Effect of host diversity and species assemblage composition on bovine tuberculosis (bTB) risk in Ethiopian cattle

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SUMMARY

Current theories on diversity–disease relationships describe host species diversity and species identity as important factors influencing disease risk, either diluting or amplifying disease prevalence in a community. Whereas the simple term 'diversity' embodies a set of animal community characteristics, it is not clear how different measures of species diversity are correlated with disease risk. We therefore tested the effects of species richness, Pielou's evenness and Shannon's diversity on bovine tuberculosis (bTB) risk in cattle in the Afar Region and Awash National Park between November 2013 and April 2015. We also analysed the identity effect of a particular species and the effect of host habitat use overlap on bTB risk. We used the comparative intradermal tuberculin test to assess the number of bTB-infected cattle. Our results suggested a dilution effect through species evenness. We found that the identity effect of greater kudu – a maintenance host – confounded the dilution effect of species diversity on bTB risk. bTB infection was positively correlated with habitat use overlap between greater kudu and cattle. Different diversity indices have to be considered together for assessing diversity–disease relationships, for understanding the underlying causal mechanisms. We posit that unpacking diversity metrics is also relevant for formulating disease control strategies to manage cattle in ecosystems characterized by seasonally limited resources and intense wildlife–livestock interactions.

Key words: evenness, diversity, greater kudu, identity effect, maintenance hosts, multi-host disease ecology, habitat use overlap.

INTRODUCTION

Bovine tuberculosis (bTB), caused by *Mycobacterium bovis*, is an important zoonotic disease affecting many mammal species, and mainly spreads via aerosol transmission (Skuce *et al.* 2012). The World Health Organisation (WHO, 2012) identified bTB as one of the eight worldwide neglected zoonoses needing urgent attention, especially in developing countries. The disease is endemic in sub-Saharan African cattle (de Garine-Wichatitsky *et al.* 2013), and cattle are the main host for *M. bovis* (Cosivi *et al.* 1998). A wide range of domestic and wildlife mammals, but also humans can be infected with bTB (Munyeme *et al.* 2008). Although control programmes have eliminated or nearly eliminated this disease from domestic animals in some developed countries, bTB is still widespread in Great Britain, Ireland, New Zealand and many developing countries, especially in Africa (Renwick *et al.* 2007; Humblet *et al.* 2009). In fact, bTB is an important public concern, and can cause economic losses due to livestock deaths, product reduction and trade restrictions (Humblet *et al.* 2009).

Africa is recognized as a hotspot for biodiversity, but is suffering from rapid and extensive loss of that diversity (Myers *et al.* 2000; Olff *et al.* 2002; Gorenfloa *et al.* 2012; Di Marco *et al.* 2014). The continent is also a hotspot for emerging infectious diseases as illustrated by emergence of Ebola, HIV/AIDS, MERS, among others (Morens *et al.* 2004). As biodiversity loss is thought to be a major explanatory factor of the increase in emergence of infectious diseases (Keesing *et al.* 2010; Ostfeld and Keesing, 2012; Huang *et al.* 2013), it is key to investigate the links between biodiversity, and biodiversity loss on the patterns of infectious diseases in Africa. Recently, several studies have shown that a reduction in biodiversity may increase the prevalence and transmission of diseases (Keesing *et al.* 2010; Cardinale *et al.* 2012; Johnson *et al.* 2013; Myersa *et al.* 2013; Civitello *et al.* 2015). The two alternative hypotheses are the dilution and the amplification effect (Keesing *et al.* 2006; Huang *et al.* 2013; Hofmeester *et al.* 2016). The dilution effect predicts that species diversity decreases pathogen prevalence through mechanisms such as decreased host density, reduced encounters between hosts, or reduced host survival (Keesing *et al.* 2006; Huang *et al.* 2013; Johnson *et al.* 2013). In contrast, the amplification effect predicts increased pathogen prevalence with greater species diversity, through increased encounters between hosts, or through the presence of

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secondary hosts (LoGiudice *et al*. 2003; Keesing *et al*. 2006). A recent review of the relationships between species diversity and diseases reported dilution effects in up to 80% of the studies examined, and amplification effects in 12% of the studies (Cardinale *et al*. 2012; Ostfeld and Keesing, 2012). Despite the fact that the dilution effect occurs far more frequently than the amplification effect, our knowledge of which specific systems conform to the dilution effect and the mechanisms underlying the effects of diversity, is incomplete (Ostfeld and Keesing, 2012; Randolph and Dobson, 2012; Huang *et al*. 2013; Johnson *et al*. 2013; Miller and Huppert, 2013; Ostfeld, 2013; Hofmeester *et al*. 2016). Understanding the underlying mechanisms how the risk of disease relates to the level of biodiversity is important, both for predicting disease dynamics in the context of global biodiversity decline, and to provide valuable insights into successful control measures.

Most studies that examine the diversity–disease relationship focus principally on species richness as a measure of biodiversity (Keesing *et al*. 2006). In fact, biodiversity can be measured in many different ways, as the number of species (species richness), the distribution of individuals over species (species evenness), or a combination of richness and evenness, as represented by diversity indices such as the Shannon index (Magurran, 1988; Tucker and Cadotte, 2013). Many studies have argued that species richness and evenness are two independent indices (Sheldon, 1969; Smith and Wilson, 1996; Gosselin, 2006; Symonds and Johnson, 2008), and suggest treating them separately (Magurran, 1988; Legendre and Legendre, 1998). Ostfeld and Keesing (2000) stated that encounter rate is proportional to the distribution of hosts. Thus, evenness which measure how evenly the individuals are distributed in the community among different species may be most appropriate measure of biodiversity to explain disease risk, because of power to detect the probability of encounter between pathogens and each host species. Thus, despite many studies of the relationship between diversity and diseases, evaluating the effects of different diversity metrics on disease risk has proven to be rare (Chen and Zhou, 2015). Thus, these different metrics of diversity may have different predictive powers for predicting disease risk in the target population. Here we tested for the effect of different diversity metrics on bTB risk in cattle.

Several recent studies suggest that the occurrence of particular species in the animal community may play an important role in disease risk, and in determining whether biodiversity amplifies or dilutes the infectious disease (Fenton and Pedersen, 2005; Keesing *et al*. 2010; Hamer *et al*. 2011; Johnson *et al*. 2013, 2015; Oda *et al*. 2014). This effect of a particular species on pathogen transmission is known as the identity effect (Hantsch *et al*. 2013; Huang *et al*. 2014, 2016). Generally, the identity effect on pathogen transmission can be observed in two different situations (Huang *et al*. 2016). One is that a key species with particularly high or low reservoir competence may be present in communities when species diversity increases. The other situation is where a species can affect vector abundance (either positively or negatively) (Huang *et al*. 2016). To our knowledge, the generality of this pattern for directly transmitted or aerosol-borne diseases, such as bTB, has not been established. Thus, understanding the identity effect is an important step in being able to understand the expected impacts of biodiversity loss on disease dynamics. In Africa, buffalo (*Syncerus caffer*), greater kudu (*Tragelaphus strepsiceros*) and lechwe (*Kobus leche*; Cosivi *et al*. 1995) have been identified as maintenance hosts and implicated in the transmission of *M. bovis*. Warthog (*Phacochoerus africanus*) are also thought to be a potential reservoir for this bacteria in Africa (Tschopp, 2015). The presence of species such as the greater kudu and warthog are likely to affect the type of encounters with cattle, which could then alter the relation between biodiversity and disease risk. We thus tested for the existence of an identity effect of greater kudu and warthog. We predict that bTB risk increased with the occurrence of maintenance host species.

Currently, livestock and wild herbivores graze together in many arid and semi-arid rangelands of Africa, with much resource use overlap, as livestock species are ecologically similar, with similar resource requirements as several wild herbivore species (Prins, 2000; Sitters *et al*. 2009). Overlapping space use can lead to interspecific interactions, and stimulate the spread and prevalence of many diseases (Riley *et al*. 1998), as most pathogens are able to cross-infect multiple host species. Hence, in areas where wildlife and livestock co-occur, pathogens can emerge and establish in these sympatric host populations (Gortazar *et al*. 2007). For example, foot and mouth disease, rabies, anthrax, brucellosis and bTB have all been shown to be reciprocally transmissible between livestock and wildlife (Frohlich *et al*. 2002; Artois, 2003; Ward *et al*. 2006; Cooper *et al*. 2010; Proffitt *et al*. 2011). In this context, resource use overlap between host species can play an important role in pathogen transmission by increasing contact rates and environmental exposure to the agent (Roper *et al*. 2003; Böhm *et al*. 2009). How habitat use by hosts affects direct and indirect interactions among hosts is fundamental in understanding multi-host disease transmission (Cooper *et al*. 2010), and is critical for designing scientifically sound disease control strategies (Hudson *et al*. 2002). Nevertheless, the role that spatial interactions between livestock and wildlife

host play in disease transmission remains mostly unknown (Böhm *et al*. 2009; Martin *et al*. 2011; Tschopp, 2015). For instance, habitat and water resources use overlap may stimulate bTB transmission through increasing wildlife maintenance host–cattle contact, such as observed in and around Awash National Park, Ethiopia, where large numbers of livestock share their habitat with wildlife particularly during the dry season when resources are scarce. We therefore also tested whether habitat use overlap between wildlife maintenance host (greater kudu and warthog) and cattle increased bTB prevalence. Beside the role of host community composition and resource overlap, a positive effect of host (e.g. cattle) densities (Humblet *et al*. 2009; Dejene *et al*. 2016) has also been associated with bTB transmission risk. We also tested whether cattle densities were positively correlated with bTB incidence in cattle.

STUDY AREA

We carried out a cross-sectional study in Awash National Park and in the neighbouring Afar Region, Ethiopia. Awash National Park (9°20′N, 40°20′E) is situated in the Ethiopian Rift valley and had an elevation of 960–1050 m above sea level (Fig. 1). It is covered in semi-arid savanna. The Afar region is found in the northeastern part of Ethiopia (between 8°49′ to 14°30′N latitude and 39°34′ to 42°28′E longitude; Fig. 1) with an area of about 70 000 km^2 (CSA, 2008). It is characterized by an arid and semi-arid climate with low and erratic rainfall, with a mean annual rainfall of 500 mm in the semi-arid western escarpments, decreasing to 150 mm in the arid zones to the east. Study sites were included due consideration of variation in wildlife–livestock interactions, concentrations of livestock and wildlife, and the presence of common grazing and water resources (for details see Dejene *et al*. 2016).

METHODOLOGY

Study design

A cross-sectional multi-stage sampling was used to select study villages with 'sub-region' as the highest level followed by 'district' ($n = 17$; Fig. 1), and 'sub-district' ($n = 34$) at the lowest level. Study animals were obtained using a three-stage random sampling procedure. The village within the sub-district was regarded as the primary unit, the herd as secondary unit and individual animal as tertiary unit, following the method of Dejene *et al*. (2016). The desired sample size, which gave us a total of 2550 animals, was calculated following the method of Dejene *et al*. (2016). Tuberculin skin testing was performed using Purified Protein Derivative (supplied by Prionics Lelystad B.V, Lelystad, The Netherlands) to identify bTB-positive animals following the method of Dejene *et al*. (2016).

Dung counts

Plots for dung counts were established using stratified random sampling. First, sub-districts were stratified according to vegetation type. 204 plots (six in each of the 34 sub-districts) of 100×100 m^2 were laid out randomly in these vegetation types and were GPS geo-referenced. In each plot, we surveyed 50 transects of 100 m length and 2 m wide, and counted dung piles. Each pile of dung was attributed to a locally available wildlife species based on the size, shape and form of the pellets by using Stuart and Stuart (2000), and with the help of experienced local trackers. The relative abundances of wild herbivores were estimated based on the frequency of fecal droppings found in the plot transects following Vicente *et al*. (2004). We divided each 100 m transect into 10 sectors of 10 m length. We defined sign frequency as the average number of 10-m sectors with the presence of wild herbivores droppings. Based on these frequencies, we calculated for each of the species the frequency-based indirect index (FBII):

$$\text{FBII} = \frac{1}{n} \sum_{i=1}^{n} s_i$$

where s_i is the number of sign-positive sectors in the ith 100 m transect (i.e. S_i varies between 0 and 10), and n is the number of 100 m transects considered (i.e. $n = 50$ for each plot; Vicente *et al*. 2004).

Ethical statements

This study was approved by Haramaya University, Ethiopia (Reference number HUP14/559/15).

Statistical analysis

For each sub-district Pianka's Niche Overlap, mammalian species richness (S), mammalian species diversity (H′) and mammalian species evenness (𝒥′) were calculated. Habitat use overlap between cattle and greater kudu was calculated according to Pianka's Niche Overlap (Pianka, 1973). This index varies from 0, no overlap, to 1, complete overlap.

$$O_{jk} = \frac{\sum p_{ij} p_{ik}}{\sqrt{\sum p_{ij}^2 \sum p_{ik}^2}}$$

where O_{jk} is the overlapping index between species j and k, and p_{ij} and p_{ik} being the proportions of use of habitat i by the species j and k.
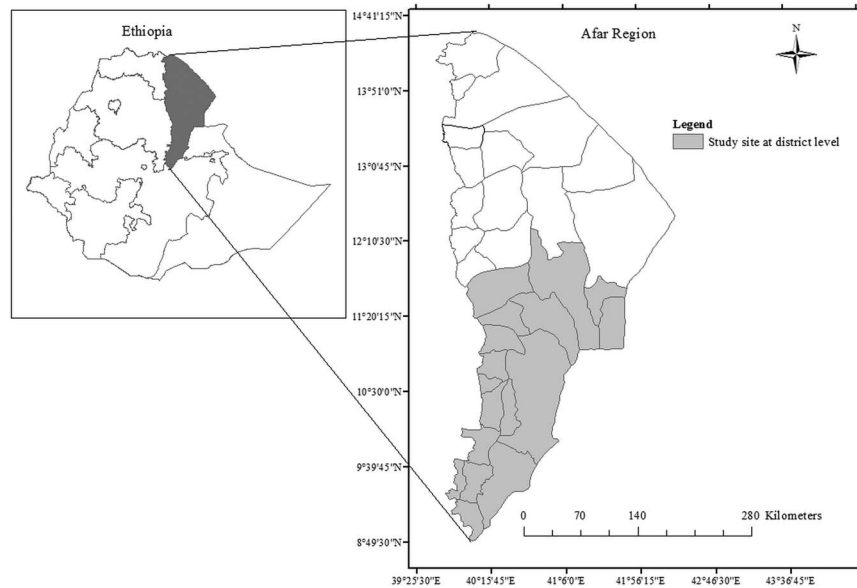
Fig. 1. Map of the study area, the Afar Region in Ethiopia (small inset) and 17 districts (larger map). The location of Awash National Park in the South is indicated by the cross-hatched area.

Shannon's diversity index (H') was used to estimate mammalian species diversity as

$$H' = -\sum_{i=1}^{S} p_i \ln p_i$$

where p_i is the proportion of species i, and S is the number of species (Hill, 1973).

Pielou's index was used to estimate mammalian species evenness (Hill, 1973), which is most widely used in ecology (Zhang *et al*. 2012).

$$\mathcal{J}' = \frac{H'}{\ln(S)}$$

where H' represents the Shannon diversity index, and S is the total number of species observed. Biodiversity metrics were calculated using package vegan of R v3.2.0 (Oksanen *et al*. 2016).

Generalized Linear Mixed Models (GLMM, family = Poisson) using package lme4 were used to examine the effects of predictors on the sub-district bTB incidence (SI- Table 1). Prior to developing our candidate models, we performed one-by-one univariate analyses to identify potential spatial risk factors, using the number of bTB-infected animals as dependent variable. Predictor variables with $P < 0.25$ recognized as potential spatial risk factors (Huang *et al*. 2013), and subsequently used to construct multiple regression models. For highly correlated independent variables, only the one causing the largest change in the Log-Likelihood added to the final global model to avoid multi-collinearity, which was assessed by using variance inflation factors. The final variance inflation factor values were all <5 and confirmed the absence of collinearity among variables. From the global model, candidate models constructed using delta AIC (<5), with the best approximating candidate model having the lowest delta AIC, as described in Burnham and Anderson (2002). Model averaging was used to construct the final model based on the lowest Akaike weights of the different candidate models (Anderson *et al*. 2000). In this analysis, we treated district as a random effect to account for repeated sampling. We carried out all analyses in R v3.2.2 (R Core Team, 2015).

RESULTS

Pielou's species evenness ($\mathcal{J}'$) and Shannon's species diversity (H') varied between 0·46–0·90 and 0·72–2·05, respectively. Habitat use overlap between cattle and kudu varied from 0, no overlap to 0·95, high overlap. The highest Pianka's Niche Overlap index between warthog and cattle was 0·84. Relative abundances of kudu and warthog ranged from 0 to 0·93 and 0 to 0·79, respectively (SI-Table 2).

Univariate analyses

Based on the results of the univariate analyses, we identified seven out of eight variables as potential risk factors, namely, mammalian species richness, Pielou's species evenness ($\mathcal{J}'$), Shannon's species diversity (H'), habitat use overlap between cattle and greater kudu, habitat use overlap between cattle and warthog, relative density of greater kudu, and relative density of warthog (Table 1). Surprisingly, density of cattle was not associated with the number of bTB-infected cattle in the sub-district (Table 1).

Table 1. Results of the one-by-one GLMM analysis of all variables and summary statistics (regression coefficient *b* with 95% confidence intervals, odds ratio (OR) with 95% confidence intervals, χ^2 and *P*-value) for all predictors against sub-district (*n* = 34) number of bTB-positive animals from the likelihood ratio test (logLik = log likelihood) and AIC value

Number of bTB-positive animals

Variables	*b* (95%CI)	OR (95% CI)	logLik	AIC	χ^2	*P*-value
Habitat use overlap with kudu	1·2 (0·6–1·7)	3·3 (1·9–5·5)	−66·0	139·5	19·6	<0·001***
Habitat use overlap with warthog	1·4 (0·7–1·9)	3·9 (2·1–6·9)	−66·8	138·0	20·9	<0·001***
Relative density of kudu	1·3 (0·6–1·8)	3·5 (1·9–6·0)	−66·1	140·2	18·9	<0·001***
Relative density of warthog	1·4 (0·8–2·0)	4·1 (2·1–7·5)	−66·6	139·1	19·6	<0·001***
Species diversity (*H'*)	0·7 (0·2–1·1)	2·0 (1·3–3·1)	−68·5	143·0	14·3	0·002**
Species evenness (*Ɉ'*)	−2·3 (−4 to 0·6)	0·9 (0·2–1·5)	−70·8	147·5	7·63	0·006**
Species richness	0·1 (0·01–0·2)	1·1 (1·0–1·2)	−70·1	146·2	9·74	0·001**
Density of cattle	0·2 (0·1–0·3)	1·0 (0·9–1·1)	−74·1	154·2	0·15	0·693
Presence of Kudu	0·9 (0·5–1·2)	2·4 (1·6–3·5)	−72·2	147·1	17·21	<0·001***

Kudu = greater kudu; *P < 0·05; **P < 0·01; ***P < 0·001.

Communities that contained greater kudu had a significantly higher bTB incidence than communities without greater kudu (Fig. 2; *b* = 0·9, 95% CI = 0·5–1·2; OR = 2·4, 95% CI = 1·6–3·5; *P* < 0·001).

The Spearman's correlation matrix showed that species richness was strongly correlated with Shannon's species diversity index. Habitat use overlap between cattle and warthog, relative density of greater kudu, and relative density of warthog were strongly correlated with habitat use overlap between cattle and greater kudu (*r* > 0·7; SI-Table 3). Therefore, we only included the latter two variables and species evenness in the multiple variable model to avoid collinearity.

Multiple variable analyses

Variables included in the multiple variable analysis were Pielou's species evenness, Shannon's species diversity and habitat use overlap between cattle and greater kudu (SI-Table 4).

The results of model averaging showed always a negative relationship between Pielou's species evenness and the number of bTB-positive cattle, but we did not find a significant relationship between Shannon's species diversity and the number of bTB-positive cattle, although the effect of species diversity was always positive in the models. In addition, our analysis also identified habitat use overlap between cattle and greater kudu as a significant risk factor for the number bTB-positive cattle in the sub-districts (Table 2; Fig. 3).

DISCUSSION

Our study showed that the bTB infection rate was negatively associated with mammalian species evenness (*Ɉ'*), in line with our predictions derived from the dilution effect hypothesis. However, contrary
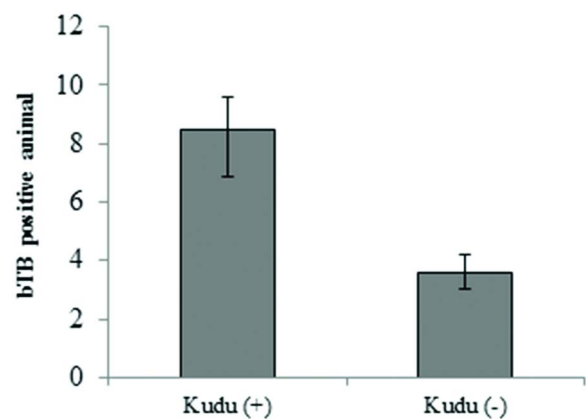

Fig. 2. Effects of the presence of a particular host species, greater kudu, on the number bTB-positive animals at the sub-district level (*n* = 34). Data shown are means with the 95% confidence intervals

to our expectation we did not find a significant relationship between mammalian species diversity (*H'*) and the number of bTB-infected cattle. There was also a positive effect of habitat use overlap between cattle and greater kudu on bTB incidence in cattle. As proposed by Ostfeld and Keesing (2000), if the encounter rate is proportional to the distribution of the host species, species evenness would seem most appropriate for disease risk, because evenness, not richness, would capture the probability of encounter between pathogens and each host species (Ostfeld and Keesing, 2000; Chen and Zhou, 2015). Our study detected a dilution effect of Pielou's species evenness on the risk of bTB prevalence, an influential aerosol-borne disease. This dilution effect is possibly explained by encounter reduction, in that the addition of alternative hosts may decrease the risk of pathogen transmission by reducing encounter rates between susceptible and infected

Table 2. *Summary statistics of the final model, obtained through model averaging, with regression coefficient* ($b \pm$ SE), *Odds Ratio (OR, 95% confidence interval) and P-value from the likelihood ratio test for the effect of species evenness ($\mathcal{J}'$), species diversity (H') and cattle-greater kudu habitat use overlap on the number of bTB-infected Ethiopian cattle in the sub-districts (n = 34)*

Number of bTB-positive animal

Variables	*b* (95% CI)	OR (95%CI)	*P*-value
Pielou's species evenness	−2·01 (−3·9 to 0·1)	0·2 (0·02–0·82)	0·036*
Shannon's species diversity	0·46 (0·3–1·2)	1·6 (0·77–3·30)	0·221
Habitat use overlap with kudu	1·14 (0·3–1·8)	2·8 (1·35–5·94)	0·008**

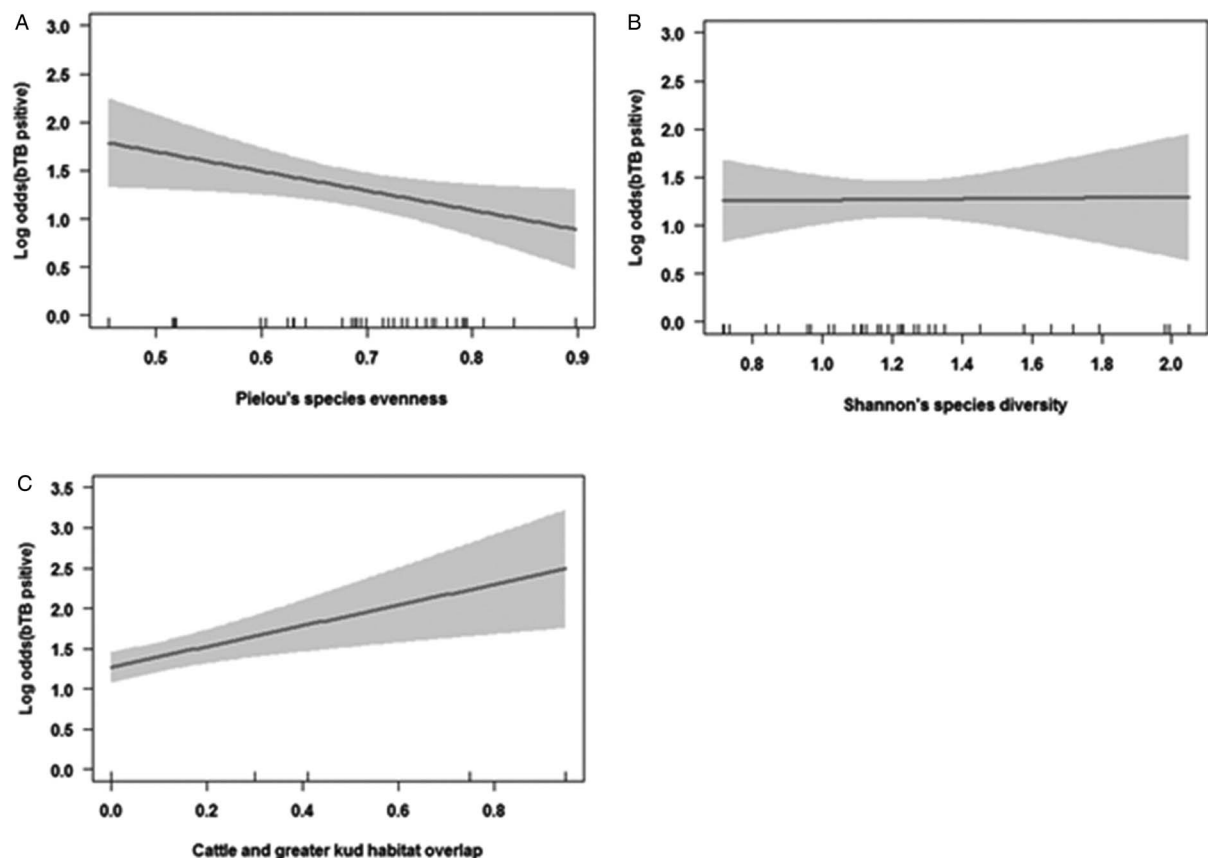Kudu = greater kudu; *P < 0·05; **P < 0·01; ***P < 0·001.

Fig. 3. GLMM results of the effects of explanatory variables on the number of bTB-positive cattle at the sub-district level (log odds scale) in relation to Pielou's species evenness (A), Shannon's species diversity (B) and resource overlap between greater kudu and cattle (C).

hosts (Keesing *et al*. 2006; Chen and Zhou, 2015). In pastoral areas of East Africa, the distribution and abundance of large grazers is negatively associated with the presence of cattle (Voeten and Prins, 1999; de Leeuw *et al*. 2001; Bonnington *et al*. 2007). For instance, de Leeuw *et al*. (2001) observed a significant reduction of species such as Oryx, gerenuk and gazelle in the presence of cattle in Kenya (de Leeuw *et al*. 2001), and Odadi *et al*. (2007) found that the preference of foraging habitat for cattle was lower in the presence of wild grazers (Odadi *et al*. 2007). Many mammal species that can be infected by bTB are spillover or dead-end hosts and do not transmit the pathogen efficiently (Corner, 2006; Renwick *et al*. 2007). The presence of these non-competent or spillover mammalian species might act as barriers to cattle herd movement and distribution, and reduce encounter rates among cattle herds by changing the grazing behaviour and habitat preference (e.g. avoidance of sites contaminated by feces or different preferences for feeding patches). Such an 'encounter reduction' (Keesing *et al*. 2006) might lead to decreased probabilities of bTB infection risk, although the exact

mechanism behind these correlations needs more attention.

We did not detect significant effects of host species diversity (H') on the bTB infection level. The lack of a significant association between host species diversity and disease risk might occur because the index we chose, the Shannon index, stresses the number of species and presence of rare species (McGarigal and Marks, 1994; Haines-Young and Chopping, 1996; Riitters *et al.* 2000; Magurran, 2004). Thus, this metric might fail to weigh in the specific importance of particular species that are not rare, which might be addressed better by focusing on the effects of host identity (Hamer *et al.* 2011). Moreover, studies also criticizing the dilution effect argued that pathogen transmission might increase in high-diversity communities (Randolph and Dobson, 2012; Wood and Lafferty, 2013; Huang *et al.* 2016) due to the increased chance of including a particular species that has a positive effect on pathogen transmission (Hantsch *et al.* 2013, 2014). For instance, a recent study on bTB suggested that the presence of buffalo increased disease risk due to its high bTB competence (Huang *et al.* 2016). Power and Mitchell (2004) also demonstrated how the identity effect of particular host species influence the diversity–disease relationship, and found that more diverse systems had higher rates of infection (i.e., amplification effect), because these species rich assemblages contained highly competent reservoir hosts (Power and Mitchell, 2004). Bouchard *et al.* (2013) found that the occurrence of white-tailed deer (*Odocoileus virginianus*), an important host for adult ticks, increase the abundance tick and thus increased the risk of tick-borne diseases (Bouchard *et al.* 2013). Similarly, we found that the presence of greater kudu and habitat use overlap between cattle and greater kudu was positively associated with the number of bTB infection. In Africa, species habitat use such as of greater kudu is not strongly affected by cattle presence (Prins, 2000), because kudus are almost exclusively browsers and the kudu-cattle dietary niche overlap is relatively small (Fritz *et al.* 1996). High habitat use overlap between cattle and kudu could increase encounter rates between them and create a positive identity effect of kudu on transmission of bTB, as a known wildlife bTB reservoir host. On the contrary, the presence of opossums created a negative identity effect on tick abundance (Keesing *et al.* 2009). Thus, high species diversity may amplify or dilute pathogen prevalence depending on the occurrence of a particular species. If the occurrence of the particular species had a negative identity effect, it may enhance the strength of the negative diversity–disease relationship; when the identity effect is positive, it may weaken the negative diversity–disease relationship and lead to a dilution effect (Huang *et al.* 2016). Another example is the influence of warthogs, which are predominantly grazers and compete with cattle for high-quality food in African savannas (Treydte *et al.* 2006). The species is also recognized as hosts for ticks, which are vectors of various diseases, including African Swine Fever in eastern Africa (Osofsky *et al.* 2005). Thus, livestock keepers tend to avoid the areas that are used by warthog for fear of diseases (Maleko *et al.* 2012). This could decrease the encounter rate between cattle and warthog, and lead to a non-significant identity effect on bTB transmission. This might be the reason for a non-significant negative diversity–disease relationship. We recognize that our conclusions are based on correlative studies and that further studies with experimental manipulation, including host behaviour change and contact rates among hosts are required to thoroughly test this hypothesis. However, our results are a necessary first step towards understanding the role of community structure on bTB risk and identifying the underlying mechanisms.

In addition to direct transmission, which requires close contact between host species, indirect transmission via environmental contamination is also possibility for bTB transmission. In the north and northeastern part of Awash National Park, particularly in the northern part of the Park at the hot spring and kudu valley areas, it is common to observe livestock grazing in close proximity with kudu during the dry season. *Mycobacterium bovis* has been detected in environmental samples in East Africa (Roug *et al.* 2014), and experimental studies have confirmed that the bacteria can survive for multiple days outside hosts (Fine *et al.* 2011). Kelly and Collins (1978) suggested that the major factors influencing survival of the bacteria in soil is soil temperature and moisture, as high temperature causes desiccation, and negatively influence survival of the bacteria. Environmental persistence of *M. bovis* has been proposed to play a role in the transmission of bTB in the UK (Courtenay *et al.* 2006). Wetlands or humid areas are also potential risk factors, and areas around pounds are generally moister, with greater amounts of shade, which are favourable conditions for *M. bovis* survival (Jackson *et al.* 1995). In Africa, flooding or soil humidity have also been suggested as propagating factors for *M. bovis* in the environment, as demonstrated in Tanzania (Cleaveland *et al.* 2007) and Zambia (Munyeme *et al.* 2009) by creating favourable conditions for *M. bovis* survival. The humid marsh–shrub wetland habitat near the hot-spring and kudu valley of Awash National Park and the surrounding water holes may act as potentially high-risk areas for *M. bovis* infection, as these areas are generally moist, with greater amounts of shade. Hence, the correlation of habitat use overlap between greater kudu and cattle with bTB infection in the GLMM analyses might not tell the whole story, as the

underlying reasons for this correlation is that it is possible that environmental transmission occurs among African wildlife and livestock. These uncertainty and complex eco-epidemiological scenarios and possible confounding factors require further investigation of the transmission network.

Our results highlight aspects of mammalian species evenness and spatial differences in species assemblage that are likely to affect the risk of disease. Our results support the idea that a greater mammalian species evenness acts as a buffer against disease outbreaks. Our findings also demonstrate that the presence of a particular reservoir hosts can affect the diversity–disease relationship. Hence, it is a prerequisite to understand the identity effect, and predict future outbreaks and minimize the risk of disease transmission. Ecologists, epidemiologists and policy makers need to understand the complex interactions among potential host species to identify risk factors for disease transmission and identify efficient management actions. In order to improve this understanding, further ecological and epidemiological research on disease transmission and contact networks is required.

SUPPLEMENTARY MATERIAL

The supplementary material for this article can be found at https://doi.org/10.1017/S0031182016002511.

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