understanding of infection dynamics within the vector or associations between tick species and probability of infection. Co-infection dynamics in the host may also be the result of co-susceptibility, i.e. related to genetic factors and/or the host's physiological condition (Henrichs et al., 2016). This is seen throughout biological systems, where environmental and genetic factors change in response to natural selection, resulting in immune response modifications (Lazzaro and Little, 2009). Molecular methods (such as SNP genotyping or sequencing) could be used to assess if there are any genetic markers correlated with higher levels or probability of infection. This has been done for some buffalo infections, such as bovine tuberculosis (Tavalire et al., 2018, 2019). On the other hand, co-infections could be the result of co-facilitation, where, for example, one *Anaplasma* spp. may suppress the host's immune system, making it easier for the second species to establish and thrive. The most famous example to illustrate this, perhaps, is that of HIV-infection in humans, facilitating infection with a range of other pathogens through immunosuppression (e.g. Devi et al., 2021). However, the mechanisms of facilitation in co-infection are not always known, and another *Anaplasma* species, *Anaplasma phagocytophilum*, has previously been implicated in facilitating infection in short-tailed field voles (*Microtus agrestis*) with a range of other pathogens, including cowpox virus, but also another haemoparasite, *Babesia microti* (Telfer et al., 2010). However, to the best of our knowledge, this

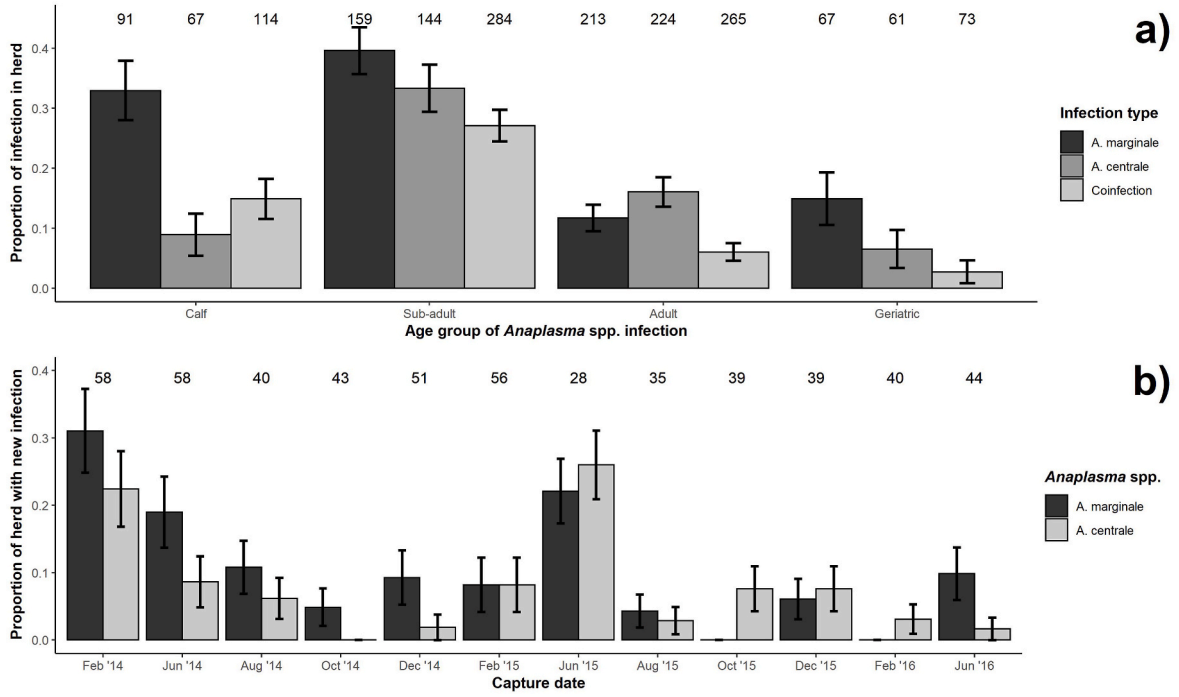

Fig. 1. The proportion of animals infected with *Anaplasma* spp. from a managed African buffalo (*Syncerus caffer*) herd from Kruger National Park, South Africa. a) The mean prevalence of animals with *A. marginale* single infection, *A. centrale* single infection, or co-infections with each other over four age groups: calves (0–1 years old), sub-adults (1–5.5 years old), adults (5.5–15 years) and geriatrics (15 years plus); b) the mean prevalence of new infections with *A. marginale* or *A. centrale* for each capture event over the two-and-a-half-year study period. Numbers at the top of figure represent sample size.

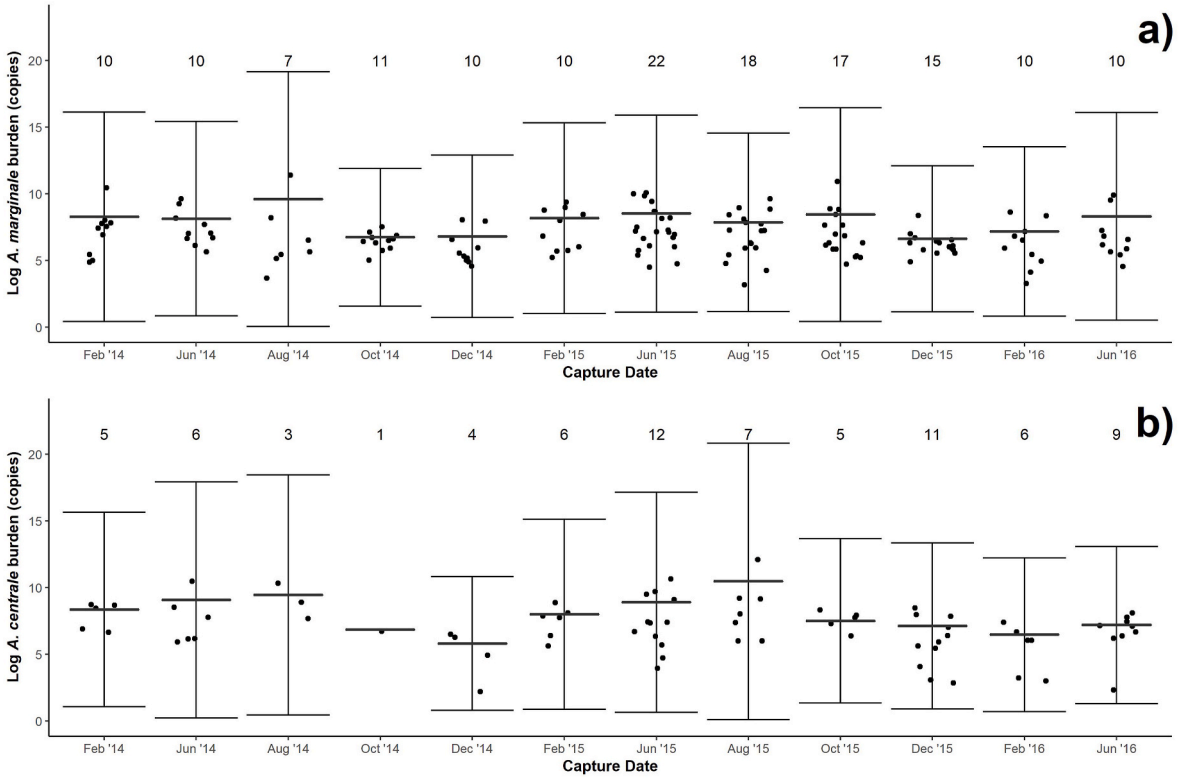

Fig. 2. Individual value plots showing the distribution of results for intensity of infection (log-transformed number of copies/reaction) with *Anaplasma marginale* (a) and *Anaplasma centrale* (b), using a real-time qPCR from a managed African buffalo (*Syncerus caffer*) herd from Kruger National Park, South Africa. Error bars represent one standard error, numbers at the top of figure represent sample size.

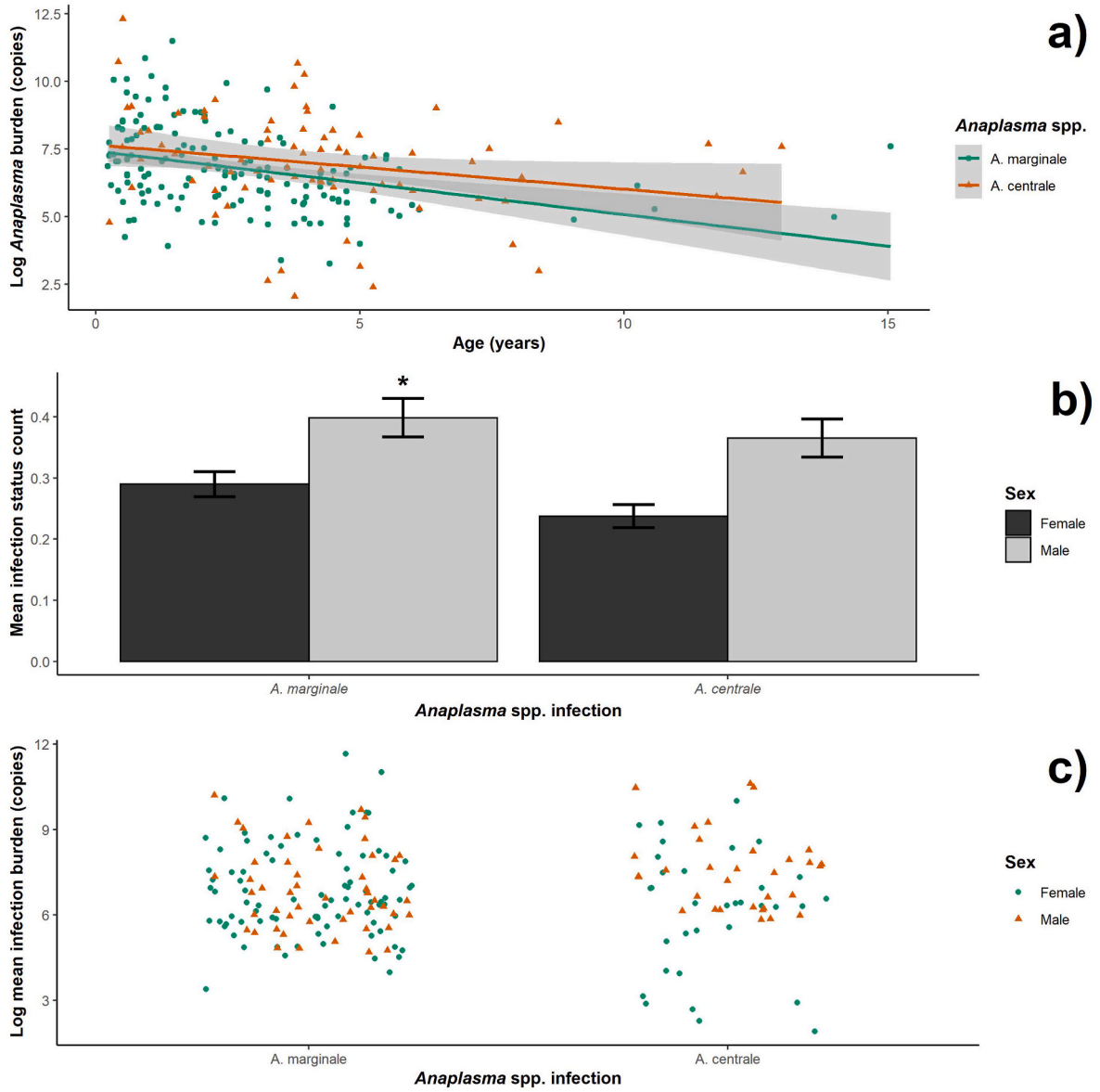

Fig. 3. Patterns of infection with *Anaplasma* spp. based on age and sex for a managed African buffalo (*Syncerus caffer*) herd from Kruger National Park, South Africa. a) The infection intensity (log-transformed number of copies/reaction) of *A. marginale* and *A. centrale* based on age (years); b) overall proportion of animals infected with *A. marginale* or *A. centrale* based on sex; c) infection intensity (log-transformed number of copies/reaction) results for *A. marginale* or *A. centrale* based on sex. * indicates statistical significance ($p < 0.05$). Error bars are standard error.

phenomenon in relation to infection intensity has not previously been described for *Anaplasma* spp. in bovines.

The observed higher infection intensity of *A. centrale* and higher probability of occurrence of *A. marginale* in buffalo sampled during the dry season could be due to alterations to immune function (Ezenwa 2004; Lochmiller et al., 1993). Seasonal variations in immune function in wild animals have previously been demonstrated (Couch et al., 2017), with extended dry periods commonly resulting in suppression of the immune response (Beechler et al., 2012). Other work suggests that *Anaplasma centrale* does not appear to evade the host immune response as successfully as *A. marginale* (Han et al., 2010), and so seasonal immunological variation may have a greater impact on its intensity of infection. The probability of infection with *Anaplasma marginale* did respond to season, but this may be an indication of risk of exposure rather than susceptibility. Exposure to *Anaplasma* could vary seasonally due to differences in infected tick numbers. A previous study found no direct association between tick burden and infection status with *A. marginale* or *A. centrale* (Henrichs et al., 2016), but it would be worth investigating this alternative hypothesis further. As discussed, little is known about infection dynamics of *Anaplasma* in ticks, how this relates to infection intensity, or how this may relate to level of exposure and infection in buffalo. However, in northern Tunisia, *A. bovis* was found to be more prevalent in small ruminants during times of higher tick activity and infestations (Belkahia et al., 2017). Subsequently, there may be undetected associations between availability of tick vectors and probability and level of infection in buffalo.

Younger buffalo had higher prevalence and intensity of infection than other age classes. This could be due to age-dependent development of acquired immunity to *Anaplasma* spp. which has previously been demonstrated in cattle (Aubry and Geale, 2011; Kocan et al., 2010; Potgieter and Stoltz, 2004). This high prevalence of infection in buffalo calves observed here may therefore also go some way towards explaining the apparent lack of clinical disease in adult African buffalo; similarly, clinical disease in cattle is relatively rare where the disease is endemic (Knowles et al., 1982). An explanation for the decreased incidence of clinical disease in adult cattle could also be related to chronic

infections, which have been documented in cattle (Grau et al., 2013; Potgieter and Van Rensburg, 1987). Depending on the sensitivity of the test, this can then result in some tests having animals not being detected when they cyclically have a very low level of infection, before the pathogen multiplies again to reach a level that can be identified (Kieser et al., 1990). A more likely reason for that in adult cattle is that their immune response is what is keeping the infection below detection levels (Kieser et al., 1990). Nonetheless, the average age of initial infection suggests that most *Anaplasma* infections were not transmitted vertically, where vertical infections would likely be via chronically infected buffalo. Previous research has indicated that tick-borne and environmentally transmitted protozoal diseases are acquired earlier than infection through other means (Combrink et al., 2020). However, five calves did test positive for infection in their first capture after birth, at which point they could be up to three-months old (being the period between captures), and vertical transmission cannot be excluded as a possible cause of infection in these animals. Although transmission through ticks is thought to be the main mode of transmission, transplacental transmission has previously been shown to occur in cattle (Grau et al., 2013; Potgieter and Stoltz, 2004).

It is possible that calves were more likely to be infected with *A. marginale* first because they were more likely to be exposed to *A. marginale*. For example, more buffalo may have been bacteraemic with *Anaplasma marginale* during the late wet and early dry season, when calves were being born; this hypothesis is somewhat supported by the increased prevalence of *A. marginale* infection during the dry season. Ticks could also preferentially transmit *A. marginale* over *A. centrale*; it is known that there are significant differences in the transmissibility of some rickettsial species and strains, including *Anaplasma* spp. (Ueti et al., 2007). As previously suggested, *A. marginale* could also be more capable of evading the immune system than *A. centrale*, and so more likely to be identified when sampling, or could more easily infect the host in the first place (Brown, 2012). Considering there appears to be a level of facilitation between the two bacterial species, the order of infection could also indicate that *A. marginale* is facilitating infection with *A. centrale*, for example through immune suppression.

Unfortunately, it is not possible to confirm the reasons behind the observed difference in rate of infection between the two species based on the data obtained as part of this study. Specific work, targeting seasonal infection dynamics in the tick vector and host immune responses related to infection with *Anaplasma* spp are needed to address these knowledge gaps.

Males experienced higher intensity of infection with *A. centrale* than females. This sex difference was also seen in young males that had not yet segregated from the herd. To the best of the authors' knowledge, a male bias in *Anaplasma* spp. infection has not previously been reported. Although segregation among male and female buffalo was not as complete as would be seen in a non-enclosed herd, males tend to spend time on the edges of the herd, and this could mean they are more likely to encounter ticks on vegetation (Prins, 1996). In addition, the observed association with sex may be a result of differences in physiology, immune response or susceptibility in males and females (Jaillon et al., 2019; López-Olvera et al., 2015; van Hooft et al., 2018). Foo et al. (2017) demonstrated a significant suppressive effect of testosterone on immune function in a range of mammal species. Further work in this area should examine sex-based rates of infection with ticks in relation to behaviour and immune response.

5. Conclusion

This study provides insights into the interactions of two closely-related parasite species, *A. marginale* and *A. centrale*, and their associations with host age, sex and season in a wildlife species, the African buffalo. Both parasite species occurred commonly, both in single and co-infections, and infection with one was positively associated with both probability and intensity of infection with the other. Prevalence of infection with *A. marginale* was highest during the dry season, as was intensity of infection with *A. centrale*. Calves generally showed infection presence with *A. marginale* before *A. centrale*. The presence and intensity of infection decreased with age for both *Anaplasma* species, and males were more likely to be infected with *A. centrale* than females, perhaps pointing to differences in susceptibility with host age and sex. Understanding infection dynamics in wild host-parasite systems may ultimately also lead to improvements of management strategies for domestic animals, because wild systems potentially provide greater insights into the basic host-parasite relationships, unaltered by human intervention.

Declaration of competing interest

The authors reported no conflicts of interest related to this research.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijppaw.2023.04.005.

References

Akaike, H., Petrov, B., Csaki, F., 1973. Information theory and an extension of the maximum likelihood principle. In: Second International Symposium on Information Theory, Tsahkadsor, Armenia, USSR, September 2-8, 1971. Akademiai Kiado, Budapest, pp. 267–281.

Aubry, P., Geale, D.W., 2011. A review of bovine anaplasmosis. Transbound. Emerg. Dis. 58, 1–30.

Bates, D., Mächler, M., Bolker, B., Walker, S., 2015. Fitting linear mixed-effects models using lme4. J. Stat. Software 67, 1–48.

Beechler, B.R., Broughton, H., Bell, A., Ezenwa, V.O., Jolles, A.E., 2012. Innate immunity in free-ranging African buffalo (*Syncerus caffer*): associations with parasite infection and white blood cell counts. Physiol. Biochem. Zool. 85, 255–264.

Beechler, B.R., Boersma, K.S., Buss, P.E., Coon, C.A., Gorsich, E.E., Henrichs, B.S., Siepielski, A.M., Spaan, J.M., Spaan, R.S., Ezenwa, V.O., Jolles, A.E., 2019. Bovine tuberculosis disturbs parasite functional trait composition in African buffalo. Proc. Natl. Acad. Sci. USA 116, 14645–14650.

Belkahia, H., Said, M.B., El Mabrouk, N., Saidani, M., Cherni, C., Hassen, M.B., Bouattour, A., Messadi, L., 2017. Seasonal dynamics, spatial distribution and genetic analysis of Anaplasma species infecting small ruminants from Northern Tunisia. Infect. Genet. Evol. 54, 66–73.

Berentsen, A.R., Miller, R.S., Misiewicz, R., Malmberg, J.L., Dunbar, M.R., 2013. Characteristics of white-tailed deer visits to cattle farms: implications for disease transmission at the wildlife-livestock interface. Eur. J. Wildl. Res. 170.

Brown, W.C., 2012. Adaptive immunity to *Anaplasma* pathogens and immune dysregulation: implications for bacterial persistence. Comp. Immunol. Microbiol. Infect. Dis. 35, 241–252.

Buddle, B.M., Vordermeier, H.M., Chambers, M.A., de Klerk-Lorist, L.M., 2018. Efficacy and safety of BCG vaccine for control of tuberculosis in domestic livestock and wildlife. Front. Vet. Sci. 5, 259.

Carelli, G., Decaro, N., Lorusso, A., Elia, G., Lorusso, E., Mari, V., Buonavoglia, C., 2007. Detection and quantification of *Anaplasma marginale* DNA in blood samples of cattle by real-time PCR. Vet. Microbiol. 124, 107–114.

Combrink, L., Glidden, C.K., Beechler, B.R., Charleston, B., Koehler, A.V., Sisson, D.R., Gasser, R.B., Jabbar, A., Jolles, A.E., 2020. Age of first infection across a range of parasite taxa in a wild mammalian population. Biol. Lett. 16, 20190811.

Couch, C.E., Movius, M.A., Jolles, A.E., Gorman, M.E., Rigas, J.D., Beechler, B.R., 2017. Serum biochemistry panels in African buffalo: defining reference intervals and assessing variability across season, age and sex. PLoS One 12, e0176830.

de la Fuente, J., Lew, A., Lutz, H., Meli, M.L., Hofmann-Lehmann, R., Shkap, V., Molad, T., Mangold, A.J., Almazan, C., Naranjo, V., Gortazar, C., Torina, A., Caracappa, S., Garcıa-Perez, A.L., Barral, M., Oporto, B., Ceci, L., Carelli, G., Blouin, E.F., Kocan, K.M., 2005. Genetic diversity of *Anaplasma* species major surface proteins and implications for anaplasmosis serodiagnosis and vaccine development. Anim. Health Res. Rev. 6, 75–89.

De Waal, D.T., 2000. Anaplasmosis control and diagnosis in South Africa. Ann. N. Y. Acad. Sci. 916, 474–483.

Debeila, E.M., 2012. Occurrence of *Anaplasma* and *Ehrlichia* Species in African Buffalo (*Syncerus caffer*) in Kruger National Park and Hluhluwe-iMfolozi Park in South Africa. Masters Thesis, University of Pretoria, pp. 1–98.

Decaro, N., Carelli, G., Lorusso, E., Lucente, M.S., Greco, G., Lorusso, A., Radogna, A., Ceci, L., Buonavoglia, C., 2008. Duplex real-time polymerase chain reaction for simultaneous detection and quantification of *Anaplasma marginale* and *Anaplasma centrale*. J. Vet. Diagn. Invest. 20, 606–611.

Devi, P., Khan, A., Chattopadhyay, P., Mehta, P., Sahni, S., Sharma, S., Pandey, Ra, 2021. Co-Infections as modulators of disease outcome: minor players or major players? Front. Microbiol. 12 https://doi.org/10.3389/fmicb.2021.664386.

Eda, S., Elliott, B., Scott, M.C., Waters, W.R., Bannantine, J.P., Whitlock, R.H., Speer, C. A., 2005. New method of serological testing for *Mycobacterium avium* subsp. paratuberculosis (Johne's disease) by flow cytometry. Foodb. Pathog. Dis. 2, 250–262.

Eygelaar, D., Jori, F., Mokopasetso, M., Sibeko, K.P., Collins, N.E., Vorster, I., Troskie, M., Oosthuizen, M.C., 2015. Tick-borne haemoparasites in African buffalo (*Syncerus caffer*) from two wildlife areas in Northern Botswana. Parasites Vectors 8, 1–11.

Ezenwa, V.O., 2004. Interactions among host diet, nutritional status and gastrointestinal parasite infection in wild bovids. Int. J. Parasitol. 34, 535–542.

Ezenwa, V.O., 2016. Helminth–microparasite co-infection in ruminants, rodents and rabbits. Parasite Immunol. 38, 527–534.

Ezenwa, V.O., Jolles, A.E., Beechler, B.R., Budischak, S.A., Gorsich, E.E., 2019. Chapter 5. The causes and consequences of parasite interactions: African buffalo as a case study. In: Wilson, K., Fenton, A., Tompkins, D. (Eds.), Wildlife Disease Ecology: Linking Theory to Data and Application, first ed. Cambridge University Press, Cambridge, pp. 129–160.

Fagbo, S., Coetzer, J.A., Venter, E.H., 2014. Seroprevalence of rift valley fever and lumpy skin disease in African buffalo (*Syncerus caffer*) in the kruger National Park and Hluhluwe-iMfolozi Park, South Africa. J. S. Afr. Vet. Assoc. 85, 1–7.

Foo, Y.Z., Nakagawa, S., Rhodes, G., Simmons, L.W., 2017. The effects of sex hormones on immune function: a meta-analysis. Biol. Rev. 92, 551–571.

Fynn, R.W., Augustine, D.J., Peel, M.J., de Garine-Wichatitsky, M., 2016. Strategic management of livestock to improve biodiversity conservation in African savannahs: a conceptual basis for wildlife–livestock coexistence. J. Appl. Ecol. 53, 388–397.

Glidden, C.K., Beechler, B., Buss, P.E., Charleston, B., de Klerk-Lorist, L.-M., Maree, F.F., Muller, T., Pérez-Martin, E., Scott, K.A., van Schalkwyk, O.L., Jolles, A., 2018. Detection of pathogen exposure in African buffalo using non-specific markers of inflammation. Front. Immunol. 8, 1944, 1944.

Glidden, C.K., Koehler, A.V., Hall, R.S., Saeed, M.A., Coppo, M., Beechler, B.R., Charleston, B., Gasser, R.B., Jolles, A.E., Jabbar, A., 2020. Elucidating cryptic dynamics of *Theileria* communities in African buffalo using a high-throughput sequencing informatics approach. Ecol. Evol. 10, 70–80.

Glidden, C.K., Coon, C.A., Beechler, B.R., McNulty, C., Ezenwa, V.O., Jolles, A.E., 2021. Co-infection best predicts respiratory viral infection in a wild host. J. Anim. Ecol. 90, 602–614.

González-Barrio, D., Ruiz-Fons, F., 2019. *Coxiella burnetii* in wild mammals: a systematic review. Transbound. Emerg. Dis. 66, 662–671.

Gorsich, E.E., Ezenwa, V.O., Jolles, A.E., 2014. Nematode–coccidia parasite co-infections in African buffalo: epidemiology and associations with host condition and pregnancy. Int. J. Parasitol.: Parasit. Wildl. 3, 124–134.

Gorsich, E.E., Etienne, R.S., Medlock, J., Beechler, B.R., Spaan, J.M., Spaan, R.S., Ezenwa, V.O., Jolles, A.E., 2018. Opposite outcomes of coinfection at individual and population scales. Proc. Natl. Acad. Sci. USA 115, 7545–7550.

Grau, H.E.G., Cunha Filho, N.A.D., Pappen, F.G., Farias, N.A.D.R., 2013. Transplacental transmission of *Anaplasma marginale* in beef cattle chronically infected in southern Brazil. Rev. Bras. Parasitol. Vet. 22, 189–193.

Han, S., Norimine, J., Brayton, K.A., Palmer, G.H., Scoles, G.A., Brown, W.C., 2010. Anaplasma marginale infection with persistent high-load bacteremia induces a dysfunctional memory CD4+ T lymphocyte response but sustained high IgG titers. Clin. Vaccine Immunol. 17, 1881–1890.

Henrichs, B., 2014. Hidden dynamics: community ecology and costs of parasite infection. Masters thesis. In: African Buffalo (*Syncerus Caffer*). Oregon State University, p. 88.

Henrichs, B., Oosthuizen, M.C., Troskie, M., Gorsich, E., Gondhalekar, C., Beechler, B.R., Ezenwa, V.O., Jolles, A.E., 2016. Within guild co-infections influence parasite community membership: a longitudinal study in African Buffalo. J. Anim. Ecol. 85, 1025–1034.

Hove, P., Khumalo, Z.T.H., Chaisi, M.E., Oosthuizen, M.C., Brayton, K.A., Collins, N.E., 2018. Detection and characterisation of*Anaplasma marginale* and *A. Centrale*in South Africa. Vet Sci 5, 26.

Jaillon, S., Berthenet, K., Garlanda, C., 2019. Sexual dimorphism in innate immunity. Clin. Rev. Allergy Immunol. 56, 308–321.

Jolles, A.E., 2007. Population biology of African buffalo (*Syncerus caffer*) at Hluhluwe-iMfolozi Park, South Africa. Afr. J. Ecol. 45, 398–406.

Jolles, A.E., Ezenwa, V.O., 2015. Ungulates as model systems for the study of disease processes in natural populations. J. Mammal. 96, 4–15.

Jolles, A., Gorsich, E., Gubbins, S., Beechler, B., Buss, P., Juleff, N., De Klerk Lorist, L., Maree, F., Perez-Martin, E., Van Schalkwyk, O.L., Scott, K., Zhang, F., Medlock, B., Charleston, B., 2021. Endemic persistence of a highly contagious pathogen: foot-and-mouth disease in its wildlife host. Science 374, 104–109.

Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., Buxton, S., Cooper, A., Markowitz, S., Duran, C., 2012. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics 28, 1647–1649.

Kieser, S.T., Eriks, I.S., Palmer, G.H., 1990. Cyclic rickettsemia during persistent Anaplasma marginale infection of cattle. Infect. Immun. 58, 1117–1119.

Knowles, R., Montrose, M., Craig, T., Wagner, G., Long, R., 1982. Clinical and serological evidence of bovine babesiosis and anaplasmosis in St. Lucia. Vet. Parasitol. 10, 307–311.

Kocan, K.M., de la Fuente, J., Guglielmone, A.A., Meléndez, R.D., 2003. Antigens and alternatives for control of *Anaplasma marginale* infection in cattle. Clin. Microbiol. Rev. 16, 698–712.

Kocan, K.M., de la Fuente, J., Blouin, E.F., Coetzee, J.F., Ewing, S., 2010. The natural history of *Anaplasma marginale*. Vet. Parasitol. 167, 95–107.

Kuznetsova, A., Brockhoff, P.B., Christensen, R.H.B., 2015. Package 'lmerTest. R Package Version 2 (0).

Lazzaro, B.P., Little, T.J., 2009. Immunity in a variable world. Philos. Trans. R. Soc. B: Biol. Sci. 364, 15–26.

Lochmiller, R.L., Vestey, M.R., Boren, J.C., 1993. Relationship between protein nutritional status and immunocompetence in northern bobwhite chicks. Auk 110, 503–510.

López-Olvera, J.R., Serrano, E., Armenteros, A., Pérez, J.M., Fandos, P., Carvalho, J., Velarde, R., Cano-Manuel, F.J., Ráez, A., Espinosa, J., Soriguer, R.C., Granados, J.E., 2015. Sex-biased severity of sarcoptic mange at the same biological cost in a sexually dimorphic ungulate. Parasites Vectors 8, 1–6.

Makgabo, S.M., Brayton, K.A., Biggs, L., Oosthuizen, M.C., Collins, N.E., 2023. Temporal dynamics of *Anaplasma marginale* infections and the composition of *Anaplasma* spp. in calves in the mnisi communal area, mpumalanga, South Africa. Microorganisms 11, 465.

Molad, T., Mazuz, M., Fleiderovitz, L., Fish, L., Savitsky, I., Krigel, Y., Leibovitz, B., Molloy, J., Jongejan, F., Shkap, V., 2006. Molecular and serological detection of *A. centrale* and *A. marginale*-infected cattle grazing within an endemic area. Vet. Microbiol. 113, 55–62.

Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D., Heisterkamp, S., Van Willigen, B., Maintainer, R., 2017. Package 'nlme'. Linear and Nonlinear Mixed Effects Models, Version 3-1.

Potgieter, F., Stoltz, W., 2004. Bovine anaplasmosis. In: Coetzer, J., Tustin, R. (Eds.), Infectious Diseases of Livestock, second ed. Oxford University Press, Cape Town, South Africa, pp. 594–616.

Potgieter, F.T., Van Rensburg, L., 1987. The persistence of colostral *Anaplasma* antibodies and incidence of in utero transmission of *Anaplasma* infections in calves under laboratory conditions. Oonderstepoort J. Vet. Res. 54, 557–560.

Prins, H., 1996. Ecology and Behaviour of the African Buffalo: Social Inequality and Decision Making, vol. 1. Chapman & Hall, London, United Kingdom, pp. 1–293.

R Core Team, 2016. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. Retrieved from. http://www.R-project.org/.

Sabey, K.A., Song, S.J., Jolles, A., Knight, R., Ezenwa, V.O., 2021. Coinfection and infection duration shape how pathogens affect the African buffalo gut microbiota. ISME J. 15 (5), 1359–1371.

Shkap, V., Leibovitz, B., Krigel, Y., Molad, T., Fish, L., Mazuz, M., Fleiderovitz, L., Savitsky, I., 2008. Concomitant infection of cattle with the vaccine strain *Anaplasma marginale* ss *centrale* and field strains of *A. marginale*. Vet. Microbiol. 130, 277–284.

Siembieda, J.L., Kock, R.A., McCracken, T.A., Newman, S.H., 2011. The role of wildlife in transboundary animal diseases. Anim. Health Res. Rev. 12, 95–111.

Sisson, D., Hufschmid, J., Jolles, A., Beechler, B., Jabbar, A., 2017. Molecular characterisation of *Anaplasma* species from African buffalo (*Syncerus caffer*) in kruger National Park, South Africa. Ticks Tick-borne Dis. 8, 400–406.

Tamekuni, K., Kano, F.S., Ataliba, A.C., Marana, E.R., Venâncio, E.J., Vidotto, M.C., Garcia, J.L., Headley, S.A., Vidotto, O., 2009. Cloning, expression, and characterization of the MSP1a and MSP1b recombinant proteins from PR1 *Anaplasma marginale* strain. Brazil. Res. Vet. Sci. 86, 98–107.

Tavalire, H.F., Beechler, B.R., Buss, P.E., Gorsich, E.E., Hoal, E.G., Le Roex, N., Spaan, J. M., Spaan, R.S., van Helden, P.D., Ezenwa, V., Jolles, A.E., 2018. Context-dependent costs and benefits of tuberculosis resistance traits in a wild mammalian host. Ecol. Evol. 8, 12712–12726.

Tavalire, H.F., Hoal, E.G., le Roex, N., Van Helden, P.D., Ezenwa, V.O., Jolles, A.E., 2019. Risk alleles for tuberculosis infection associate with reduced immune reactivity in a wild mammalian host. Proc. R. Soc. A B 286, 20190914.

Telfer, S., Lambin, X., Birtles, R., Beldomenico, P., Burthe, S., Paterson, S., Begon, M., 2010. Species interactions in a parasite community drive infection risk in a wildlife population. Science 330, 243–246.

Ueti, M.W., Reagan, J.O., Knowles, D.P., Scoles, G.A., Shkap, V., Palmer, G.H., 2007. Identification of midgut and salivary glands as specific and distinct barriers to efficient tick-borne transmission of *Anaplasma marginale*. Infect. Immun. 75, 2959–2964.

Van Hooft, P., Dougherty, E.R., Getz, W.M., Greyling, B.J., Zwaan, B.J., Bastos, A.D., 2018. Genetic responsiveness of African buffalo to environmental stressors: a role for epigenetics in balancing autosomal and sex chromosome interactions? PLoS One 13, e0191481.