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Cite this article: Beechler BR, Manore CA, Reininghaus B, O'Neal D, Gorsich EE, Ezenwa VO, Jolles AE. 2015 Enemies and turncoats: bovine tuberculosis exposes pathogenic potential of Rift Valley fever virus in a common host, African buffalo (*Syncerus caffer*). *Proc. R. Soc. B* **282**: 20142942.
http://dx.doi.org/10.1098/rspb.2014.2942

Received: 1 December 2014
Accepted: 20 February 2015

Subject Areas:
ecology, health and disease and epidemiology, immunology

Keywords:
emerging infectious disease, co-infection, parasite interactions, invasive species, disease dynamics, disease ecology

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Electronic supplementary material is available at http://dx.doi.org/10.1098/rspb.2014.2942 or via http://rspb.royalsocietypublishing.org.

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Enemies and turncoats: bovine tuberculosis exposes pathogenic potential of Rift Valley fever virus in a common host, African buffalo (*Syncerus caffer*)

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The ubiquity and importance of parasite co-infections in populations of free-living animals is beginning to be recognized, but few studies have demonstrated differential fitness effects of single infection versus co-infection in free-living populations. We investigated interactions between the emerging bacterial disease bovine tuberculosis (BTB) and the previously existing viral disease Rift Valley fever (RVF) in a competent reservoir host, African buffalo, combining data from a natural outbreak of RVF in captive buffalo at a buffalo breeding facility in 2008 with data collected from a neighbouring free-living herd of African buffalo in Kruger National Park. RVF infection was twice as likely in individual BTB+ buffalo as in BTB− buffalo, which, according to a mathematical model, may increase RVF outbreak size at the population level. In addition, co-infection was associated with a far higher rate of fetal abortion than other infection states. Immune interactions between BTB and RVF may underlie both of these interactions, since animals with BTB had decreased innate immunity and increased pro-inflammatory immune responses. This study is one of the first to demonstrate how the consequences of emerging infections extend beyond direct effects on host health, potentially altering the dynamics and fitness effects of infectious diseases that had previously existed in the ecosystem on free-ranging wildlife populations.

1. Introduction

Anthropogenic changes to the environment—such as shifts in biotic assemblages, altered climate patterns and reduced environmental predictability—have led to alterations in disease patterns worldwide [1,2]. These altered patterns include the emergence of new pathogens and parasites, often via spillover from one species to another, and pre-existing pathogens and parasites increasing in geographical range [1]. These changing infection patterns can cause cascading effects through the host population, such as population declines [3] due to increased mortality (as seen in rinderpest outbreaks in sub-Saharan Africa) [4] or decreased fecundity (as experienced by koala bears infected with chlamydia) [5]. Not only do infectious diseases have direct effects on host populations, but they may also alter the spread and fitness effects of other pathogens within the host population due to mechanisms such as change in susceptibility to infection, increased mortality [6] or decreased fecundity [7] of co-infected individuals, thereby altering established host–parasite dynamics.

Recent literature has shown that a native pathogen community may alter the success of an invading infectious disease [8,9]. For instance, European eels with higher micro-parasite and macro-parasite richness were more likely to be

infected by the invading parasite *Anguillicoloides crassus* [10]. A two-parasite disease model showed that native nematodes might facilitate the invasion of bovine tuberculosis (BTB) in African buffalo [11]. However, very little work has investigated how the presence of an emerging pathogen may alter the dynamics of previously existing native infections. For the purposes of this paper, we define an emerging disease as the World Health Organization does, 'a disease that has appeared in the population for the first time or that might have previously existed but is rapidly increasing in incidence or geographical range'. We use the term native disease to mean a disease that existed in the ecosystem and host species prior to the emerging disease.

Emerging and native parasites can interact via the host immune system [12]. An emerging pathogen may erode host defences against native infections, increasing transmission risk of the native infection in infected individuals [13]. Alternatively, the emerging infection may remove susceptible animals from the pool by cross-protective immune response [12], changes in host behaviour [14] or mortality [6], reducing the transmission opportunities for native infections. If the immune response mounted to one parasite is cross-protective to another, then infection with one parasite can prevent the other from establishing. By contrast, immune responses may be mutually antagonistic [12,15]. An immune response to one type of parasite may allow infection of another by preventing an appropriate immune response [16,17], creating a facilitative effect.

We studied an outbreak of Rift Valley fever (RVF), a native pathogen, in African buffalo (*Syncerus caffer*) infected with *Mycobacterium bovis* (causal agent of BTB), which is an emerging disease in the area of study, Kruger National Park (KNP). We investigated whether animals with BTB have differential risk of acquiring RVF, and compared the fitness effects of co-infection with single infections. We hypothesized that interactions between *M. bovis* and RVF virus may be mediated via their effects on, and responses to, host immunity.

BTB is not native to sub-Saharan Africa and is considered to be an emerging infection in African wildlife [18]. BTB emerged into the landscape in either the 1960s [19] or 1980s [20] and was first detected in the 1990s [21] in KNP. Since that time BTB has been spreading northward in the park, with prevalence increasing over time throughout the park [22] and just recently crossing the northern boundary of the park, into Zimbabwe [23]. BTB in African buffalo is an excellent system to study immune-mediated interactions between parasites because BTB has moderate effects on the survival of African buffalo [22,24], but modifies the host immune system to ensure its survival within the host for the lifetime of the buffalo [25,26]. For instance, there is evidence that cattle with BTB have a suppressed innate immune response [27]. In addition to an altered innate immune response, BTB affects the cell-mediated acquired immune system, with an increase in inflammatory cytokines (Th1 skew) that is linked to increased pathology associated with BTB infection [28].

RVF is a mosquito-transmitted intracellular viral disease with numerous mammalian hosts, including African buffalo. RVF is considered native to South Africa, having existed in the ecosystem prior to BTB and been identified as a spillover infection from animals to people in 1952 [29]. Outbreaks are known to occur in domestic animals every 5–7 years during the wet season [29], but the virus may cycle undetected in wildlife populations during the interepidemic period [30,31]. It has mild effects on African buffalo, primarily causing a short-term illness that passes within 2–3 days—much like a seasonal cold in humans—with severe effects primarily limited to occasional abortion [29]. The ability of hosts to resist RVF infection is dependent on a strong innate immune response [32]. Since BTB can suppress the innate immune response, we hypothesize that animals previously infected with BTB may be more susceptible to infection with RVF.

We investigated the role of BTB in a natural RVF outbreak in a captive population of African buffalo at a breeding facility, and an adjacent free-ranging population in KNP. We analysed data from the captive population to determine whether animals with BTB were more or less likely to become infected with RVF during the outbreak and to suffer fitness consequences in the form of abortions. We then tested whether patterns found in the captive population were mirrored in the free-ranging buffalo population. To investigate potential mechanisms mediating epidemiological patterns, we investigated whether buffalo with BTB have an altered immune response that may affect the likelihood of acquiring RVF or suffering abortion during an outbreak. Finally, we used a mathematical model to determine how observed changes in individual susceptibility could scale up to alter population-level patterns of RVF transmission.

2. Material and methods

(a) Co-infection patterns

(i) Rift Valley fever outbreak in the captive population

In 2008, an outbreak of RVF occurred in and around KNP [33]. We collected data on the captive animals from a buffalo breeding facility on the southern boundary of KNP, the Nkomazi area, on RVF infection prior to, during and after outbreak. During the year prior to the outbreak (2007), the buffalo breeding facility was free of RVF. The outbreak, first noted in the facility on 14 January 2008, was contained by the end of February when the entire herd was vaccinated for RVF. Prior to vaccination, but after the outbreak, blood was collected from each individual and was serologically tested for RVF using a haemagglutination-inhibition (HAI) titration assay at Onderstepoort Veterinary Institute in Pretoria, South Africa [30,34]. The breeding facility had both BTB+ buffalo and BTB− buffalo, but animals were known to be brucellosis free, were on a deworming schedule to prevent gastrointestinal helminth infection and were regularly treated with antiparasitic dips to reduce ticks and tick-borne infections. Animals were assigned BTB status based on the results of multiple caudal fold skin tests prior to the outbreak; all had been tested at least once in the prior year. This assay is described in the OIE terrestrial manual (2012) and has been used in African buffalo [6,35]. Briefly, animals are intradermally injected with bovine tuberculin and the swelling response is measured 72 h later, with a swelling response greater than 2 mm considered positive. BTB− buffalo were certified disease-free based on the results of two prior BTB tests. The sensitivity and specificity of caudal fold skin BTB tests are, respectively, 80–91% and 95–100% in cattle [36–38], and 80.9% and 90.2% in African buffalo (J. P. Raath 2005, unpublished data). BTB+ and BTB− buffalo were maintained in separate, but similar bomas (enclosures approx. 0.25 km apart), and had no direct contact with one another. While these bomas did not allow direct contact, they were close enough for infected vectors to fly from one to the other—although whether they did is not a variable we assessed.

To determine the cause of mortality in the juvenile and adult buffalo during the outbreak state, veterinarians performed full necropsies and noted the presence of lesions concordant with

RVF infection [29]. Infection was confirmed with immunohistochemical staining [39]. Aborted fetuses were also collected, and necropsies and immunohistochemistry were once again used for confirmation of RVF infection. Additional RVF confirmatory tests on fetuses were performed using RT-PCR [40] of fetal blood samples. All immunohistochemistry and PCR analyses were conducted at the Onderstepoort Veterinary Institute.

To assess whether abortion rates were different in co-infected and singly infected individuals, we first determined what proportion of individuals should have been pregnant on the buffalo breeding facility prior to the outbreak. Previous non-outbreak years' pregnancy and birthing data were used to determine an interbirth interval on the buffalo breeding facility of 462 days (from 1999 to 2007, $n = 756$) and an average pregnancy rate of 73% in adult female cows, which did not differ between BTB+ and BTB− buffalo. When calculating abortion rates in the captive population, we used a denominator of 73% of the total reproductive females. We then assessed whether abortion rates were different between the four disease groups (co-infected, single RVF infection, single BTB infection, uninfected) using a non-parametric Kruskal–Wallis ANOVA with Dunn multiple comparisons.

(ii) Rift Valley fever outbreak in the free-ranging population

To evaluate whether BTB/RVF co-infection patterns found in the buffalo breeding facility were mirrored in a free-living population, we sampled 96 free-living young female buffalo in the southern portion of KNP (where BTB prevalence is approx. 50% [24,41]) near the buffalo breeding facility in October 2008 (approx. seven months after the outbreak of RVF) as part of a larger disease study [24]. Animals were chemically immobilized with etorphine hydrochloride, azaperone and ketamine by darting from a helicopter. After immobilization, age, body condition and pregnancy status were determined. Animal ages were assessed from incisor emergence patterns for buffalo 2–5 years old and from tooth wear of the first incisor for buffalo 6 years of age and older [42]. Body condition was measured by visually inspecting and palpating four areas on the animal where fat is stored in buffalo: ribs, spine, hips and base of tail. Each area was scored from 1 (very poor) to 5 (excellent) and a body condition score calculated as the average of all four areas [20]. This index is correlated with the kidney fat index [43]. Pregnancy status was assessed by rectal palpation [6,42,44], performed by an experience wildlife veterinarian. Blood was collected by jugular venipuncture into lithium heparinized tubes (for BTB diagnostics) and tubes with no additive (RVF diagnostics), and transported back the laboratory on ice within 8 h of collection. Faeces was collected rectally and transported back to the laboratory on ice for faecal egg counts of strongyle nematodes and coccidia (for specific methods, see [44]). Following data collection, immobilization was reversed using M5050 (diprenorphine). Animals were chemically restrained for no longer than 60 min. Time of capture and duration of anaesthesia were initially included in all statistical models but were never found to be important predictors.

We determined RVF serostatus with the virus neutralization test, which has a sensitivity and specificity of nearly 100% [45] and can be used to look for antibodies in serum. Tuberculosis infection status was determined using a standard whole blood gamma interferon assay protocol (BOVIGAM) [46]. In brief, this assay is performed by comparing the *in vitro* IFNγ response to *M. bovis* antigen (bovine tuberculin) with the IFNγ response to an avian tuberculin antigen and background IFNγ levels in the absence of antigenic stimulation. This assay has been optimized for use in African buffalo [47], and blood cells from buffalo infected with *M. bovis* show a pronounced spike in IFNγ production in response to bovine but not avian tuberculin, whereas bovine tuberculin challenge does not induce IFNγ production in the blood of unexposed animals [47]. We implemented the gamma interferon assay with the BOVIGAM enzyme-linked immunosorbent assay kit (Prionics), which has a sensitivity of 86% and a specificity of 92% in African buffalo [47]. We used the BOVIGAM test instead of the skin test used at the buffalo breeding facility because the skin test was impractical in our field setting; the skin test requires two captures in 3 days, whereas the BOVIGAM test can be performed on whole blood collected in one capture.

We performed a Fisher exact test to determine whether animals with BTB were more likely to be seropositive for RVF than their BTB counterparts in the free-ranging population. The majority of these RVF+ animals probably converted in the 2008 outbreak: most animals were between 2 and 5 years old, whereas the most recent identified RVF outbreak in the area, prior to 2008, occurred in 1999, before these animals were born. We calculated an RVF prevalence ratio with and without BTB (i.e. prevalence ratio = prevalence in BTB+ buffalo/prevalence in BTB− buffalo). To further evaluate the correlation between BTB and RVF, we performed a generalized linear model with binomial distribution to evaluate whether BTB status predicted RVF status, after accounting for buffalo age, body condition, pregnancy, faecal egg count of GI nematodes and coccidia in the free-ranging population. We were unable to assess whether co-infected animals in the free-ranging population were more likely to abort than singly infected individuals, as we demonstrated in the population at the buffalo breeding facility, for two reasons. First, the population of buffalo sampled was primarily pre-reproductive (less than 4 years of age), and second, sampling did not exactly coincide with the RVF outbreak, and it is likely that any animal that did abort due to RVF during the outbreak was pregnant again at the time our sampling took place (approx. seven months later).

(b) Immune mechanisms

The 96 free-living individuals described in the methods above were followed subsequently for 4 years. Each individual was marked with a radio-collar and recaptured every six months (2008–2012). Any animal that died during the study period was replaced by a similarly aged animal to maintain a constant sample size of approximately 100 individuals at each recapture. At each capture period, the same data were collected, including age, body condition and BTB status, as described above. We also collected information on a pro-inflammatory cytokine (IL12) and general innate immune capability as measured by the bactericidal assay on subsets of these animals, as described below.

(i) Bactericidal assay

We performed the bactericidal assay as a measure of innate immune capability. The assay measures the proportion of bacteria (*E. coli*, in this case) killed by whole blood during a 30 min period of interaction between blood and bacterial broth. Killing mechanisms include protein-mediated killing (e.g. complement, acute-phase proteins) and cell-mediated killing (e.g. phagocytosis by macrophages, neutrophils). This assay was performed as described in [44], with replicate plates between July 2010 and July 2011, for 97 individual buffalo, some of which were the same individuals as reported above for the RVF outbreak in the free-living population ($n = 34$) and some of which were added to the study after the outbreak ($n = 63$). Briefly, for experimental tubes whole blood and bacteria were mixed together and incubated for 30 min. For control tubes, the same quantity of bacteria and phosphate-buffered saline (PBS) were mixed. After 30 min, the mixture was plated onto agar and the bacteria allowed to grow at 37°C for 12 h. After 12 h, the number of bacteria colonies on each plate was counted. The number of colonies killed by whole blood was determined by subtracting the number of colonies on the experimental plate from the control plate. This was used as the independent variable in statistical analyses, and we account for day-to-day variation in

Table 1. Age and sex patterns of RVF seroconversion of captive buffalo during a natural outbreak in 2008.

	number seroconverted	total number tested	percentage seroconverted
adult cows	40	124	32.26
adult bulls	3	19	15.79
calves under 1 year	21	26	80.77

growth by including the number of colonies on the control plates as an offset term in all statistical models [44]. A generalized linear model (quasi-poisson distribution, log link) was used to determine if the number of colonies killed by whole blood differed by BTB status, body condition, age or any two-way interaction effects.

(ii) IL12 assay

Cytokines are immunologically active proteins that aid in cell signalling during a host immune response and have been proposed as an excellent way to simplistically and realistically describe the immune profiles for the purpose of understanding within-host–parasite interactions [48]. IL12 is known to be important in immune defence against viruses and is a key pro-inflammatory cytokine [49]. We assessed IL12 production in whole blood in response to *in vitro* stimulation with two mitogens, pokeweed and live RVF virus. Pokeweed is a general immune stimulant that is often used to induce cytokine and cell proliferation; the strain of RVF we used was a modified live strain used in vaccines (Smithburn strain). After return from the field, whole blood in lithium heparinized tubes was pipetted into 1.5 ml aliquots. Into each aliquot, we added 50 µl of mitogen (30 000 live RVF virus units from Onderstepoort Biologicals or 15 µg of pokeweed; Sigma L9379, rehydrated in PBS) into experimental tubes and 50 µl of PBS into control tubes. Whole blood and mitogen (PBS for controls) were incubated at 37°C for 24 h. After 24 h, the plasma was pipetted off the top of the tube, placed in cryovials and stored at −20°C until analysis. The quantity of IL12 in each sample was measured using a sandwich ELISA following established protocols [50] with a commercially available antibody designed for bovines (Abd Serotec, #MCA1782EL & MCA2173B) and recombinant bovine IL12 for the standard curve (Kingfisher, RP0077B). All samples were performed in duplicate on a 96-well plate and the mean optical density was calculated for each set of duplicate wells at a wavelength of 405 nm. The mean OD was calculated for each set of duplicate wells with an average variation between wells of 5.76%. Sample concentrations were calculated using a linear standard curve and are expressed as pg ml^{-1}. The difference in IL12 detected between control and experimental tubes was used as the dependent variable in statistical analyses.

To assess whether animals with BTB differed from those without BTB in IL12 production after stimulation with the non-specific mitogen (pokeweed), we performed a generalized linear mixed model (Gaussian family, log link, dependent variable was log of the difference between IL12 in the stimulated samples and IL12 in the non-stimulated sample) on 118 individual buffalo captured between June 2008 and August 2010 that we had repeated IL12 measurements on for a total of 419 IL12 data points. The random effects in the model were the number of plates the sample was run on and buffalo individual (to avoid pseudo-replication of repeated measures on the same individual). We evaluated the fixed effects, including all two-way interactions of age, year of capture, BTB status, animal body condition and the amount of IL12 already in the blood before stimulation (circulating IL12). We found no significant two-way interactions and so presented the main effects model.

We then evaluated IL12 production in response to RVF virus (Smithburn strain) in a subset of 27 animals captured in September/October 2011. We calculated a proportional change in IL12 production ((IL12 in tubes with mitogen-IL12 in control tubes)/IL12 in control tubes) and assessed whether BTB+ individuals also had higher IL12 response to RVF than BTB− individuals using a two-tailed *t*-test on arcsine-square-root-transformed data.

(c) Mathematical model

We can infer, from the collected data, an individual buffalo's differential risk for contracting RVF during the outbreak based on their BTB status. However, we hypothesize that the presence of BTB increases the risk of RVF infection not only in BTB+ buffalo but in the whole herd, and that the presence of BTB could change the dynamics of RVF at the population level. To test this hypothesis, we modified a mathematical model of RVF transmission in free-living buffalo [51] to explore how the altered risk of RVF infection in BTB-infected individuals may change epidemic dynamics of RVF (see electronic supplementary material, appendix 1 for details). We tested sensitivity of the model output to the proportion of the herd infected with BTB and to the magnitude of change in susceptibility to RVF for BTB + buffalo.

We altered the model to account for BTB presence by dividing the herd into BTB+ and BTB− groups, changing one parameter in the model to account for increased susceptibility of BTB+ buffalo to RVF infection via infected mosquito bite. Since we have no evidence for a difference in buffalo-to-mosquito transmission probability, we leave the probability of a susceptible mosquito acquiring the virus after biting an infectious buffalo unchanged. The available data are for a single RVF outbreak, so we modelled RVF spread in one rainy season, and did not explicitly include BTB transmission dynamics or buffalo population dynamics in the model. This simple and interpretable model provides a framework with which to assess the population-level effects of BTB on a single RVF outbreak in the herd.

3. Results

(a) Descriptive statistics of Rift Valley fever outbreak in captive and free-living buffalo

Two hundred and thirty-five captive buffalo were tested for RVF before and during the 2008 outbreak. Of these, 60 were calves under 1 year of age, 156 were adult cows and the remaining 19 were adult bulls. There were 82 new cases of RVF recorded during the 2008 outbreak at the breeding facility (i.e. a seroconversion rate of 34.9%; table 1). Our sample of free-living buffalo consisted of 96 female buffalo between the ages of 2 and 14. We measured a RVF seroprevalence rate of 39.6% (38/96) in the free-ranging population. Of the 38 RVF+ buffalo, only five were born prior to the previous outbreak of RVF recorded in the area, in 1999.

Clinical signs associated with RVF infection were noted during the outbreak in the captive population. One adult female buffalo and one young calf died from RVF. Eight

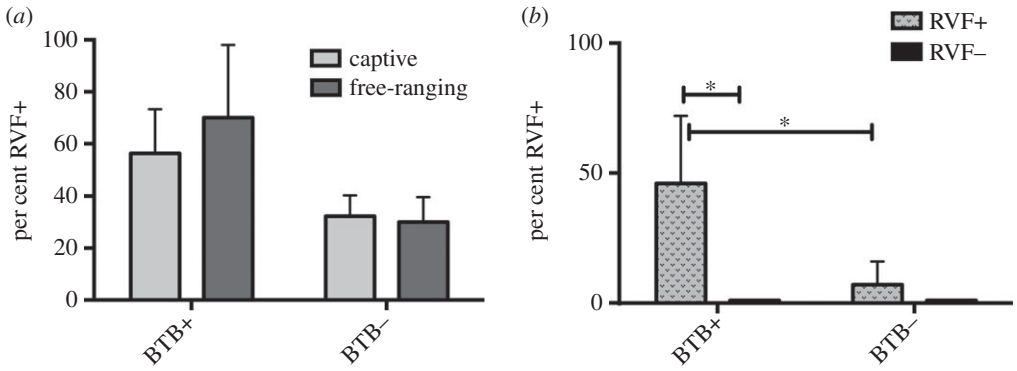

Figure 1. Effect of BTB on (*a*) RVF incidence and (*b*) abortion. (*a*) BTB+ buffalo were more likely to acquire RVF infection (Fisher exact test, $p = 0.0147$) during an outbreak in the captive herd (light grey) and are more likely to be seropositive (Fisher exact test, $p = 0.03$) in a free-ranging herd (dark grey). (*b*) Animals with BTB were more likely to abort from RVF than those without BTB. No animals without RVF aborted; a line was placed just above 0 on the *y*-axis for visibility. Stars represent significant differences on a Kruskal–Wallis ANOVA with Dunn pairwise comparisons.

female buffalo aborted (gestation period of buffalo is 11 months): two individuals aborted 10-month-old fetuses, three aborted 4–5-month-old fetuses, one a 3–4-month-old fetus, and the age of the fetus was not recorded for the other two abortions.

(b) Co-infection patterns

In the captive population, individual BTB+ buffalo had a relative risk of acquiring RVF that was 1.744 (CI 1.171–2.596) times higher than their BTB− counterparts. Whereas 56.25% ($n = 124$) of the BTB+ adult female buffalo seroconverted during a natural outbreak in a buffalo breeding facility, only 32.26% ($n = 86$) of the BTB− adult female buffalo seroconverted (figure 1*a*). In the free-ranging population, BTB+ buffalo ($n = 10$) had a relative risk of being seropositive for RVF that was 2.326 (CI 0.89–6.056) times higher than their BTB− counterparts ($n = 32$; Fisher exact test, $p = 0.03$) (figure 1*a*). Neither age, body condition nor GI parasite egg counts correlated with RVF serostatus, nor altered the direction and magnitude of the correlation between RVF serostatus and BTB infection (table 2).

In the captive population, buffalo with BTB were more likely to abort due to RVF than those without BTB ($K = 50.36$, $p < 0.00001$; figure 1*b*; pairwise comparisons: co-infected versus RVF only $p < 0.001$, co-infected versus BTB only $p < 0.001$, co-infected versus uninfected $p < 0.001$, no other significant pairwise differences), while buffalo without RVF did not suffer any abortions. While 7% (2/29) of the pregnant buffalo infected with only RVF aborted, 46% (6/14) of the co-infected animals aborted, so the relative risk of abortion was 6.57 times greater in co-infected individuals than those infected only with RVF. No buffalo infected with only BTB aborted. In previous years, there was no difference between abortion rates in the BTB+ and BTB− individuals (B. Reininghaus 2009, unpublished data).

(c) Immune mechanisms

Animals with BTB had significantly lower bactericidal ability of whole blood, a proxy for innate immune function, compared with those without BTB (figure 2*a*; est = −0.52, s.e. = 0.24, $p = 0.03$). This difference was robust to accounting for animal body condition (GLM, est = −0.41, s.e. = 0.2, $p = 0.4$) and age (est = 0.02, s.e. = 0.04, $p = 0.49$). We also investigated whether there was any evidence that buffalo infected with BTB had altered immune profiles that could worsen the fitness consequences of RVF infection. Buffalo with BTB mounted stronger IL12 responses to an *in vitro* stimulus with a non-specific mitogen (pokeweed) than those without BTB (figure 2*b* and table 3) and a marginally stronger IL12 response to *in vitro* challenge with RVF live viral particles (figure 2*b*; two-tailed *t*-test, $t = 1.54$, $p = 0.14$; table 3).

Table 2. A generalized linear model (binomial distribution, log link, d.f. = 92) was performed to further evaluate the correlation between BTB status and RVF seropositivity in free-ranging African buffalo. Age, pregnancy status, overall body condition, faecal nematodes and coccida did not alter the positive association between BTB and RVF. Asterisk indicates statistically significant *p*-value at a level of $p < 0.05$.

	estimate	s.e.	*p*-value
age	0.20	0.12	0.105
BTB status (+)	1.51	0.76	0.046*
pregnancy status (yes)	−0.21	0.77	0.785
body condition	0.11	0.37	0.768
nematodes eggs per gram	0.001	0.001	0.429
coccidia oocysts per gram	−0.001	0.004	0.660

(d) Mathematical model

We used a mathematical model to determine whether these individual changes in the likelihood of acquiring RVF could affect RVF epidemics at the herd level. We varied two key parameters: the additional RVF transmission factor for mosquitoes to BTB+ buffalo and the prevalence of BTB in the herd. We varied the increased risk of RVF infection for BTB+ animals, χ_{TB}, from 1.0 to 4.4. An increase in transmission from infected mosquitoes to BTB+ buffalo of $\chi_{TB} = 3.4$ best represented the approximately two times greater RVF prevalence in BTB+ buffalo observed in our free-ranging and captive populations. This value depends on the assumed BTB prevalence and whether there is immunity to RVF from previous exposure. We varied BTB prevalence, $\varnothing_{TB}$, from 0 to 1.

In agreement with the outbreak data, the model predicts higher RVF seroprevalence in BTB+ buffalo than in BTB− at the end of the outbreak. However, we found that increasing

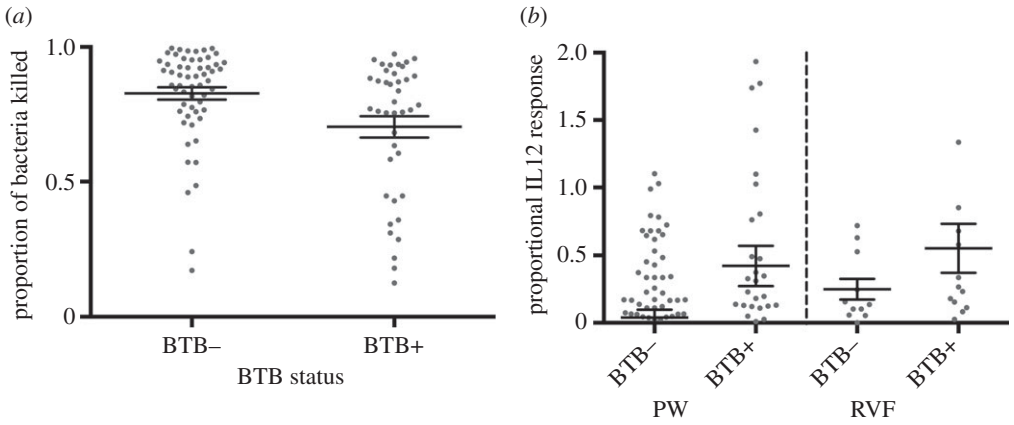

Figure 2. Immunologic effects of BTB. Animals with BTB had (*a*) reduced bactericidal ability of whole blood and (*b*) increased IL12 response. Each point is an individual animal's proportion of bacteria killed (*a*) or IL12 response to mitogen (*b*) with the mean and s.e.m. represented by the line and error bars respectively.

Table 3. Animals with BTB had stronger IL12 response to pokeweed even after accounting for animal body condition, year of capture and baseline IL12. The table contains estimates, s.e. and *p*-values for the model parameters in a generalized linear model (Gaussian family, log link) with formula log IL12 difference∼IL12 plate + IL12 base circulating level + animal body condition + BTB status, with animal ID and IL12 plate number as random effects. Asterisks indicate statistically significant *p*-values at a level of $p < 0.05$.

	estimate	s.e.	*p*-value
circulating IL12	−0.003	0.0002	<0.01*
capture year	−0.001	0.001	0.56
animal body condition	−0.0003	0.0008	0.72
BTB status (+)	0.002	0.008	0.04*

BTB prevalence within a herd increased both the overall magnitude of an RVF outbreak and the RVF seroprevalence in BTB− individuals (figure 3). This implies that the presence of BTB increases RVF infection risk for all members of the herd, not just those infected with BTB. Outbreak size responded nonlinearly to increased BTB prevalence at a fixed transmission factor, with outbreak size increasing more rapidly as BTB prevalence increased (figure 3). The relative effect of BTB prevalence and the transmission factor on RVF dynamics (time to peak and length of outbreak) varied across the parameter ranges explored (figure 4).

4. Discussion

Our results suggest that an emerging pathogen, such as BTB, may have not only direct effects on the host, but also indirect effects by altering the infection patterns of diseases previously existing within the host population. Buffalo in both the free-ranging and captive populations were approximately twice as likely to acquire RVF when previously infected with BTB, providing strong evidence that BTB affects host susceptibility to other pathogens. Because these patterns were duplicated in two independent populations, we investigated possible mechanisms behind the correlations.

BTB in cattle causes dynamic alterations to the host immune response over time [52], whereby animals may have reduced ability to mount immune responses to protect them from microparasites such as RVF [53]. Pirson *et al.* [54] suggested that receptors and function of antigen-presenting cells were suppressed in BTB infection, which would decrease the host's ability to respond to an insult from a pathogen. Concordant with these findings, we saw that animals with BTB had suppressed innate immune responses, as measured by the bactericidal ability of whole blood. This reduced ability to respond to a pathogen with a strong innate response may increase the likelihood that animals with BTB become infected with other pathogens that require suppression by the innate immune system, such as most acute viral pathogens.

We used a mathematical model to show that changes in host susceptibility to RVF due to BTB infection in individual buffalo could increase the intensity of RVF epidemics in the entire herd. As the prevalence of BTB increased, the size of RVF epidemics in buffalo increased, with more disease occurring in both BTB+ and BTB− buffalo. The response of RVF outbreak size to BTB prevalence was nonlinear, with outbreak size increasing more rapidly at higher BTB prevalence, indicating a complex relationship between RVF population-level dynamics and co-infection (figure 3; electronic supplementary material, appendix 1). At BTB prevalence above 20%, with a transmission factor increase of 3.4 (which best represented our data from the free-living and captive populations), BTB significantly alters the spread of RVF. At medium BTB prevalence (40–50%), such as we see in the southern KNP, with a transmission factor increase of 3.4 the size of an RVF outbreak in buffalo more than doubles. In addition, the presence of BTB changed the shape of the epidemic curve depending on the transmission factor and BTB prevalence (figure 4). At BTB prevalence of 40–50%, a transmission factor increase of 3.4 increases the time to peak and overall length of the outbreak. Within KNP, BTB prevalence ranges from 0 to 1% in the northern section of the park to 50% in the southern section of the park, where BTB first was found [55]. As BTB continues to move north within KNP, crossing into Zimbabwe [56], RVF epidemics in African buffalo may increase in size. Whether this potential increase in RVF-infected buffalo will increase the risk of outbreaks extending into humans, domestic livestock or free-ranging ruminants needs to be investigated.

Animals with BTB had greatly increased rates of abortion due to RVF, with abortions an estimated six times higher

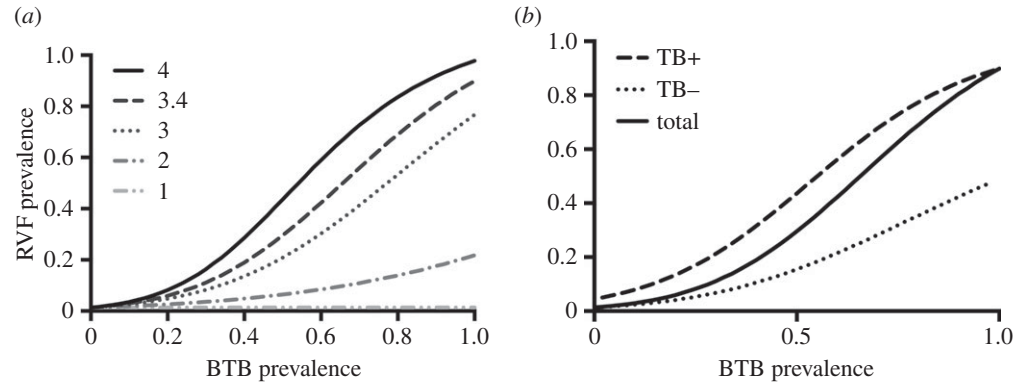

Figure 3. Effect of BTB on RVF epidemic size. (*a*) As BTB prevalence increases, so does the total RVF outbreak size. The extent of the increase depends on the factor by which transmission is increased due to BTB (transmission factors 1, 2, 3 and 3.4 and 4 are shown in the figure). A transmission factor increase of 3.4 best represented the data from the captive and free-living herds. (*b*) When the transmission factor for BTB+ buffalo was fixed at 3.4 times the rate in BTB− buffalo, increasing BTB prevalence resulted in increased predicted RVF prevalence for both BTB+ and BTB− buffalo. At BTB prevalence of 40–50%, as seen in the southern KNP, the RVF outbreak is predicted to be more than twice as large as in herds without BTB.

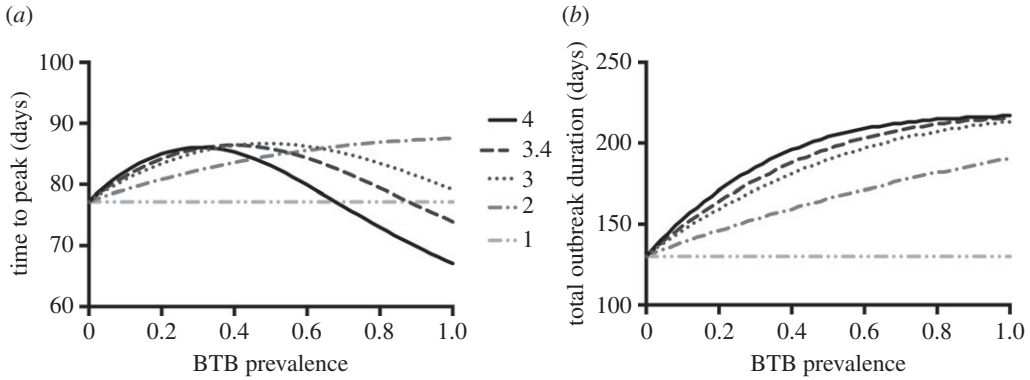

Figure 4. BTB prevalence and the relative increase in risk of RVF transmission for BTB+ buffalo, χTB, changed the shape of the epidemic curve for RVF. Higher transmission risk changed both (*a*) the time to peak RVF prevalence and (*b*) the total duration of the outbreak. At low BTB prevalence, increasing BTB prevalence results in a longer time to peak RVF prevalence. However, at high BTB prevalence, increasing BTB prevalence results in a faster outbreak and a shorter time to peak RVF prevalence.

in the BTB+ individuals than the BTB− individuals. While BTB alone may have only minor population-level effects on buffalo [6,22], it may exacerbate the effects of other diseases such as RVF, which could therefore influence the impact on host population dynamics. Future work should focus on understanding whether the alteration of individual-level buffalo–RVF interactions scales up to affect buffalo population dynamics.

The increase in RVF abortion in animals previously infected with BTB may be due to an immune-mediated interaction between BTB and RVF. We investigated this idea by comparing the production by BTB+ and BTB− buffalo of a pro-inflammatory cytokine (IL12) in response to *in vitro* challenge with RVF virus or a generic stimulant, pokeweed. IL12 is produced in response to microparasite infection, as part of a cell-mediated or T-helper cell type 1 (Th1)-mediated response [49]. IL12 is a key cytokine involved in ramping up the inflammatory response that allows intracellular microparasites to be eliminated from the host's body. While inflammation is an important component of the animal's repertoire of anti-microparasite defences, it also incurs substantial costs in the form of collateral damage, or immunopathology. For example, Thacker *et al.* [28] found that BTB-infected cows with systemically increased Th1 cytokine mRNA expression had increased pathology associated with BTB infection. Studies with other pathogens have also found that cows with a pro-inflammatory (Th1) skew to their immune systems suffered increased abortions [57,58]. In our study, buffalo with BTB produced more IL12 than uninfected buffalo, in response to *in vitro* stimulation with RVF vaccine and pokeweed. This suggests BTB infection modifies buffalo immunity towards a Th1 or pro-inflammatory skew, similar to previous observations in cows with BTB [27]. Once infection has occurred, this skew towards a Th1 immune response may help eliminate the pathogen more quickly, but may come at a great cost to the individual—increasing the likelihood of abortion or other clinical signs in co-infected individuals. Mechanistic work, including experimental infections, will be needed to clarify whether the observed pro-inflammatory skew in BTB+ buffalo is indeed causal of exacerbated fitness consequences during co-infection with RVF.

It is also possible that other mechanisms besides immunity may be important in driving the patterns noted here. For example, the patterns could be resource-mediated, but this seems unlikely since the parasites do not use the same resources in the host. Other members of the parasite could also play a role. Future work will need to investigate parasite and pathogen communities beyond two-way interactions, and evaluate whether the altered immune dynamics are the primary mechanism for increased susceptibility to RVF in BTB+ individuals, and to what extent other mechanisms may play a role.

In conclusion, we found that buffalo previously infected with BTB had increased risk of acquiring RVF and also had an increased risk of aborting due to RVF. BTB also magnified the intensity of RVF outbreaks in a mathematical model, which has implications for spillover of this zoonotic infection to livestock and people. Our study points to a new frontier—understanding how emerging pathogens modify disease dynamics and health outcomes of previously established infections. If new enemies expose the pathogenic potential of old diseases, emerging infections may pose more significant risks for population health than anticipated.

Ethics statement. Protocols for animal work were approved by the Animal Care and Use Committees at Oregon State University and the University of Georgia. Approval for all protocols were also obtained from SANParks and DAFF in South Africa.

Acknowledgements. The work would not have been possible without the assistance of Veterinary Wildlife Services at Kruger National Park, the State Veterinary office in Skukuza and our field technicians Robert and Johannie Spaan.

Funding statement. Funding for the field and laboratory work associated with the free-ranging buffalo study was provided by NSF EID DEB-1102493/EF-0723928, EF-0723918. Funding for B.R.B. was provided by Morris Animal Foundation grant ID D12ZO-409. Funding for C.A.M. was provided by NSF SEES grant CHE-1314029 and by NIH MIDAS grant no. U01-GM097661–01.

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