Co-infection best predicts respiratory viral infection in a wild host

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Abstract

1. The dynamics of directly transmitted pathogens in natural populations are likely to result from the combined effects of host traits, pathogen biology, and interactions among pathogens within a host. Discovering how these factors work in concert to shape variation in pathogen dynamics in natural host–multi-pathogen systems is fundamental to understanding population health.
2. Here, we describe temporal variation in incidence and then elucidate the effect of hosts trait, season and pathogen co-occurrence on host infection risk using one of the most comprehensive studies of co-infection in a wild population: a suite of seven directly transmitted viral and bacterial respiratory infections from a 4-year study of 200 free-ranging African buffalo *Syncerus caffer*.
3. Incidence of upper respiratory infections was common throughout the study—five out of the seven pathogens appeared to be consistently circulating throughout our study population. One pathogen exhibited clear outbreak dynamics in our final study year and another was rarely detected.
4. Co-infection was also common in this system: The strongest indicator of pathogen occurrence for respiratory viruses was in fact the presence of other viral respiratory infections. Host traits had minimal effects on odds of pathogen occurrence but did modify pathogen–pathogen associations. In contrast, only season predicted bacterial pathogen occurrence.
5. Though a combination of environmental, behavioural, and physiological factors work together to shape disease dynamics, we found pathogen associations best determined infection risk. Our study demonstrates that, in the absence of very fine-scale data, the intricate changes among these factors are best represented by co-infection.

Keywords: bovine respiratory disease complex; co-infection; infection dynamics; upper respiratory disease

1 INTRODUCTION

A central goal of epidemiology and disease ecology was to understand and predict the dynamics of infectious diseases in host populations. Work in these fields has emphasized the fundamental role of fluxes in the proportion of susceptible, infected, and recovered hosts in determining disease dynamics by reproducing the range of temporal patterns seen in nature—from cyclic to endemic—with elegantly simple models. However, beyond modelling general disease dynamic patterns, understanding variation in infection risk remains challenging (Germann et al., 2006; Hall et al., 2006).

Even in the simplest case of direct pathogen transmission between hosts, variation in disease dynamics is likely to result from the interplay between variation in host behaviour and physiology, as well as factors affecting pathogen viability outside the host. Contact patterns among hosts can change dramatically in response to seasonal fluctuations in aggregation (e.g. during breeding seasons) or changes in the distribution of resources (e.g. convening near water during dry periods), driving variability in pathogen transmission. For example: rotavirus, measles and *Streptococcus pneumoniae* outbreaks have all been linked to aggregation of children during the fall school term (Cook et al., 1990; Dowell et al., 2003; Fine & Clarkson, 1982). Likewise, raccoons *Procyon lotor* with spatially aggregated food resources had higher prevalence of directly transmitted parasites than those populations with dispersed resources (Wright & Gompper, 2005). Host susceptibility may also change with seasonal or annual shifts in immune and reproductive status (Jolles et al., 2015). For instance: outbreaks of ovine pneumonia in bighorn sheep*Ovis canadensis*tend to co-occur with the peak of the mating season in the fall (Cassirer et al., 2013). Finally, pathogen viability outside the host can change with humidity, temperature, and exposure to UV radiation which allows risk forecasting based on meteorological information for some pathogens, such as cholera (Colwell, 1996) and influenza (Lowen et al., 2007).

Though less well-understood, interactions (e.g. competition or facilitation) among pathogens infecting the same hosts can add yet another layer of complexity to patterns of disease transmission and infection risk. Effects of interactions on the transmission dynamics of one or both co-infecting pathogens have been demonstrated (Gorsich et al., 2018; Susi et al., 2015): in some cases co-infecting pathogens appear to equal or outweigh environmental and host factors in importance as predictors of infection (e.g. Telfer et al., 2010).

Interactions between free-living species are frequently context-dependent (Chamberlin et al., 2014). Although understudied in wild host–parasite systems, parasite interactions are likely to be context-dependent as well. Co-occurring pathogens can interact through competition for shared resources or through immune-mediated interactions (Graham, 2008; Griffiths et al., 2011); as such strength of interactions may change with host physiological status. Furthermore, significant species associations may not be the product of true interactions, but correlated responses to changes in host physiology or pathogen exposure (Blanchet et al., 2020). In these cases, in the absence of detailed information on host traits and environment, co-occurring pathogens may still be useful in capturing variation in of odds of infection.

Discovering whether or how host, pathogen, and environmental factors combine to shape variation in infection risk in typical but complex multi-pathogen systems (Cox, 2001; Pedersen & Fenton, 2007) is fundamental to the development of adaptive disease management strategies in rapidly changing environments. Yet, the breadth and depth of data needed to dissect the causes of multi-pathogen disease dynamics in natural populations are rarely attainable: longitudinal studies assessing multiple, potentially co-infecting pathogens, and detailed data on host physiological status and proxies for behaviour across different seasons.

Here we present such data from a 4-year study of 200 free-ranging, female African buffalo *Syncerus caffer* in a population where half of the individuals were treated with an anthelmintic drug (Ezenwa & Jolles, 2015). Every 6 months, the same buffalo were captured, screened for seven directly transmitted respiratory pathogens (Table 1), and their physical and reproductive status evaluated (Table 2). We monitored host factors that have been shown to correlate with disease susceptibility and contact frequency (body condition, reproductive status, age, herd association, co-infection, Table 2; Gorsich et al., 2015; Keeling & Rohani, 2008; Rodwell et al., 2001). Our focal pathogens (Table 1) typically co-infect domestic cattle during shipment and form the bovine respiratory disease complex (Earley et al., 2017; Griffin, 1997; Lillie, 1974). While the pathology of each virus and bacteria is well-studied in livestock (Table 1), risk factors for infection—especially the relative importance of various independent and interacting host, environmental and co-infection factors—are poorly understood. Further, while pathogens associated with the respiratory disease complex have been identified in wildlife (e.g. Bauer & Condy, 1981), their ecology, epidemiology and importance to wildlife health are unknown. Notably, while not a focal pathogen, we include bovine tuberculosis status (bTB) in our analysis as infection by this chronic bacterial pathogen influences buffalos' immune profile (Ezenwa et al., 2010).

In this study, we:

1. Characterize temporal variation in respiratory pathogen incidence within this wild population.
2. Next we use a unified analytical framework (conditional random fields: Clark et al., 2018) to ask: What is the relative importance, both independent and interacting, of host traits, season, and pathogen associations on occurrence of seven respiratory pathogens?

Table 1. Biology of respiratory pathogens included in this study, and annual incidence in African buffalo

Pathogen (abbreviation)	Family (subfamily)	Type	Description	Average annual incidence ± *SD*
Mycoplasma bovis (MB)	Myco-plasmataceae	Bacterial, opportunistic	MB commonly causes calf pneumonia, mastitis, and arthritis, while occasionally causing swelling of various reproductive organs and abortion in cattle. Infected cattle can shed the bacteria for months to years (Maunsell et al., 2011; Smith et al., 2019)	20.2 ± 2.1%
Mannheimia haemolytica (MH)	Pasteurellaceae	Bacterial, opportunistic	Though MH can act as a commensal and commonly exists in the upper respiratory tract of healthy ruminants, it is also associated with pneumonia and bovine respiratory disease complex in cattle after a hosts' immune defences have been compromised by stress or another infection (Rice et al., 2007)	11.0 ± 1.8%
Bovine Adenovirus-3 (AD-3)	Adenoviridae (mastadenovirus)	Viral, acute	The pathogenic effects of AD-3 alone remain controversial; but when associated with disease can cause oculonasal discharge, colic, diarrhoea, enteritis and fever in cattle (Coetzer et al., 2006)	13.3 ± 3.8%
Bovine Parainfluenza-3 (Pi-3)	Paramyxoviridae (respirovirus)	Viral, acute	When interacting with other disease-causing factors, clinical signs of Pi-3 include: coughing, pyrexia, nasal discharge and inappetence. Cattle develop a strong immune response, but sterile immunity is short lived (Maclachlan & Dubovi, 2010)	12.0 ± 3.2%
Bovine Herpesvirus-1 (BHV)	Herpesviridae (Alphaherpes-virinae)	Viral, acute or chronic (latent)	Also known as infectious bovine rhinotracheitis, BHV affects the respiratory and reproductive tracts of bovids; reactivation of latent infections may play an important role in transmission in cattle (Maclachlan & Dubovi, 2010)	11.3 ± 5.2%
Bovine Respiratory Syncytial Virus (BRSV)	Paramyxovirus (pneumovirus)	Viral, acute or chronic (latent)	While most infections are unapparent, some BRSV-infected animals present with fever, coughing, and upper respiratory discharge. Most severe disease is reported in calves under 6 months. Persistent infections have been suggested based on epidemiological and experimental data in cattle (Valarcher et al., 2001; Van Vuuren, 1994). Re-infection is also common; in fact, cows can be infected more than once per year (Van der Poel et al., 1994)	12.1 ± 15.0%
Bovine Viral Diarrhoea Virus (BVDV)	Flaviviridae (pestivirus)	Viral, acute or chronic (latent)	In cattle, BVDV can present as clinically inapparent, a mild acute diarrheal disease, a fatal mucosal disease, or as an in utero infection from which persistent infections can develop (Smith et al., 2019)	1.9 ± 0.4%

Table 2. Within-host traits and environmental variables included in our conditional random field analysis

Variable	Variable type	Collection information, data transformation and units
Season	Categorical	Pathogen exposure regimes, pathogen viability, host behaviour and host immunocompetence may fluctuate with season: thus, we included season at sampling to detect seasonality of pathogen occurrence and hypothesize about seasonally variables not explicitly defined within our model. Wet = November–April; Dry = May–October
Age	Continuous	Approximate age of each animal (months) based on teeth regressions as per Jolles et al. (2005)
Capture herd	Binomial	This variable refers to the herd in which the buffalo was found during the given capture: Crocodile Bridge or Lower Sabie
Condition	Categorical	Visualization and palpation of the ribs, spine, hips and the base of the tail was scored on a scale of 1 (very poor) to 5 (excellent); overall body condition score was calculated as the average of these four scores. Condition at the beginning of the interval—that is, at the previous capture—was used in analyses (Ezenwa et al., 2009)
Age-horn residual	Continuous	The regression residuals of age at first capture on horn width (cm) was collected at the previous capture. In female buffalo, the variable is a marker of GI parasite infection with higher residuals indicating lower parasite richness, as well as lower coccidia occurrence and intensity (Ezenwa & Jolles, 2008)
Pregnancy status	Binomial	Pregnancy status is based on palpation by a veterinarian via rectal palpation
Lactation status	Binomial	Lactation status was assessed by manual milking of all four teats (Jolles, 2007)
calf-at-heel status	Binomial	This variable indicates whether there was a calf at the mother's side during visual surveys when animals were being picked out for darting
Bovine tuberculosis status (bTB) convert	Binomial	bTB is typically a chronic, subclinical disease of the lung and upper respiratory tract in African buffalo. bTB interactions with host traits and other pathogens have been well-characterized in African buffalo. Due to the chronicity of infection, we included if the animal converted to bTB as a host trait
Anthelmintic bolus treatment	Binomial	As part of another study (Ezenwa & Jolles, 2015), half of the buffalo in each herd were administered a slow-release, oral anti-helminthic treatment (Fenbendazole aka Panacur) at every capture

2 MATERIALS AND METHODS

2.1 Study area

Kruger National Park (KNP) is located in the north-eastern corner of South Africa between 22.5 and 25.5°S, and 31.0 and 31.6°E (Figure S1). The area of the KNP is 19,485 km^2, but since 2002, the area available to wildlife has effectively doubled due to the removal of fences between private game reserves in the west and Mozambique in the east. The population of African buffalo in the park is about 37,000 animals (SANPARKS, 2010–2011). Our 4-year project was restricted to buffalo in the southern KNP and took place between June 2008 and June 2012.

On average, 84% of KNP's total rainfall is concentrated between November and April (Zambatis, 2003) with approximately 600 mm of rainfall per year in the southern KNP (Venter & Gertenbach, 1986). The dry season typically occurs between May and October. Rather than using calendar year in our analyses, we used rainfall year, hereafter referred to as 'year', with year commencing in November.

2.2 Sampling regime

Female African buffalo between 2 and 5 years old were captured as part of a study on parasite interactions in free-ranging buffalo (Ezenwa & Jolles, 2015). The first 100 buffalo were captured from the Lower Sabie herd between 23 June and 5 July 2008 (Figure S1). The second 100 buffalo were captured from the Crocodile Bridge herd between 1 and 8 October 2008 (Figure S1). Buffalo were recaptured approximately every 6 months after this initial capture through June 2012. Any buffalo that died or emigrated from the study area during the study period was replaced with an animal of similar age so that a near-constant sample size of 200 was maintained at each capture (additional detail in Spaan et al., 2019).

2.3 Sample and data collection

Buffalo were located and identified via radiocollars (seven GPS collars to locate herds, 193 VHF collars to identify individuals). At capture, buffalo were chemically immobilized with etorphine hydrochloride (M99) and ketamine by darting from a truck or helicopter. Following sample and data collection, immobilization was reversed using diprenorphine (M5050). All immobilizations were performed by South African National Park's (SANPark) veterinarians and registered project staff, and all procedures were approved by Oregon State University, University of Georgia and SANPark's Institutional Animal Care and Use Committees.

While buffalo were immobilized, blood and host-trait data were collected from each animal. Blood samples for serological assays were collected via jugular venipuncture in sterile tubes containing no anticoagulant. Blood was placed on ice and stored in a cooler box within 5 min for transportation back to the laboratory. At the laboratory, serum was collected after centrifugation for 20 min at 2,000 g and stored at −20°C until analysis. Host-trait measures included age, body condition, horn width, pregnancy status, lactation status, and calf-at-heel status using previously published methods (Table 2).

Half of the studied buffalo in each herd were administered an oral anthelmintic treatment in the form of a Panacur® slow-release bolus at every capture. The bolus contains the active ingredient fenbendazole. Nematode egg shedding is effectively eliminated in buffalo for ~160 days after a single administration (Ezenwa et al., 2010) and alters African buffalo response to infection by microparasites (Ezenwa & Jolles, 2015). The other half of each herd did not receive Panacur® at any capture.

2.4 Serology

2.4.1 Serological assays

Buffalo serostatus for each of the respiratory pathogens were determined using commercially available assays after each capture as previously described (Glidden et al., 2018). Briefly, monoclonal antibodies specific to the F protein of bovine respiratory syncytial virus (BRSV) and the NS3 protein bovine viral diarrhoeal virus (BVDV) were detected in serum using separate competitive ELISA kits (Bio-X Diagnostics), while the serostatus of bovine herpesvirus-1 (BHV), Pi-3 (bovine parainfluenza-3), AD-3 (bovine adenovirus-3), MB (*Mycoplasma bovis*) and MH (*Mannheimia haemolytica*) were assessed using direct ELISA test kits (Bio-X Diagnostics). For the first set of tests MB and MH were not included on the assay. Buffalo were tested for bTB using the BOVIGAM ELISA kit (Prionics) which is a standard whole blood interferon-gamma (IFNγ) assay (Schiller et al., 2009; Wood & Jones, 2001). This kit in particular has been previously optimized for use in African buffalo (Michel et al., 2011). All serum samples were stored in the same laboratory (KNP Veterinary Wildlife Services).

2.4.2 Classifying occurrence

All animals were recruited as adults, so it was impossible to determine whether the first detected increases in antibody titres were due to a primary exposure, re-exposure or recrudescence (Combink et al., 2020). For this reason, we define pathogen 'occurrence' to include all three possibilities. We expect occurrence, representing all three possibilities, to represent initiation of active, transmissible infections. Identical to Glidden et al. (2018), BRSV and BVDV were tested using a competitive ELISA which give scores of 0%–100% positive. Samples were deemed positive if ELISA scores were >50%, per manufacturer instructions. If the animal was tested six or more times with only one, weakly positive (<65%) result, we assumed the test result was a false positive. Occurrence of BRSV or BVDV was counted if test results went from negative (<50%) to positive (>50%) or if positive animals had a 15+% increase in their competitive ELISA score from one capture to the next (Glidden et al., 2018). We observed low occurrence of BVDV and thus excluded it from further analyses (Figure 1). For BHV, Pi-3, AD-3, MH and MB, ELISAs were scored on a 0–5 scale and occurrence was counted if the ELISA score increased by 2 or more points between two captures, per manufacturer instructions. ELISA results were previously shown to correlate with other markers of inflammation and infection (Glidden et al., 2018).

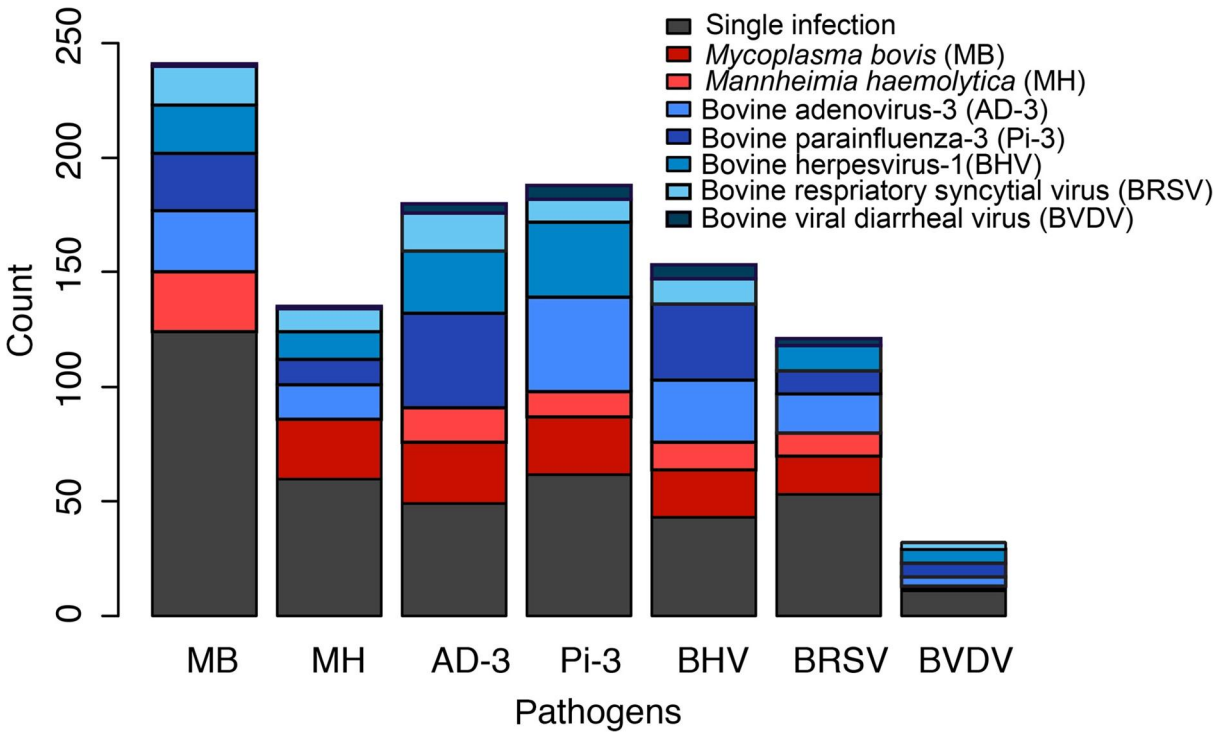

Figure 1.

Infection by multiple pathogens in the same time period was common. This figure shows the proportion of observations of each infection as a single infection (dark grey) and as a co-infection with other focal pathogens (colour corresponds to exact co-infection)

To determine bTB conversion, we considered an animal's full IFNγ bTB dataset (all sampling points): an animal was considered to have become infected with bTB (bTB+) only if we observed at least two consecutive negative tests followed by at least two consecutive positive tests (Ezenwa & Jolles, 2015). In total, 80 animals converted to bTB+ throughout the study.

2.5 Statistical analyses

All analyses were run using r version (v 4.0.2).

2.5.1 Characterizing monthly and yearly variation in incidence

We examined population-level temporal trends in the number of new cases of each pathogen (incidence) per month of the study using general additive models in the r package mgcv (Wood, 2011). For the purposes of the model, a new case was defined identically to 'occurrence' in Section 2.5.1. For each month of the study and for each pathogen, we summed the number of new cases and used this as the dependent variable (Poisson distribution) in each model (six models total, one per pathogen). We included calendar month and rainfall year as independent, smooth terms. For month, the penalized smoothing basis was a cyclic cubic regression spline. We used a cyclic cubic regression spline because it allows us to account for months occurring in a loop since environmental conditions in the last month of the year (December) are similar to the first month of the year (January;

Kiguchi & Minami, 2012). For year, the penalized smoothing basis was a P-spline. Number of animals sampled per month-year was included as a fixed effect to account for sampling effort. For each pathogen, we considered the model with the lowest second-order Akaike information criteria (AICc: Hurvich & Tsai, 1989) to have the best fit. We further evaluated model fit using deviance explained (Wood, 2017). Model diagnostics were evaluated using the 'gam.check' function in the mcgv package which plots quantile–quantile plots of residuals, the linear predictor versus residuals, the histogram of residuals and the plot of fitted values versus response. We also used 'gam.check' to examine whether the basis dimension for the smooth term was adequate. We omitted the first study rainfall year from the analyses because there were only 2 months of data for that year, which left a total of 43 sampling time points for these analyses.

2.5.2 Determining the relative importance of host traits and pathogen associations on within-host pathogen occurrence of respiratory pathogens

We conducted a conditional random field (CRF) analysis using the r package MRFcov (Clark et al., 2018) to estimate the independent and interacting effect of host traits, season, and pathogen associations (co-occurrence) on log-odds of pathogen occurrence. We initially included six focal pathogens (Pi-3, AD-3, BHV, MH, MB and BRSV) and bTB (described as infected status, as opposed to occurrence) as nodes and host traits (Table 2) and season as covariates. Continuous covariates were standardized to standard deviations from the mean. CRFs enable one to determine if each variable pair is conditionally dependent upon one another given their relationship with all other variables, as well as determine if pairwise associations vary with covariates (Clark et al., 2018). CRFs are better at estimating true species interactions than typical null-model randomization approaches as they account for indirect associations when estimating species co-occurrence, although co-occurrences could still arise if presence of two pathogens responds to a variable not accounted for in the model (Clark et al., 2018). The framework is described and referenced in detail in Clark et al., 2018. Briefly:

The log-odds of occurrence of pathogen *j* given covariate *x* and the presence–absence of species *k* can be modelled as:

$$\log\left(\frac{P(y_j = 1|y_{\setminus j}, x)}{1 - P(y_j = 1|y_{\setminus j}, x)}\right) = \alpha_{j0} + \beta_j^T x + \sum_{k:k\neq j}\left(\alpha_{jk0} + \beta_{jk}^T x\right) y_k, \quad (1)$$

where $\mathbf{y_j}$ is a vector of binary occurrence records for species *j* (1 for occurrence, 0 for no occurrence), $\mathbf{y}_{\setminus j}$ is a vector of binary occurrence records for all other species apart from *j*, α_{j0} is the pathogen level intercept and $\beta_j^T x$ is the coefficient of covariate *x* on species *j* occurrence probability. α_{jk0} and $\beta_{jk}^T x$ represent pathogen associations, where α_{jk0} represent conditional dependencies between pathogens and $\beta_{jk}^T x$ represent the effect of covariate x on this dependence; if $\alpha_{jk0} \neq 0$ but $\beta_{jk}^T x = 0$ then occurrence probabilities are conditionally dependent but do not vary with covariate x, in contrast, if $\alpha_{jk0} \neq 0$ and $\beta_{jk}^T x \neq 0$ the strength of the dependence varies with covariate *x*. Parameterization of the likelihood is estimated using logistic regression, thus, regression coefficients describe the effect of the predictor on the pathogen's conditional log-odds.

For each pathogen-specific regression, sparsity is added using L_1 regularization (i.e. LASSO), which forces coefficients with minimal effect to zero. With *MRFcov*, optimization of the regularization parameter is conducted internally using the cv.glmnet function in the R package GLMNET (Friedman et al., 2010), which uses k-fold cross-validation to choose a penalty that minimizes cross-validated error (15-fold cross-validation for pathogens except MH and BRSV, where leave-one-out cross-validation was used due to <150 observations where occurrence = 1). After LASSO variable selection, α_{jk0} and $\beta_{jk}^T x$ were symmetrized (i.e. $\alpha_{jk0} = \alpha_{kj0}$ and $\beta_{jk}^T x = \beta_{kj}^T x$) following Cheng et al. (2014). Consequently, parameters are approximated from a unified graphical network after maximizing the conditional log-likelihood for each pathogen (Lee & Hastie, 2015). To account for our repeated measures study design, we used the smoothconstruct2() function in the R package MGCV (Wood, 2011) to construct smooth splines for the effect of animal ID and estimate a penalized coefficient for each level of animal ID. The effects of interspecific associations and host traits on pathogen occurrence were estimated after accounting for the effect of animal ID.

Only complete datasets were used in initial analyses such that we used 705 samples from 192 animals. After observing that MB and MH had no associations or conditional associations with other pathogens (see Section 3), we ran an analysis omitting MB and MH which permitted the addition of a number of additional animals such that we used 858 samples and 226 animals in our second run of the analysis. Priority effects may occur where MH and MB proliferate following respiratory tract damage from viral infection (Rice et al., 2007; Srikumaran et al., 2007). Thus, we tested for signatures of priority effects by running an additional analysis that included MH and MB observations at time $t + 1$; however, we did not find any evidence of priority effects, so do not discuss this further (Appendix S1). For each analysis, we estimated a model that included and did not include covariates. For each model, we assessed the fit to the observed data by calculating the proportion of observations that each model successfully classified. In short, we fit models using a training partition of data (90% observations, randomly selected) and calculated linear predictions of pathogen occurrence for a test partition containing the remaining 10% of observations. We repeated this procedure 10 times: for each test prediction we calculated sensitivity (proportion of positives correctly identified), specificity (proportion of negatives correctly identified) and overall proportion of true predictions. The model containing covariates was then fit to 100 bootstrapped versions of the observed data to capture uncertainty in model parameters (e.g. Fountain-Jones et al., 2019). Using bootstrap estimates, we further quantified the relative importance of pathogen associations, host traits and the conditionally dependent pathogen associations by taking the square of the mean coefficient divided by the sum of all squared mean coefficients.

3 RESULTS

Occurrence of respiratory infections in buffalo was common, but variable among pathogens: in a given 6-month capture interval, nearly 50% of our study animals acquired at least one respiratory infection with annual incidence rate varying between 2% (BVDV) and 20% (MB) among pathogens (Table 1). Co-infection was also common: buffalo had between 0 and 4 pathogen occurrences at each sampling event with an average of 1.34 occurrences of respiratory infections per animal per year (Figure 1).

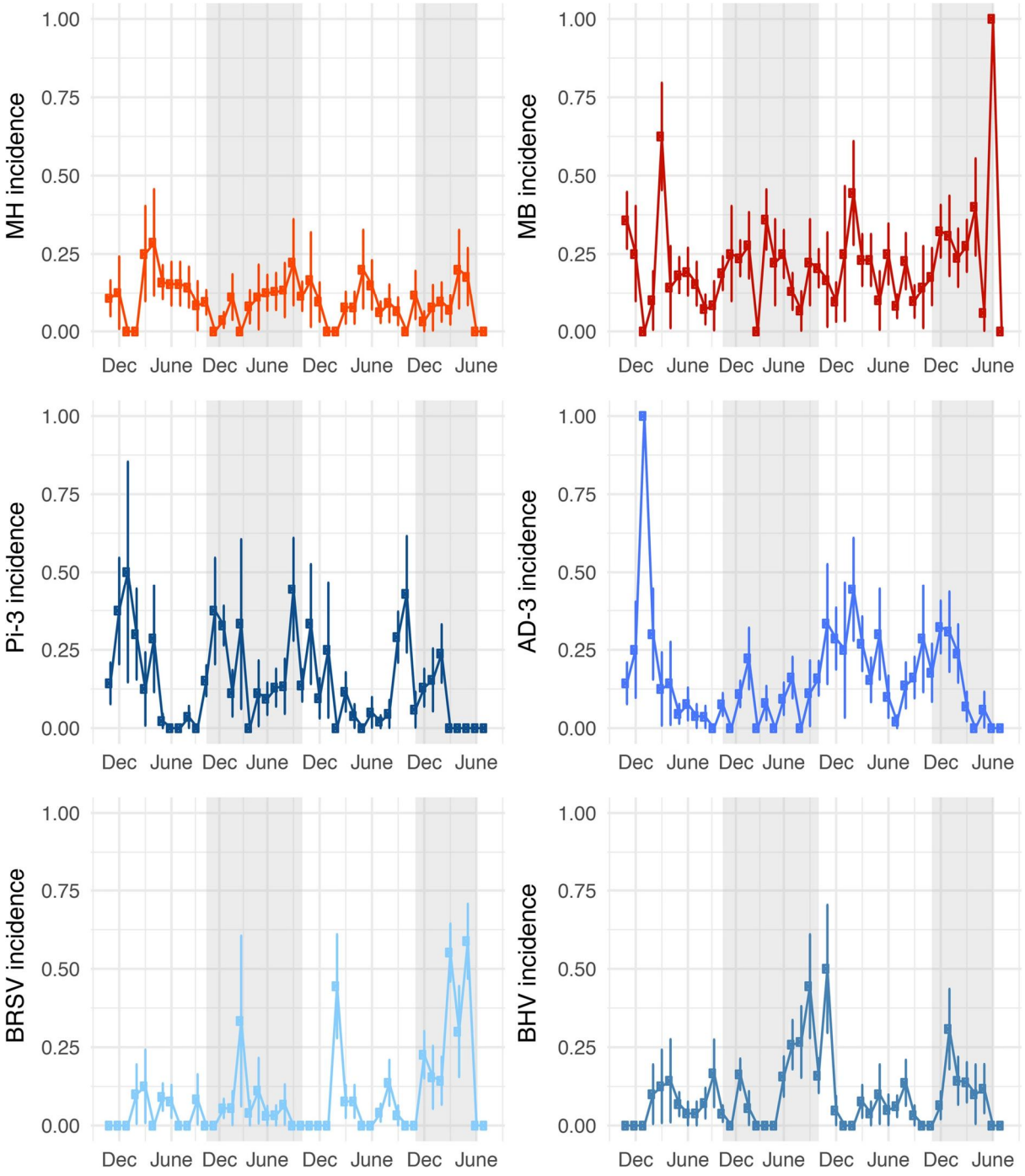

Figure 2

Incidence (number of new cases detected/number of animals sampled) by study month. Bars represent standard errors. Standard errors were calculated by (√(incidence*(1 − incidence))/number of animals sampled. Rainfall years are shaded by alternating white and grey backgrounds

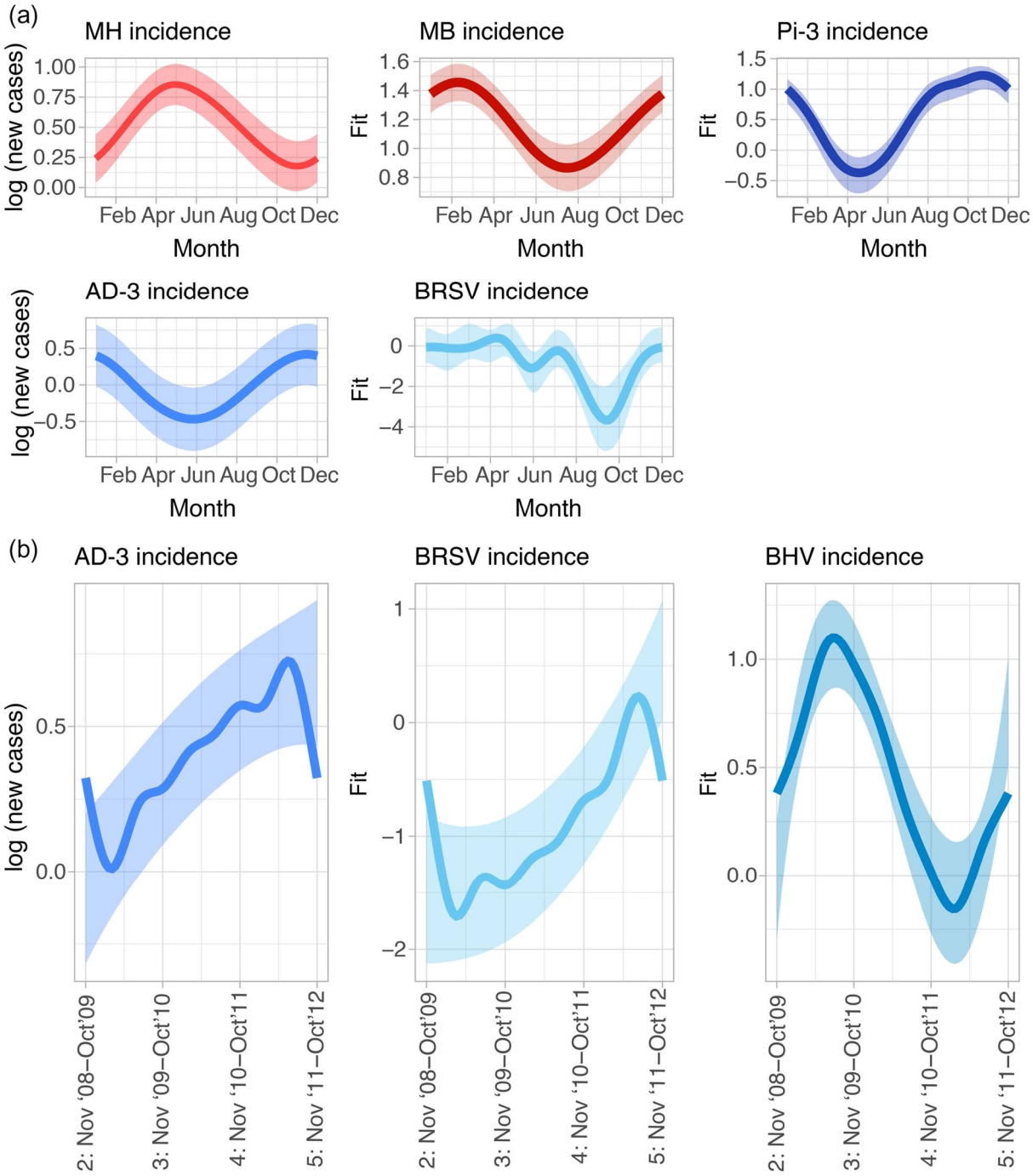

Figure 3

GAM predictions for number of new cases per calendar month after controlling for sample number. Dynamics are depicted for pathogens where (a) the calendar month or (b) rainfall year was significant in our final model. For AD-3 and bovine respiratory syncytial virus, values for month are predicted during rainfall year 1 (November 2008–October 2009) and values for year are predicted during June

3.1 Characterizing monthly and yearly variation in population-level disease dynamics

We characterized monthly and yearly variation in incidence using generalized additive models. Month had a significant effect on the number of new cases detected of MH, MB, Pi-

3, AD-3 and BRSV after controlling for sampling effort (Figure 2 and Figure 3; Table S1). The number of new cases of Pi-3, AD-3 and MB peaked during the wet season months (Pi-3: November, AD-3: December, MB: February), whereas MH peaked at the transition between the wet and dry seasons (May). The number of new cases detected of BRSV—after controlling for sampling effort—decreased in October, suggesting that the fewest new cases occurred during October.

Year had a significant effect on the number of new cases detected of AD-3, BRSV, and BHV after controlling for sampling effort (Figures 2 and 3; Table S1). BRSV exhibited one large outbreak during the final year of the project. The study population also appeared to have two outbreaks of BHV with a larger outbreak occurring within the November 2009–October 2010 rainfall year and a smaller outbreak occurring within the November 2011–December 2012 rainfall year. Though AD-3 occurrence exhibited cyclical seasonal dynamics with a consistent peak in the wet season months, our model suggests that peaks in incidence were higher towards the end of the project. Our time series is too short to ascertain whether these inter-annual peaks in BHV and AD-3 are indicative of independent outbreaks or form part of a biennial infection cycle.

Deviance explained, which summarizes model fit, was quite high for each model (MH = 55.00%, MB = 65.40%, Pi-3 = 61.10%, AD-3 = 59.40%, BRSV = 74.80%, BHV = 48.60%, Table S1). However, a large portion of deviance explained may be attributed to sample size which we included as a covariate to account for sampling effort.

3.2 Determining the relative importance of host traits and pathogen associations on within-host pathogen occurrence of respiratory pathogens

Pathogen co-occurrence (i.e. pathogen associations) had the largest effect on odds of pathogen occurrence for the viruses Pi-3, AD-3 and BHV, with host traits only important in influencing the strength of pathogen–pathogen associations (Figure 4; Table 3). The final virus, BRSV, was primarily influenced by season and partially by host age (Figure 4; Table 3). Neither pathogen co-occurrence nor host traits had an effect on odds of occurrence for our focal bacteria, MH and MB (although odds of MB occurrence was slightly higher in the wet season; Figure 4; Table 3). We found similar results when testing for priority effects on MH and MB occurrence (supp materials).

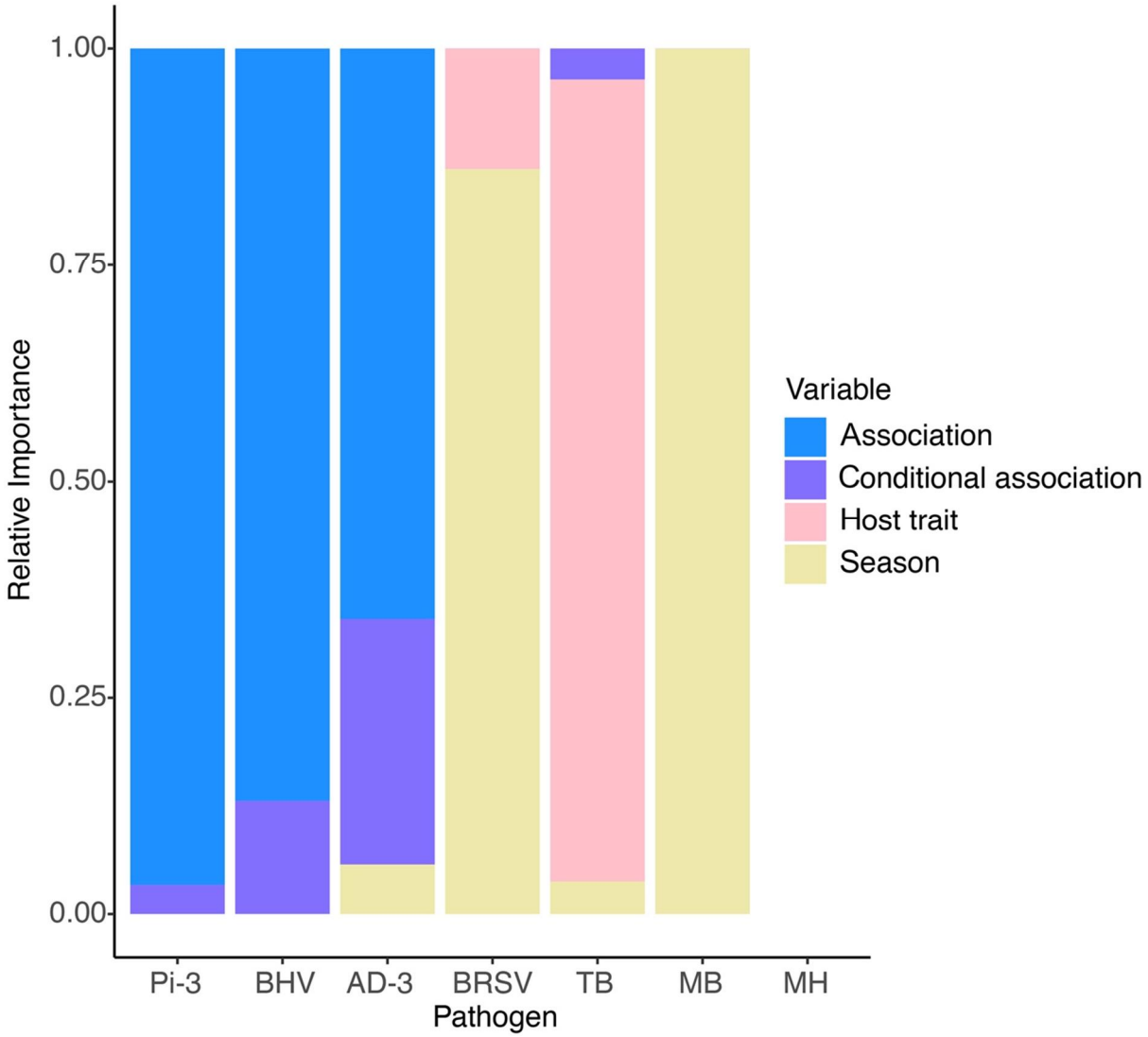

Figure 4

Relative importance of pathogen associations, conditional associations, host traits and season in predicting pathogen occurrence. Each coefficient was transformed to relative importance by dividing the mean coefficient squared by the sum all coefficients squared. The relative importance of coefficients within each category [i.e. association, conditional association (i.e. pathogen × covariate interaction where $\alpha_{jk0} \neq 0$ and $\boldsymbol{\beta}_{jk}^{T}\boldsymbol{x} \neq 0$), host trait, season)] was then summed to get the relative importance per category; only coefficients with a relative importance >0.1 were included in the figure

Table 3. Conditional random field regression coefficients for each pathogen's occurrence probability. Coefficients are interpreted identically to logistic regression. Only predictors where the relative importance is >0.01 are reported; the full estimates containing all parameters where the confidence interval does not overlap 0 are reported in Table S2

Pathogen	Predictor	Coefficient (95% CI)	Relative importance
	AD-3 occurrence	0.68 (0.64–0.72)	0.52
Pi-3	BHV occurrence	0.63 (0.60–0.66)	0.44
	$Season_{RL=dry}$ × BHV occurrence	0.17 (0.17–0.18)	0.03
	Pi-3 occurrence	0.63 (0.60–0.66)	0.76
	AD-3 occurrence	0.23 (0.17–0.27)	0.1
BHV	$Season_{RL=dry}$ × Pi-3 occurrence	0.17 (0.17–0.18)	0.06
	$Lactating_{RL=non-lactating}$ × AD-3 occurrence	0.17 (0.08–0.21)	0.06
	$Herd_{RL=Croc\ Bridge}$ × Pi-3 occurrence	0.09 (0.08–0.10)	0.02
	Pi-3 occurrence	0.68 (0.64–0.72)	0.58
	$Pregnant_{RL=non-pregnant}$ × TB status	−0.44 (−0.54 to −0.36)	0.24
AD-3	BHV occurrence	0.23 (0.17–0.27)	0.07
	$Season_{RL=dry}$	0.21 (0.15–0.26)	0.06
	$Lactating_{RL=non-lactating}$ × BHV occurrence	0.17 (0.08–0.21)	0.04
BRSV	$Season_{RL=dry}$	0.2 (0.18–0.24)	0.86
	Age	0.08 (0.07–0.09)	0.14
	Age	2.14 (1.92–2.40)	0.83
	$Lactating_{RL=non-lactating}$	0.59 (0.51–0.69)	0.06
bTB status	$Season_{RL=dry}$	0.45 (0.40–0.53)	0.04
	$Pregnant_{RL=non-pregnant}$ × AD-3 soccurrence	−0.44 (−0.54 to −0.36)	0.03
	Horn width – age residuals	0.38 (0.27–0.53)	0.03
MB[a]	$Season_{RL=dry}$	0.11 (0.001–0.19)	1
MH[a]	*No significant predictors*	—	—

Abbreviations: AD-3, bovine adenovirus-3; BHV, bovine herpesvirus-1; BRSV, bovine respiratory syncytial virus; bTB, bovine tuberculosis status; MB, *Mycoplasma bovis*; MH, *Mannheimia haemolytica*; Pi-3, bovine parainfluenza-3.

[a] Estimated from 705 samples and 192 animals, all others estimated from 858 samples and 226 animals.

In terms of specific pathogen associations and interacting covariates (Table 2), we found that odds of Pi-3 occurrence, and vice versa, were higher when animals were infected with AD-3 and BHV (i.e. odds of AD-3 and BHV were higher when animals were infected with Pi-3; Figure 5). We found that the strength of the association between Pi-3 and BHV was higher in the wet season and in the Lower Sabie herd (Table 3). Additionally, we found that the odds of AD-3 occurrence, and vice versa, were higher when animals with infected with BHV (Figure 5), with the association between these pathogens stronger when animals were lactating (Table 3). We found that odds of AD-3 occurrence were slightly higher in animals that were bTB+ and that were not pregnant (coefficient = 0.03 (95% CI: 0.003–0.05, Figure 5;

Table S2); however, this association shifted to negative (i.e. odds were lower) and became stronger when animals were pregnant (Table 3).

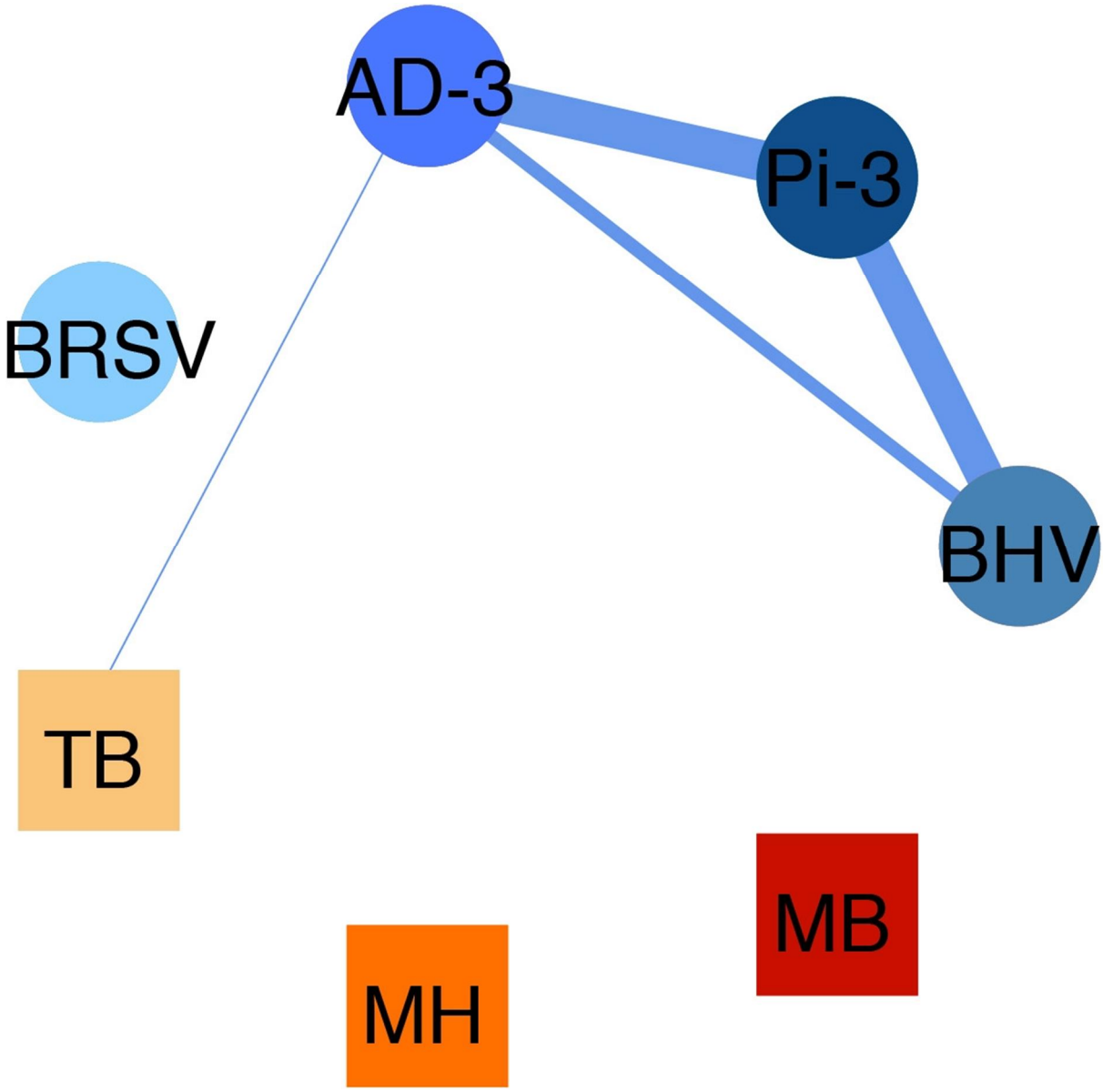

Figure 5

Pathogen association network. Each node represents a pathogen (viruses are circles, squares are bacteria) and edges are weighted by association coefficients (blue positive, red negative). Associations represent associations at reference level = 0; see Table 3 for association strengths when reference level = 1

When evaluating model fit (i.e. calculating sensitivity, specificity and proportion true predictions) of models that included only pathogen associations versus models that included pathogen associations and covariates (host traits, season: Table 2), the ability for our model to classify observations correctly was only slightly higher when we included covariates than when we did not include covariates (Table 4). These results support our primary conclusion that pathogen associations are the best predictors of pathogen occurrence.

Table 4. Predictive performance of MRF (without model covariates) and CRF (with model covariates) models. Table includes results from partial (omitting MB and MB: N_{sample} = 858, $N_{animals}$ = 226) and full (all pathogens: N_{sample} = 705, $N_{animals}$ = 192) datasets

	Sensitivity (95% CI)	Specificity (95% CI)	Proportion true predictions (95% CI)
Partial dataset (MB and MH omitted)			
MRF	0.363 (0.278–0.458)	0.995 (0.986–1.00)	0.896 (0.867–0.922)
CRF	0.384 (0.304–0.463)	0.999 (0.996–1.00)	0.904 (0.881–0.926)
Full dataset (all pathogens included)			
MRF	0.275 (0.221–0.329)	0.995 (0.989–1.00)	0.885 (0.864–0.902)
CRF	0.277 (0.219–0.345)	0.998 (0.995–1.00)	0.888 (0.865–0.912)

Abbreviations: CRF, conditional random field; MB, *Mycoplasma bovis*; MH, *Mannheimia haemolytica*; MRF, Markov random field.

4 DISCUSSION

In this study, we used a unique dataset on seven respiratory infections in free-ranging, adult female African buffalo to characterize temporal dynamics as well as tease apart the relative importance of host traits, season, and co-infection on odds of infection. We detected variation in pathogen incidence over time with pathogen incidence commonly peaking in the late dry season or wet season. We found that, on their own, host traits were poor predictors of occurrence (see Section 2.4.2: new infection, re-infection, recrudescence) of all focal pathogens in our population. In contrast, pathogen co-occurrence was the strongest predictor of virus occurrence, whereas season was the only predictor of bacteria occurrence.

We found that concomitant infections were more important predictors of infection risk (i.e. occurrence) than host traits, especially for AD-3, Pi-3, and BHV. As our study is correlation based, these three pathogens might be associated because of actual facilitative pathogen interactions within the host (e.g. via immune suppression); alternatively, their association might reflect (constitutively or temporarily) correlated susceptibility and/or exposure to this suite of viruses. Pi-3 is viewed as a permissive infection for other viral infections in cattle, enabling viruses to circumvent host defences (Smith et al., 2019). In addition, the seasonal peak in Pi-3 infection preceded peak occurrence of AD-3 and BHV. As such, positive associations between Pi-3, AD-3, and BHV may point to a facilitative effect of Pi-3 infection on AD-3 and BHV. We found the association between Pi-3 and BHV was slightly stronger in the wet season and the Lower Sabie herd. As hosts were generally in better condition in the wet season and Lower Sabie herd (Gorsich et al., 2015), the strengthening of the Pi-3–BHV association could be driven by a rise in correlated exposure rates via increased contact around shared resources, as opposed to change in host physiology. We found that AD-3 and BHV had a small, positive association that increased by sixfold when animals were lactating. Spaan (2018) found that infection by AD-3 in buffalo causes a rise in stress hormones (fecal glucocorticoid metabolites). As herpesvirus recrudescence is attributed to stress in other systems (Yan et al., 2020), our data suggest the combined or synergistic effects of AD-3 infection and energetic demands of lactation (Speakman, 2007) may amplify BHV recrudesce in buffalo. We found a slight positive association between AD-3 occurrence and

bTB infection in non-pregnant animals, but a stronger negative association in pregnant animals illuminating that associations can switch direction under certain conditions. Immune profiles shift during pregnancy (Pazos et al., 2012) as well as bTB infection (Ezenwa et al., 2010). Perhaps the combined shift alters odds of occurrence by AD-3. However, it is unclear if our analysis is detecting a causal relationship or correlated response between AD-3 and bTB.

Collectively, our data and these hypotheses suggest that infection risk is driven by fine-scale changes in host physiology, host contact rates, and the interacting effects of co-infections. However, in absence of fine-scale data, intricate changes among these factors are best broadly represented by co-infection with interacting and/or functionally similar viruses, as opposed to general host traits. In the future, one virus in this suite could be used as an indicator for other infections (e.g. Fleishman et al., 2005) when forecasting infection risk. Additionally, if the underlying mechanisms driving associations is determined, reducing transmission of this triad viral pathogens could be done simultaneously (e.g. if Pi-3 indeed facilitates viruses, vaccinate for Pi-3).

We did not find any effect of host traits or pathogen co-occurrence of opportunistic bacteria (MH and MB). Odds of MB occurrence was slightly higher in the wet season (characterized by high temperatures and humidity, Venter & Gertenbach, 1986; Zambatis, 2003) and population-level incidence peaked towards the end of the wet season. MB persists in the environment for long durations (McAuliffe et al., 2006) and, in humans, mycoplasma incidence has been shown to increase with temperature and humidity (Onozuka et al., 2009). Consequently, MB occurrence may be best explained by environmental exposure. MH and MB are normally found in the respiratory tract of bovids (Ayling et al., 2000; Cozens et al., 2019) and pathogenic subtypes proliferate under stressful conditions in cattle (Cozens et al., 2019; Maunsell et al., 2011). We found that host traits associated with stress in buffalo (body condition, horn residuals, Spaan, 2018) did not influence occurrence of bacteria. Additionally, BRSV is well-known for damaging host tissue and facilitating replication of pathogenic bacteria in cattle (Rice et al., 2007; Srikumaran et al., 2007). We may have detected priority effects of BRSV on pathogen occurrence if we had data following the BRSV outbreak (occurring in the final year of our project), or if we sampled on shorter intervals. Equally as likely, BRSV may not have the same pathological effects in buffalo as it does in cattle; thus, we do not observe tissue damage-mediated facilitation in this wild system. Overall, there are different triggers of bacterial proliferation in buffalo than in cattle. Similar to viruses, our analysis indicates that fine-scale data are necessary to predict occurrence of opportunistic bacterial infections. Perhaps when only coarse data can be collected, co-infection by more functionally or phylogenetically similar bacteria would be the best predictor of infection by MH or MB. Future research could identify and test more suitable indicator taxa.

The sensitivity of our analysis was low indicating that there are a number of other processes influencing respiratory pathogen disease dynamics. As most of these pathogens cause short-lived infections, detection of variables associated with occurrence may necessitate more frequent sampling intervals. Moreover, host traits may have a small effect on pathogen occurrence, but strong predictors of the ability to clear a pathogen once infected or infection status (e.g. bTb and *Brucellosis* in African buffalo (Ezenwa & Jolles, 2015, Gorsich

et al., 2015)). Serology data limit our ability to characterize multiple different infection outcomes (i.e. time to clearance, pathogen intensity); however, future work could use different diagnostic techniques (e.g. quantitative PCR) to weigh the effect of host traits on different outcomes of infection. Furthermore, we did not take into account long-term infection history (i.e. timing of waned immunity from previous exposure)—which (at both the individual and population level (i.e. number of susceptible, infected and recovered host) would likely increase model sensitivity. However, tracking long-term infection history is impracticable in a long-lived mammal.

In this study, we identified co-infection as a key factor in determining infection risk by respiratory viruses with additional research necessary to determine factors influencing odds of bacteria occurrence. We demonstrate that pathogen associations can be context-dependent and may change with shifts in host contact rates or physiology. Ultimately, this work illustrates that studying pathogens in wild systems as communities, as opposed to single infections, may better our understanding of disease processes and the ability to predict infection risk. It is our hope that uncovering the environmental, behavioural, and physiological processes behind variation in disease dynamics can yield mechanistically based models that are more resilient to shifting environmental baselines.

ACKNOWLEDGEMENTS

The authors thank colleagues at the Veterinary Wildlife Services department at Kruger National Park, the State Veterinary Office in Skukuza, and our field and laboratory technicians Robert and Johannie Spaan. They additionally thank Rob Spaan for providing the map. They also thank Eilea Delgadillo for insightful discussion on bovine disease complex epidemiology and pathogenesis as well as Dr Nicholas Clark for help using *MRFcov*. This study was supported by a National Science Foundation Ecology of Infectious Diseases grant (EF-0723918/DEB-1102493, EF-0723928 to V. Ezenwa and A. Jolles) and by the USDA-NIFA AFRI grant (2013-67015-21291 awarded to A. Jolles) and by the UK Biotechnology and Biological Sciences Research Council grant (BB/L011085/1 awarded to A.E.J.) as part of the joint USDA-NSF- NIH-BBSRC Ecology and Evolution of Infectious Diseases program. C.M. was supported by the NIH (T32 OD10999, PI: Dr Dale E. Bjorling). C.A.C.C. was supported financially and academically by a Post-Doctoral Fellowship from the University of Pretoria and Professor Moritz van Vuuren respectively. C.K.G. was supported by an NSF-GRFP and ARCS Fellowship. We declare no conflict of interests.

AUTHORS' CONTRIBUTIONS

C.K.G., B.R.B., C.M., V.O.E. and A.E.J. collected the data; C.K.G. and C.A.C.C. analysed the data and led writing of the manuscript. All the authors conceived the ideas, designed the methodology, contributed critically to the drafts and gave final approval for publication.

REFERENCES

Ayling, R., Baker, S., Nicholas, R., Peek, M., & Simon, A. (2000). Comparison of *in vitro* activity of danofloxacin, florfenicol, oxytetracycline, spectinomycin and tilmicosin against *Mycoplasma mycoides* subspecies *Mycoides* small colony type. *Veterinary Record*, 146(9), 243– 245.

Bauer, D. J., & Condy, J. B. (1981). Isolation and characterisaion of bovine adenoviruses types 2,4 and 8 from free-living African buffaloes (*Syncerus caffer*). *Research in Veterinary Science*, 31(1), 69– 75.

Blanchet, F. G., Cazelles, K., & Gravel, D. (2020). Co-occurrence is not evidence of ecological interactions. *Ecology Letters*, 23, 1050– 1063. https://doi.org/10.1111/ele.13525

Cassirer, E. F., Plowright, R. K., Manlove, K. R., Cross, P. C., Dobson, A. P., Potter, K. A., & Hudson, P. J. (2013). Spatio-temporal dynamics of pneumonia in bighorn sheep. *Journal of Animal Ecology*, 82, 518– 528. https://doi.org/10.1111/1365-2656.12031

Chamberlain, S. A., Bronstein, J. L., & Rudgers, J. A. (2014). How context dependent are species interactions? *Ecology Letters*, 17, 881– 890. https://doi.org/10.1111/ele.12279

Cheng, J., Levina, E., Wang, P., & Zhu, J. (2014). A sparse Ising model with covariates. *Biometrics*, 70, 943– 953. https://doi.org/10.1111/biom.12202

Clark, N. J., Wells, K., & Lindberg, O. (2018). Unravelling changing interspecific interactions across environmental gradients using Markov random fields. *Ecology*. https://doi.org/10.1002/ecy.2221

Coetzer, J. A. W., Thomson, G. R., & Tustin, R. C. (2006). Infectious diseases of livestock – With special reference to Southern Africa. Oxford University Press.

Colwell, R. R. (1996). Global climate and infectious disease: The cholera paradigm. *Science*, 5295(274), 2025– 2031. https://doi.org/10.1126/science.274.5295.2025

Combrink, L., Glidden, C. K., Beechler, B. R., Charleston, B., Koehler, A. V., Sisson, D., Gasser, R. B., Jabbar, A., & Jolles, A. E. (2020). Age of first infection across a range of parasite taxa in a wild mammalian population. *Biology Letters*, 16(2), 20190811. https://doi.org/10.1098/rsbl.2019.0811

Cook, S., Glass, R., LeBaron, C., & Ho, M.-S. (1990). Global seasonality of rotavirus infections. *Bulletin of the World Health Organization*, 68, 171.

Cox, F. (2001). Concomitant infections, parasites and immune responses. *Parasitology*, 122, S23– S38. https://doi.org/10.1017/S003118200001698X

Cozens, D., Sutherland, E., Lauder, M., Taylor, G., Berry, C. C., & Davies, R. L. (2019). Pathogenic *Mannheimia haemolytica* invades differentiated bovine airway epithelial cells. *Infection and Immunity*, 87(6), e00078–19. https://doi.org/10.1128/IAI.00078-19

Dowell, S. F., Whitney, C. G., Wright, C., Rose, C. E. Jr, & Schuchat, A. (2003). Seasonal patterns of invasive pneumococcal disease. *Emerging Infectious Diseases*, 9, 573– 579. https://doi.org/10.3201/eid0905.020556

Earley, B., Buckham Sporer, K., & Gupta, S. (2017). Invited review: Relationship between cattle transport, immunity and respiratory disease. *Animal: an International Journal of Animal BioScience*, 11(3), 486– 492. https://doi.org/10.1017/S1751731116001622

Ezenwa, V. O., Etienne, R. S., Luikhart, G., Beja-Pereira, A., & Jolles, A. E. (2010). Hidden consequences of living in a wormy world: Nematode-induced immune suppression facilitates invasion in the African buffalo. *The American Naturalist*, 176, 613– 624.

Ezenwa, V. O., & Jolles, A. E. (2008). Horns honestly advertise parasite infection in male and female African buffalo. *Animal Behaviour*, 75, 2013– 2021. https://doi.org/10.1016/j.anbehav.2007.12.013

Ezenwa, V. O., & Jolles, A. E. (2015). Opposite effects of anthelmintic treatment on microbial infection at individual versus population scales. *Science*, 347, 175– 177. https://doi.org/10.1126/science.1261714

Ezenwa, V. O., Jolles, A. E., & O'Brien, M. P. (2009). A reliable body condition scoring technique for estimating condition in African buffalo. *African Journal of Ecology*, 47, 476– 481. https://doi.org/10.1111/j.1365-2028.2008.00960.x

Fine, P. E., & Clarkson, J. A. (1982). Measles in England and Wales—I: An analysis of factors underlying seasonal patterns. *International Journal of Epidemiology*, 11, 5– 14. https://doi.org/10.1093/ije/11.1.5

Fleishman, E., Thomson, J., Nally, R., Murphy, D., & Fay, J. (2005). Using Indicator Species to Predict Species Richness of Multiple Taxonomic Groups. *Conservation Biology*, 19(4), 1125– 1137. https://doi.org/10.1111/j.1523-1739.2005.00168.x

Forbes, K. M., Mappes, T., Sironen, T., Strandin, T., Stuart, P., Meri, S., Vapalahti, O., Henttonen, H., & Huitu, O. (2016). Food limitation constrains host immune responses to nematode infections. *Biology Letters*, 12, 20160471. https://doi.org/10.1098/rsbl.2016.0471

Fountain-Jones, N. M., Clark, N. J., Kinsley, A. C., Carstensen, M., Forester, J., Johnson, T. J., Miller, E., Moore, S., Wolf, T. M., & Craft, M. E. (2019). Microbial associations and spatial proximity predict North American moose (*Alces alces*) gastrointestinal community composition. *Journal of Animal Ecology*, 89, 817– 828.

Friedman, J., Hastie, T., & Tibshirani, R. (2010). Regularization paths for generalized linear models via coordinate descent. *Journal of Statistical Software*, 33(1), 1– 22. https://doi.org/10.18637/jss.v033.i01

Germann, T. C., Kadau, K., Longini, I. M., & Macken, C. A. (2006). Mitigation strategies for pandemic influenza in the United States. *Proceedings of the National Academy of Sciences of the United States of America*, 103, 5935– 5940. https://doi.org/10.1073/pnas.0601266103

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. *Frontiers in Immunology*, 8, 1944. https://doi.org/10.3389/fimmu.2017.01944

Glidden, C. K., Coon, C. A. C., Beechler, B. R., McNulty, C., Ezenwa, V. O., & Jolles, A. E. (2020). Data from: Co-infection best predicts respiratory viral infection in a wild host [Dataset]. *Dryad Digital Repository*, https://doi.org/10.5061/dryad.hmgqnk9fj

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. *Proceedings of the National Academy of Sciences of the United States of America*, 115(29), 7545– 7550. https://doi.org/10.1073/pnas.1801095115

Gorsich, E. E., Ezenwa, V. O., Cross, P. C., Bengis, R. G., & Jolles, A. E. (2015). Context-dependent survival, fecundity and predicted population-level consequences of brucellosis in African buffalo. *Journal of Animal Ecology*, 84, 999– 1009. https://doi.org/10.1111/1365-2656.12356

Graham, A. L. (2008). Ecological rules governing helminth–microparasite co-infection. *Proceedings of the National Academy of Sciences of the United States of America*, 105, 566–570. https://doi.org/10.1073/pnas.0707221105

Griffin, D. (1997). Economic impact associated with respiratory disease in beef cattle. *Veterinary Clinics of North America: Food Animal Practice*, 7(13), 367– 377.

Griffiths, E. C., Pedersen, A. B., Fenton, A., & Petchey, O. L. (2011). The nature and consequences of co-infection in humans. *Journal of Infection*, 63, 200– 206. https://doi.org/10.1016/j.jinf.2011.06.005

Hall, A. J., Jepson, P. D., Goodman, S. J., & Härkönen, T. (2006). Phocine distemper virus in the North and European Seas-Data and models, nature and nurture. *Biological Conservation*, 131, 221– 229.

Hurvich, C. M., & Tsai, C.-L. (1989). Regression and time series model selection in small samples. *Biometrika*, 76, 297– 307.

Jolles, A. E., Beechler, B. R., & Dolan, B. P. (2015). Beyond mice and men: Environmental change, immunity and infections in wild ungulates. *Parasite Immunology*, 37, 255– 266.

Jolles, A. E., Cooper, D. V., & Levin, S. A. (2005). Hidden effects of chronic tuberulosis in African buffalo. *Ecology*, 86, 2358– 2364.

Keeling, M. J., & Rohani, P. (2008). Modeling infectious diseases in humans and animals. Princeton University Press.

Kiguchi, R., & Minami, M. (2012). Cyclic cubic regression spline smoothing and analysis of CO_2 data at Showa Station in Antarctica. In Proceedings of International Biometric Conference 2012, 40pp. Retrieved from https://higherlogicdownload.s3.amazonaws.com/BIOMETRICSOCIETY/713ac962-588b-42d5-940f-47ae32f0b28c/UploadedImages/Documents/Past_Events/2012_IBC/IBC2012-Scientific-Programme.pdf

Lee, J. D., & Hastie, T. J. (2015). Learning the structure of mixed graphical models. *Journal of Computational and Graphical Statistics*, 24, 230– 253.

Lillie, L. E. (1974). The bovine respiratory disease complex. *The Canadian Veterinary Journal*, 15, 233– 242.

Lowen, A. C., Mubareka, S., Steel, J., & Palese, P. (2007). Influenza virus transmission is dependent on relative humidity and temperature. *PLoS Pathgens*, 3, e151.

Maclachlan, N. J., & Dubovi, E. J. (2010). Fenner's veterinary virology. Academic Press.

Maunsell, F., Woolums, A., Francoz, D., Rosenbusch, R., Step, D., Wilson, D. J., & Janzen, E. (2011). *Mycoplasma bovis* infections in cattle. *Journal of Veterinary Internal Medicine*, 25(4), 772– 783. https://doi.org/10.1111/j.1939-1676.2011.0750.x

McAuliffe, L., Ellis, R. J., Miles, K., Ayling, R. D., & Nicholas, R. A. J. (2006). Biofilm formation by mycoplasma species and its role in environmental persistence and survival. *Microbiology*, 152(4), 913– 922. https://doi.org/10.1099/mic.0.28604-0

Michel, A. L., Cooper, D., Jooste, J., De Klerk, L.-M., & Jolles, A. (2011). Approaches towards optimising the gamma interferon assay for diagnosing *Mycobacterium bovis* infection in African buffalo (*Syncerus caffer*). *Preventive Veterinary Medicine*, 98, 142– 151. https://doi.org/10.1016/j.prevetmed.2010.10.016

Onozuka, D., Hashizume, M., & Hagihara, A. (2009). Impact of weather factors on *Mycoplasma pneumoniae* pneumonia. *Thorax*, 64, 507– 511. https://doi.org/10.1136/thx.2008.111237

Pazso, M., Sperling, R. S., Moran, T. M., & Kraus, T. A. (2012). The influence of pregnancy on systemic immunity. *Immunology Research*, 54, 254– 261.

Pedersen, A. B., & Fenton, A. (2007). Emphasizing the ecology in parasite community ecology. *Trends in Ecology & Evolution*, 22, 133– 139. https://doi.org/10.1016/j.tree.2006.11.005

Rice, J., Carrasco-Medina, L., Hodgins, D., & Shewen, P. (2007). Mannheimia haemolytica and bovine respiratory disease. *Animal Health Research Reviews*, 8, 117– 128.

Rodwell, T. C., Whyte, I. J., & Boyce, W. M. (2001). Evaluation of population effects of bovine tuberculosis in free-ranging African buffalo (*Syncerus caffer*). *Journal of Mammalogy*, 82, 231– 238.

SANPARKS. (2010–2011). Kruger National Park Biodiversity Statistics. Retrieved from https://www.sanparks.org/parks/kruger/conservation/scientific/ff/biodiversity_statistics.php

Schiller, I., Waters, W. R., Vordermeier, H. M., Nonnecke, B., Welsh, M., Keck, N., Whelan, A., Sigafoose, T., Stamm, C., Palmer, M., Thacker, T., Hardegger, R., Marg-Haufe, B., Raeber, A., & Oesch, B. (2009). Optimization of a whole-blood gamma interferon assay for detection of *Mycobacterium bovis*-infected cattle. *Clinical and Vaccine Immunology*, 16, 1196– 1202. https://doi.org/10.1128/CVI.00150-09

Smith, B. P., Van Metre, D. C., & Pustrela, N. (2019). Large animal internal medicine (6th ed.). Elsevier.

Spaan, R. S., Epps, C. W., Ezenwa, V. O., & Jolles, A. E. (2019). Why did the buffalo cross the park? Resource shortages, but not infections, drive dispersal in female African buffalo (*Syncerus caffe*r). *Ecology & Evolution*, 9, 5651– 5663. https://doi.org/10.1002/ece3.5145

Spann, J. (2018). Stress physiology in free-ranging female African buffalo (*Syncerus caffer*): Environmental drivers, and immunological and infection consequences (PhD dissertation). Oregon State University.

Speakman, J. R. (2007). Physiological costs of reproduction in small mammals. *Philosophical of Transactions of the Royal Society B*, 363, 375– 398.

Srikumaran, S., Kelling, C. L., & Ambagala, A. (2007). Immune evasion by pathogens of bovine respiratory disease complex. *Animal Health Research Reviews*, 8, 215– 229. https://doi.org/10.1017/S1466252307001326

Susi, H., Barrès, B., Vale, P. F., & Laine, A.-L. (2015). Co-infection alters population dynamics of infectious disease. *Nature Communications*, 6. https://doi.org/10.1038/ncomms6975

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. https://doi.org/10.1126/science.1190333

Valarcher, J.-F., Bourhy, H., Lavenu, A., Bourges-Abella, N., Roth, M., Andreoletti, O., Ave, P., & Schelcher, F. (2001). Persistent infection of B lymphocytes by bovine respiratory syncytial virus. *Virology*, 291, 55– 67. https://doi.org/10.1006/viro.2001.1083

Van der Poel, W., Brand, A., Kramps, J., & Van Oirschot, J. (1994). Respiratory syncytial virus infections in human beings and in cattle. *Journal of Infection*, 29, 215– 228. https://doi.org/10.1016/S0163-4453(94)90866-4

Van Vuuren, M. (1994). Bovine respiratory syncytial virus infection. In J. A. W. Coetzer (Ed.), Infectious diseases of livestock (pp. 769– 772). Oxford University Press. Retrieved form https://www.amazon.com/Infectious-Diseases-Livestock-3-Set/dp/019578202X

Venter, F., & Gertenbach, W. (1986). A cursory review of the climate and vegetation of the Kruger National Park. *Koedoe*, 29, 139– 148. https://doi.org/10.4102/koedoe.v29i1.526

Wood, P., & Jones, S. (2001). BOVIGAM TM: An in vitro cellular diagnostic test for bovine tuberculosis. *Tuberculosis (Edinb)*, 81, 147– 155. https://doi.org/10.1054/tube.2000.0272

Wood, S. N. (2011). Fast stable restricted maximum likelihood and marginal likelihood estimation of semiparametric generalized linear models. *Journal of the Royal Statistical Society: Series B (Statistical Methodology)*, 73(1), 3– 36. https://doi.org/10.1111/j.1467-9868.2010.00749.x

Wood, S. (2017). Generalized additive models. Chapman and Hall/CRC. https://doi.org/10.1201/9781315370279

Wright, A. N., & Gompper, M. E. (2005). Altered parasite assemblages in raccoons in response to manipulated resource availability. *Oecologia*, 144, 148– 156. https://doi.org/10.1007/s00442-005-0018-3

Yan, C., Luo, Z., Li, W., Dallman, R., Kurihara, H., Li, Y.-F., & He, R.-R. (2020). Disturbed Yin-Yang balance: Stress increases the susceptibility to primary and recurrent infections of herpes simplex virus type 1. *Acta Pharmeceutica Sinica B*, 10(3), 383– 398.

Zambatis, N. (2003). Determinants of grass production and composition in the Kruger National Park (MSc (Agric.) dissertation). University of Natal.