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Original Contribution

Pathogen Exposure in Cattle at the Livestock-Wildlife Interface

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Abstract: Land use is an important driver of variation in human infectious disease risk, but less is known about how land use affects disease risk in livestock. To understand how land use is associated with disease risk in livestock, we examined patterns of pathogen exposure in cattle across two livestock ranching systems in rural Kenya: private ranches with low- to medium-intensity cattle production and high wildlife densities, and group ranches with high-intensity cattle production and low wildlife densities. We surveyed cattle from six ranches for three pathogens: *Brucella* spp., bovine viral diarrhea virus (BVDV) and *Leptospira* serovar Hardjo. We found that exposure risk for *Leptospira* was higher on private ranches than on group ranches, but there was no difference in exposure by ranch type for *Brucella* or BVDV. We hypothesize that variation in livestock and wildlife contact patterns between ranch types may be driving the pattern observed for *Leptospira* exposure and that the different relationships we found between exposure risk and ranch type by pathogen may be explained by differences in transmission mode. Overall, our results suggest that wildlife–livestock contact patterns may play a key role in shaping pathogen transmission to livestock and that the magnitude of such effects likely depend on characteristics of the pathogen in question.

Keywords: Ranching, Pastoralist, *Leptospira*, *Brucella*, Bovine viral diarrhea virus

INTRODUCTION

Anthropogenic land use is an important and complex driver of infectious disease dynamics (Patz et al. 2004). Land use can influence pathogen transmission by altering environmental conditions that affect parasite and vector development rates [e.g., human malaria, (Afrane et al. 2005)]; changing the abundance and distribution of hosts critical for transmission [e.g., Lyme disease, (LoGiudice et al. 2008)]; and affecting contact patterns among hosts [e.g., Nipah virus, (Pulliam et al. 2012)]. Although studies have examined relationships between land use and disease risk for a range of human pathogens (Patz et al. 2004; Karesh et al. 2012), less is known about how land use is associated with patterns of infection in animals, especially domestic species (Perry et al. 2011). Livestock diseases pose a significant direct health risk to humans (Cleaveland et al. 2001) and can also impact humans indirectly through economic and subsistence losses, particularly in resource-

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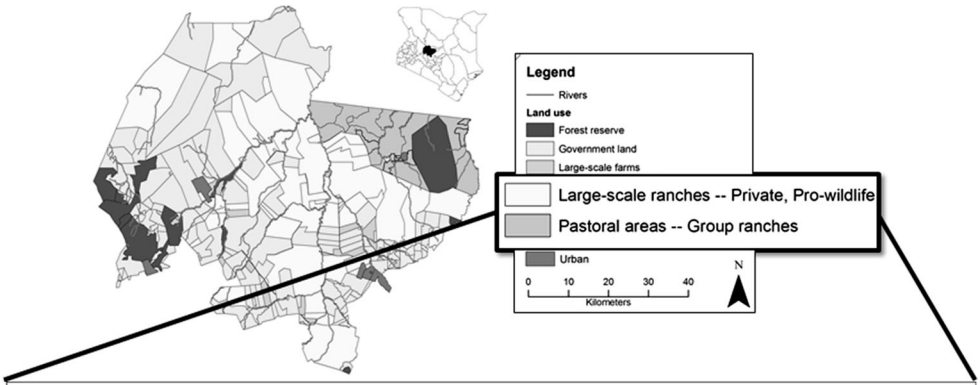
poor livestock production systems (Molyneux et al. 2011). Therefore, studying how land use relates to infectious disease risk in livestock can translate into a better understanding of disease impacts on both human and animal populations.

In many regions of the world, human communities depend heavily on livestock for their livelihoods (Molyneux et al. 2011). Critically, land used for livestock production in many systems is often also utilized by wildlife (Kock 2005), with potential implications for pathogen transmission between the two groups. For example, in Laikipia County, Kenya, livestock production takes two main forms, group and private ranching, each involving land use practices that have implications for the transmission of livestock pathogens. Group ranches typically share resources (i.e., pasture) among multiple individuals in a pastoralist framework and the majority of land is dedicated to livestock production, whereas private ranches often couple livestock production with tourism or conservation objectives, resulting in higher wildlife densities (Georgiadis et al. 2007; Sundaresan and Riginos 2010; Kinnaird and O'Brien 2012). These practices result in a particularly distinctive pattern in which ratios of wildlife to livestock can be up to 14 times higher on private ranches (Georgiadis et al. 2007; Kinnaird and O'Brien 2012; see Figure 1). Common wildlife occurring across ranches in Laikipia include 19 species of ungulates (e.g., African buffalo (*Syncerus caffer*), eland (*Taurotragus oryx*), impala (*Aepyceros melampus*), Grant's gazelle (*Nanger granti*), waterbuck (*Kobus defassa*); Georgiadis et al. 2007; Kinnaird and O'Brien 2012; Ogutu et al. 2016), many of which are known carriers of pathogens that infect livestock (Bengis et al. 2004). Importantly, because wild ungulate populations on these ranches are unmanaged, there is opportunity for substantial indirect contact between livestock and wildlife via shared grazing and water sources. Moreover, given the asymmetry in wildlife/livestock ratios between group and private ranches, higher livestock–wildlife contact rates on private ranches could translate into higher pathogen prevalence among livestock. This is particularly relevant to pathogens in which indirect contact is sufficient for cross-species transmission to occur.

In this study, we tested the hypothesis that differences in ranching style (i.e., group vs. private ranching) affect pathogen transmission to livestock. We used cattle as a focal livestock host to compare the seroprevalence of three pathogens with different transmission characteristics across ranch types. Cattle comprise approximately 60–80% of livestock biomass in Laikipia (Georgiadis et al. 2007; Ogutu



Study	Herbivore Demographics			
	Land use	Livestock	Wildlife	Summary
Georgiadis et al. 2007[1]	Private	2.46	1.74	Ratio of wild herbivores to cattle ~ 0.7:1
	Group	2.06-3.93	0.11-0.64	Ratio of wild herbivores to cattle ~ 0.05:1 - 0.2-1
Kinnaird and O'Brien 2012[2]	Private	10-20	-0.017	Low to medium livestock stocking rates and low wild herbivore abundance
	Group	>25	-0.289	Medium to high livestock stocking rates and lower wild ungulate abundance compared to private ranches.

[1] biomass densities of wild herbivores and cattle across land use types using 25 years of county wide aerial survey data
[2] total livestock units and relative abundance indexes for wild ungulates estimated via camera trapping from 2008-10

Figure 1. Map of major land use types in Laikipia, Kenya. The table summarizes key differences in herbivore demographics between private and group ranches. Map adapted from Sundaresan and Riginos 2010.

et al. 2016), and our target pathogens, *Brucella* spp, bovine viral diarrhea virus (BVDV) and *Leptospira* spp. serovar Hardjo, have well-known impacts on livestock production (Grooms 2006; McDermott et al. 2013). All three pathogens commonly infect cattle in Kenya (Njeru et al. 2016; Callaby et al. 2016; de Vries et al. 2014) and are also known to infect wild ungulates across sub-Saharan Africa, including species that are common in Laikipia (e.g., *Brucella*: Ducrotoy et al. 2015; BVDV: Vilcek and Nettleton 2006; Walz et al. 2010; *Leptospira*: de Vries et al. 2014). The transmission of *Brucella* in cattle is largely associated with direct contact with reproductive fluids or tissues released by infected animals (Olsen and Tatum 2010), while transmission of BVDV is driven by close contact with persistently infected individuals (Lindberg and Houe 2005). *Leptospira* transmission occurs through direct contact with urine of infected individuals or frequently via indirect contact with contaminated water and pasture (Vijayachari et al. 2008). Given asymmetries in wildlife–livestock contact rates between ranch types, and transmission differences among these three pathogens, we predicted that the relationship between ranch type and pathogen risk would vary by pathogen. For *Leptospira,* which has a significant indirect transmission component, we expected that exposure risk would be higher on private ranches as a function of higher ratios of wildlife to livestock. In contrast, for *Brucella* and BVDV, which are primarily directly transmitted, we expected little difference in pathogen exposure between ranch types given that wildlife–livestock contact may not contribute substantially to transmission. Rather, exposure risk to *Brucella* and BVDV might depend more on cattle density which varies between ranch types to a much smaller degree than do wildlife densities (Figure 1).

Methods

Study Sites

Laikipia County covers an area greater than 9000 km^2 and is made up of a mosaic of land use types including government, private and communally owned properties (Figure 1). The majority of the county is comprised of cattle ranches, and these properties are important for livestock production and wildlife conservation (Sundaresan and Riginos 2010). Ranches were classified into two categories, private or group, based on information on land ownership and use, livestock management and attitudes toward wildlife (Georgiadis et al. 2007; Kinnaird and O'Brien 2012). Private ranches are designated as properties owned by an individual or trust with centralized livestock management and active investment in and/or conservation of wildlife since the early 1990s. Group ranches are designated as areas with a community structure (e.g., chief and distinct homesteads) where multiple individuals own livestock, but resources are shared (e.g., communal grazing). These sites were also characterized by little to no active wildlife conservation. Extensive past work has quantified patterns of livestock and wild herbivore densities across these ranch types (Georgiadis et al. 2007; Kinnaird and O'Brien 2012; see Figure 1). We sampled cattle originating from five ranches in Laikipia County and one in adjacent Isiolo County. Cattle originating from the one ranch in Isiolo were sampled in Laikipia having been very recently transferred from a group ranch in Isiolo to a private ranch in Laikipia. These animals were assigned to Group Site 3 (Table 1), based on the characteristics of their property of origin. Informed oral consent was obtained from all livestock owners prior to sampling. To maintain owner confidentiality, we do not report the exact location and names of our sampling sites.

Sampling

We sampled 415 East African Zebu cattle from three private and three group ranches in June–July 2012. Between 40 and 75 individual cattle were sampled at each site, and the number of herds sampled per site varied from 1 to 4 on private ranches and from 1 to 20 on group ranches (Table 1). This variation was the result of differences in herd structure between land use types: Private ranches typically have fewer, larger herds, while group ranches have multiple, small herds. Prior to sampling, we confirmed that vaccination campaigns for the focal pathogens had not been conducted on group ranches and verified directly the vaccination status of herds with owners on private ranches. One herd sampled on Private Site 1 had been vaccinated for *Brucella* ($n = 23$), so these animals were excluded from the *Brucella* analyses.

Sampling was restricted to cattle over 1 year of age. When available, specific age data were obtained based on animal branding patterns designating the month and year of animal birth. Detailed age data were not available for animals on group ranches so we used an age threshold of 1 year for sampling at these sites to exclude any juvenile cattle. For the animals with age data, the age range of sampled individuals was 32–179 months. Both sexes were

Table 1. Description, Number of Animals Sampled, Sampling Dates, Number of Herds Sampled and Average Herd Size for Each Site.

Type	Description	Animals sampled	Herds sampled	Average herd size
Private 1	Conservancy; managed primarily for wildlife research with a ranch component; multiple cattle herds	75	3	93
Private 2	Single-owner ranch; managed for leisure; both wildlife and cattle	40	1	100
Private 3	Single-owner ranch; managed commercially for livestock production; no active wildlife management; stocked with multiple herds of cattle	75	4	120
Group 1	Small group ranch; community structure with an appointed chief and shared grazing; distinct homesteads but with a single communal cattle herd	75	1	100
Group 2	Peri-urban community area; community structure with an appointed chief and shared grazing; distinct homesteads and herds with lower numbers of cattle	75	20	5
Group 3	Community conservancy; managed for livestock and wildlife through a pro-wildlife trust; community structure with shared grazing; herd composition and structure unknown	75	U	U

U denotes cases where information was unknown.

Table 2. Summary of Pathogen Seroprevalence for Cattle at Six Study Sites, Including # Positive/# Number Tested, Prevalence and 95% Confidence Interval.

Type	*Brucella*		BVDV		*Leptospira*	
	+/tested	% Prevalence (CI)	+/tested	% Prevalence (CI)	+/tested	% Prevalence (CI)
Private 1	0/52	2.0 (0.0–5.9)	72/75	97.6 (92.0–99.9)	44/68	66.7 (52.9–79.5)
Private 2	0/39	2.7 (0.0–8.0)	23/40	58.4 (40.7–75.1)	11/36	28.3 (12.1–46.5)
Private 3	4/75	2.9 (0.1–9.2)	71/74	97.6 (92.0–99.9)	41/65	65.0 (50.9–78.6)
Group 1	5/75	3.4 (0.1–10.4)	25/71	33.1 (20.4–46.4)	14/73	14.7 (4.3–26.3)
Group 2	2/75	1.9 (0.1–6.0)	57/74	81.1 (68.9–92.0)	2/75	2.0 (0.1–6.7)
Group 3	24/74	29.8 (17.5–42.9)	56/74	85.6 (73.6–95.9)	26/74	36.7 (23.5–50.6)
Avg. Private		2.5		84.3		53.3
Avg. Group		11.7		66.6		17.8
Avg. Overall		7.1		75.6		35.6

Suspect samples were excluded from the prevalence calculations.

sampled, but at all sites, females made up the majority of the herds. The proportion of females sampled at each site ranged from 67 to 100%, but there was no significant difference across ranch type in the proportion of females sampled (Mann–Whitney U test: $U = 1.23$, $p = 0.27$).

For pathogen testing, we collected blood from the jugular vein into 10-ml vacutainer tubes using 18-gauge needles. Immediately after filling each blood tube, a heparinized 100-μL capillary tube was filled to measure packed cell volume (PCV). Blood samples were transported back to the laboratory and centrifuged at 3300 rpm for 20 min to harvest serum. Serum samples were stored at −20°C until processing. Capillary tubes were centrifuged for 10 min at 11,000 rpm, and PCV was measured using a hematocrit reader card. For each animal sampled, a single observer collected a series of morphometric measurements, including heart girth (circumference of body at the shoulders), body length (length from point of rump to point of muzzle with neck extended) and height (distance from pin bone to hoof). PCV and morphometric measures were used to ac-

count for potential differences in cattle body condition across ranch types. Such body condition differences could arise due to socioeconomic differences between ranch types (Georgiadis et al. 2007; Sundaresan and Riginos 2010), with potential effects on pathogen prevalence.

Serological Testing

We tested for antibodies to three pathogens using commercially available enzyme-linked immunosorbent assay (ELISA) kits. To detect exposure to *Brucella* spp., we used the IDEXX Brucellosis Serum Ab Test (Sensitivity/Specificity (Se/Sp): 96/97, IDEXX), which detects antibodies for both *Brucella abortus* and *Brucella melitensis*. For BVDV, we used the IDEXX BVDV Total Ab Test (Se/Sp: 96.7/97.1; Lanyon et al. 2013). For *Leptospira* spp., we used the Linnodee Lepto Kit (Se/Sp: 94.8/94.1; Linnodee Animal Care), which tests for antibodies to *Leptospira* serovar Hardjo, a common serovar found in cattle in sub-Saharan Africa (Myburgh et al. 1989; Niang et al. 1994; Schoonman and Swai 2010). All assays were performed according to the manufacturers' specifications.

Statistical Analyses

Diagnostic results were calculated using cutoff values provided by each manufacturer. For the BVDV and *Leptospira* assays, some samples fell within the range of suspect values, and as per the manufacturers' specifications, we reran these samples. In some cases, rerunning the sample did not resolve the issue (29/77 for *Leptospira*, 11/19 for BVDV), so we excluded these samples from further analysis since they could not be classified as seropositive or seronegative as per the cutoff criteria.

To examine seroprevalence patterns across sites, we calculated herd-level prevalence for each site using the prevalence package (Devleesschauwer et al. 2014) in R version 3.2.3. This method uses a Bayesian approach to account for test uncertainty and varying sensitivity and specificity when estimating true prevalence. Given high reported sensitivity and specificity values for the diagnostic tests we used, we set a uniform distribution of sensitivity and specificity values between 90 and 95%.

We used data on PCV and size-corrected mass to test for differences in cattle condition across ranch types. Variation in PCV values is indicative of nutritional stress and active parasite infection in livestock (Grace et al. 2007; Marufu et al. 2010); thus, an animal with lower PCV may be in poorer condition or showing signs of infections. Size corrected for mass can reflect differences in weight that are not a function of skeletal size. Individuals that have higher (positive) size–mass residuals weigh more than expected for their body size and therefore may be in better condition (Schulte-Hostedde et al. 2005). We calculated size-corrected mass from the regression of heart girth, a common proxy for mass in livestock (Goe et al. 2001; Kashoma et al. 2011), on body length, a size metric. We used linear mixed-effect models (LMM) to examine associations between the two condition metrics and ranch type. Each condition measure was the response variable in a separate model, sex was included as a fixed effect, and site was included as a random effect.

We investigated the effect of ranch type on pathogen exposure risk, quantified as individual serostatus for each pathogen, using generalized linear mixed-effects models (GLMM) with a binomial distribution (0 = seronegative, 1 = seropositive). For each model, ranch type (private vs. group) was the main predictor, with sex and PCV included as covariates. Site was included as a random effect to account for the fact that multiple individuals were sampled at each site. PCV was included in the models to account for the potential effects of variation in host condition on infection patterns. Since our two measures of condition were significantly and positively correlated (PCV vs. size–mass residuals: $r = 0.204$, $n = 395$, $p < 0.001$), we only included one (PCV) and not both as a covariate in the GLMMs. Sex was included in the models to account for possible sex biases in infection patterns. All mixed-effects models were implemented using the lme4 package in R [v. 1.1-9, (Bates et al. 2015)]. For all significant factors in the models, odds ratios and confidence intervals (CIs) were calculated by taking the exponent of the coefficient of the fixed effects and the associated upper and lower CIs of these coefficients (i.e., e^x where x is either the coefficient, upper CI or lower CI).

Finally, because the seroprevalence of *Brucella* at Group Site 3 was an outlier compared to all other values (see Table 2), and because the animals from this site originated from a group ranch but then spent a time on a private ranch prior to sampling (see 'Study Sites' section above), we reran the exposure risk models for all three pathogens excluding this site to evaluate its impact on our conclusions. We also examined whether our approach of excluding suspect samples (see above) affected the results of our exposure risk models. To do this, we reran the affected models [*Leptospira* ($n = 29$ suspect samples) and BVDV ($n = 11$ suspect samples)] including the suspect samples as either all seropositive or all seronegative.

Results

Pathogen Seroprevalence and Host Condition Across Ranch Types

Across all sites, average prevalence by pathogen was 7.1% for *Brucella*, 35.6% for *Leptospira* and 75.6% for BVDV (Table 2). However, prevalence varied among sites, ranging between 2.0 and 29.8% for *Brucella*, 2.0–66.7% for *Leptospira* and 33.1–97.6% for BVDV. Prevalence also varied by ranch type. Group ranches had a higher seroprevalence of *Brucella* (11.7 vs. 2.5% on private ranches), although this pattern was driven by the high seroprevalence at a single location (Group Site 3) (Table 2). Private ranches had a higher seroprevalence of both BVDV (84.3 vs. 66.6% on group ranches) and *Leptospira* (53.3 vs. 17.8% on group ranches) (Table 2). With respect to condition, both PCV and size–mass residuals were higher among individuals on private ranches compared to group ranches (Figure 2); however, the size–mass residual effect was only marginally significant (GLMM: PCV, $n = 396$ observations, 6 sites; ranch type: estimate ± s.e (private) = 4.59 ± 1.35, $p = 0.027$; sex: estimate ± s.e (male) = −0.370 ± 0.596, $p = 0.535$; size–mass residuals, $n = 414$ observations, 6 sites; ranch type: estimate ± s.e (private) = 6.47 ± 2.87, $p = 0.088$; sex: estimate ± s.e (male) = −0.370 ± 0.596, $p = 0.535$).

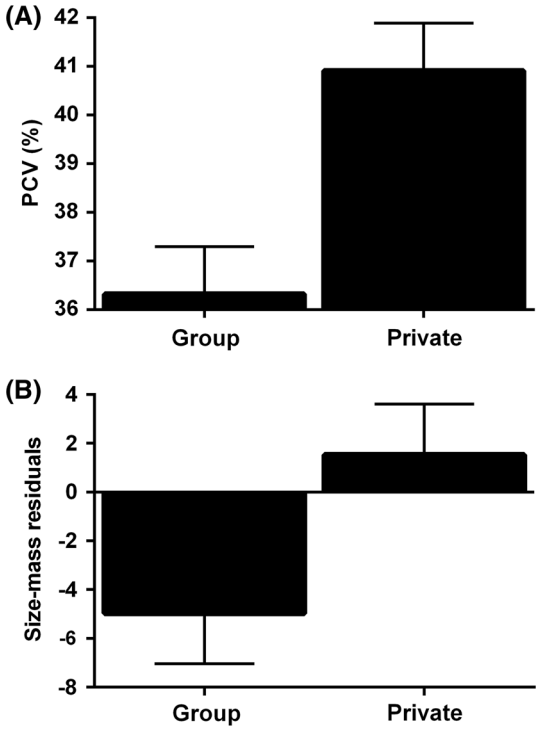


Figure 2. Mean ± standard error of condition measures on group and private ranches: **a** PCV and **b** size–mass residuals.

Predictors of Individual Exposure Risk

Across all sites, and controlling for sex and condition (measured as PCV), we found that *Brucella* exposure risk in cattle was marginally lower on private ranches (OR = 0.05, CI = 0.002–1.12, $p = 0.059$; Table 3a). However, this trend disappeared after we excluded Group Site 3 (OR = 0.24, CI = 0.02–3.05, $p = 0.276$; Table 3b), which had an overall *Brucella* seroprevalence ∼ 10 times higher than any other site (Table 2). As with *Brucella*, there was no association between ranch type and exposure to BVDV (Table 3a,b). In contrast, the risk of being exposed to *Leptospira* was significantly higher on private ranches compared to group ranches, and this effect was consistent across analyses including all sites (OR = 9.6, CI = 2.86–32.4, $p = 0.0002$; Table 3a) and excluding Group Site 3 (OR = 14.6, CI = 4.91–43.2, $p < 0.0001$; Table 3b).

Finally, the *Leptospira* and BVDV models that included suspect cases as either all seropositive or all seronegative, showed results that were qualitatively similar to those generated from models that excluded the suspect cases (Table S1 of ESM). These results suggest that the way in which suspect cases were dealt with in our analyses did not affect our conclusions about *Leptospira* and BVDV exposure risk.

Discussion

We compared pathogen exposure in cattle across two ranch types that represent distinct forms of land use for livestock production in rural Kenya. Broadly, our results align with previous pathogen serosurveys of cattle across Kenya. For example, we reported an average prevalence of 35.6% for *Leptospira* and 7% for *Brucella*, which are both similar to previously reported numbers of 25–34% for *Leptospira* (de Vries et al. 2014) and 0.8–16% on smallholder farms for *Brucella* (Njeru et al. 2016). Importantly, private and group ranches in our study region differ in ways that influence wildlife and livestock demographics and may create significant variation among species contact patterns across sites. We predicted that these differences could potentially explain variation in pathogen exposure rates between ranch types. Because private ranches have been reported to support up to 14 times more wildlife compared to livestock

Table 3. Predictors of Individual Pathogen Exposure Risk in Cattle Across All Sites (3a) and Excluding Group Site #3 (3b).

Risk factor	Est ± std error *Brucella* (n = 372)	Z	P value	Est ± std error BVDV (n = 386)	Z	P-value	Est ± std error *Leptospira* (n = 367)	Z	P value
3a									
Ranch type (private)	−3.00 ± 1.59	−1.89	0.059	1.51 ± 0.99	1.53	0.126	2.26 ± 0.61	3.66	**0.0002**
PCV	0.09 ± 0.05	1.74	0.082	0.006 ± 0.03	0.16	0.870	0.036 ± 0.03	1.11	0.267
Sex (male)	1.98 ± 0.72	2.74	**0.006**	−0.41 ± 0.46	−0.89	0.370	−0.92 ± 0.43	−2.14	**0.032**

Risk factor	Est ± std error *Brucella* (n = 299)	Z	P value	Est ± std error BVDV (n = 318)	Z	P-value	Est ± std error *Leptospira* (n = 300)	Z	P value
3b									
Ranch type (private)	−1.39 ± 1.28	−1.09	0.276	1.93 ± 1.15	1.67	0.095	2.68 ± 0.55	4.83	**0.000001**
PCV	0.087 ± 0.09	0.934	0.350	−0.006 ± 0.04	−0.17	0.868	0.06 ± 0.04	1.45	0.146
Sex (male)	2.02 ± 0.75	2.689	**0.007**	−0.41 ± 0.46	−0.88	0.377	−0.89 ± 0.43	−2.07	**0.038**

Binomial GLMMs include site as a random effect.
Bold values are statistically significant at $p < 0.05$.

than do group ranches (Figure 1), we expected that this trait could elevate cross-species contact rates on private when compared to group ranches. We expected that any effect of enhanced wildlife contact on pathogen exposure would be particularly apparent for indirectly transmitted pathogens that do not require direct contact between species in order for transmission to occur. In support, we found that *Leptospira* exposure in cattle was significantly higher on private ranches compared to group ranches. In contrast, *Brucella* and BVDV exposure were not associated with ranch type. Our results suggest that wildlife–livestock contact may play a key role in shaping pathogen transmission to livestock in this system, although the magnitude of such effects likely depends on characteristics of the pathogen in question.

Leptospira seroprevalence was over three times higher on private ranches than on group ranches (53.5 vs. 17.8%), and cattle on private ranches had significantly higher exposure, with over nine times higher odds of being seropositive. Given higher wildlife densities on private ranches compared to group ranches, our results suggest that overlap between cattle and wild species may be an important risk factor for *Leptospira* infection. Importantly, indirect contact plays a major role in the transmission of *Leptospira*. In cattle, contaminated pasture, water sources and co-grazing with other infected livestock species (e.g., sheep and goats) are known risk factors for *Leptospira* infection (Mazeri et al. 2013). Since wildlife are important reservoirs for multiple *Leptospira* serovars (Bengis et al. 2004), contamination of water sources by these species may also be a major risk factor for cattle (Vijayachari et al. 2008; Siembieda et al. 2011). Indeed, a recent study in a pastoralist setting in Tanzania showed that infection prevalence of *Leptospira* serovar Hardjo was highest in cattle (17%) and wild buffalo (8%), low in goats (2%) and undetected in rodents (Assenga et al. 2015b). These results suggest not only that wild ungulates can act as a potential source of infection for cattle, but that in some systems, they may play a more important role than other livestock species.

Unlike *Leptospira*, BVDV seroprevalence did not differ substantially between private and group ranches (84.3 vs. 66.6%), and individual exposure risk to BVDV was not associated with ranch type. Similarly, although *Brucella* seroprevalence was over four times higher on group compared to private ranches (11.7 vs. 2.5%), this difference was driven by a single site which had over 20% prevalence (Group Site 3), and when this site was excluded from analysis, there was no significant difference in *Brucella* exposure risk between ranch types. The lack of an effect of ranch type on exposure risk to BVDV and *Brucella* suggests first that the relatively small differences in cattle densities between ranch types may not be sufficient to drive differences in within-species (cattle) transmission of these pathogens, and second that the nature in which these pathogens are transmitted might limit between-species (cattle–wildlife) transmission. For BVDV, transmission is largely driven by key individuals that are persistently in-

fected (PI) throughout their lifetimes and continuously shed virus in their bodily secretions (Lindberg and Houe 2005; Nelson et al. 2015). Moreover, the virus does not survive long in the environment, particularly at high temperatures (Nelson et al. 2015), necessitating close spatial and/or temporal overlap between individuals for effective transmission. Thus, close contact with specific key hosts likely drives most of the variation in exposure to BVDV. Although persistent infection is known to occur in wild ungulates, including in eland (Nelson et al. 2015), which are widely distributed across our study region, close contact between livestock and wildlife is rare in our system, so the likelihood that cattle would acquire infection from PI wildlife is probably low. For this reason, it seems unlikely that wildlife would make a significant contribution to BVDV infection in cattle. High BVDV prevalence in cattle herds has often been attributed to the presence of PI individuals within specific herds (Courcoul and Ezanno 2010), and it is possible that the high (close to 100%) seroprevalence of BVDV we observed on some of the ranches in our study can be explained by variation in the frequency of persistent infection. PI individuals cannot be detected using antibody tests so we were unable to evaluate how the frequency of PI individuals varied among herds or by ranch type in our study, but this would be an interesting focus for future work.

As with BVDV, the mode of transmission of *Brucella* may limit the opportunities for cattle to be infected directly by wildlife in our study system. While *Brucella* spp. have been isolated from several wild ungulate species that occur in our study region, such as buffalo (Daliner and Staak 1973; Gradwell et al. 1977), impala (Schiemann and Staak 1971) and waterbuck (*Kobus ellipsiprymnus;* Groocock and Staak 1969), and past serological studies in Kenya have reported relatively high seroprevalence rates in buffalo (30%; Waghela and Karstad 1986), the level of close contact between these species and cattle that would facilitate transmission probably only rarely occurs. Thus, it is likely that cattle–cattle interactions drive the majority of *Brucella* transmission across our study sites. It is notable, however, that there was only minimal variation in *Brucella* seroprevalence (1.9–3.4%) among five out of six of our sites, a pattern which suggests that cattle contact rates may be relatively equivalent across sites. By contrast, the one outlying site, Group Site 3, which had a seroprevalence of nearly 30%, was unusual in that cattle from this site were relocated a long distance prior to sampling, a factor which might have contributed to the elevated *Brucella* exposure compared to other sites. More generally, although a few studies have reported evidence of *Brucella* co-circulation among sympatric cattle and wildlife (mainly buffalo) in African rangeland systems (e.g., Assenga et al. 2015a, Tanner et al. 2015), little is known about the extent to which cross-species transmission is occurring for this pathogen. Recently, genetic and genomic tools have been applied to understanding similar issues in the Greater Yellowstone Ecosystem of the USA (Beja-Pereira et al. 2009; Kamath et al. 2016; O'Brien et al. 2017), a system in which wildlife act as a key reservoir of *Brucella* for cattle. Similar techniques could be applied to African rangeland systems to help clarify situations in which wildlife play a role in *Brucella* transmission to livestock.

Quantifying contact rates between livestock and wildlife is an important first step toward understanding whether and how wildlife contribute to pathogen transmission in livestock. In this study, we could not directly quantify wildlife and livestock densities or contact rates at each study site, so we used ranch type as a proxy for this critical value. We based our assumption of asymmetric contact between livestock and wildlife on private versus group ranches on two studies that rigorously quantified domestic and wild herbivore densities across ranch types in the study region. Our results suggest that for at least some pathogens, ranch type is strongly associated with exposure risk to cattle. Future work will have to tease apart whether this effect is due to differential contact with wildlife, as we hypothesize here, or other factors we failed to consider in this study. Nevertheless, we attempted to account for at least two important confounders in our analysis: body condition and sex. Body condition has the potential to skew infection patterns across ranch types because of known differences in forage quality and veterinary care which could translate into differences in body condition and pathogen susceptibility. Indeed, we found that body condition, measured as both PCV and size–mass residuals, was higher on private ranches. However, the significant effect of ranch type we observed for *Leptospira* was independent of host condition. Likewise, since sex biases in pathogen infection are commonly observed in mammals (Moore and Wilson 2002), we also accounted for sex in all of our analyses. Although we found a female bias in *Leptospira* infection, the effect of ranch type was independent of sex.

Conclusion

Overall, we found that ranch type was associated with the risk of cattle exposure for one out of three focal pathogens

in our study. Specifically, cattle on private ranches were significantly more likely to be exposed to *Leptospira* serovar Hardjo, but not to BVDV or *Brucella* spp. For *Leptospira*, where environmental contamination is a major route of transmission, the strong positive association between pathogen risk and private ranching suggests that contact with wild ungulate species may be a significant driver of differences in exposure. For *Brucella* and BVDV, the lack of an association suggests that differences in cattle densities across sites are not sufficiently large to drive variation in transmission for these pathogens and that contact with wildlife also does not contribute to variation in exposure. In conclusion, interspecific contact relevant to pathogen transmission is a difficult phenomenon to quantify. In this study, we used previously reported differences in livestock and wild herbivore densities between two common ranching systems in rural Kenya, as a proxy for livestock–wildlife contact rates. Our study reveals intriguing variation in the relationship between ranch type and pathogen exposure by pathogen type. Ultimately, this is a useful first step toward understanding the factors that influence infectious disease transmission at the wildlife–livestock interface.

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References

Afrane YA, Lawson BW, Githeko AK, Yan G (2005) Effects of microclimatic changes caused by land use and land cover on duration of gonotrophic cycles of Anopheles gambiae (Diptera: Culicidae) in western Kenya highlands. *Journal of Medical Entomology* 42:974–980

Assenga JA, Matemba LE, Muller SK, Malakalinga JJ, Kazwala RR (2015) Epidemiology of Brucella infection in the human, livestock and wildlife interface in the Katavi-Rukwa ecosystem. *Tanzania. BMC Veterinary Research* 11(1):189

Assenga JA, Matemba LE, Muller SK, Mhamphi GG, Kazwala RR (2015) Predominant leptospiral serogroups circulating among humans, livestock and wildlife in Katavi-Rukwa ecosystem. *Tanzania. PLoS Neglected Tropical Diseases* 9(3):e0003607

Bates D, Maechler M, Bolker B, Walker S (2015) Fitting Linear Mixed-Effects Models Using lme4. *Journal of Statistical Software* 67:1–48

Beja-Pereira A, Bricker B, Chen S, Almendra C, White PJ, Luikart G (2009) DNA Genotyping Suggests that Recent Brucellosis Outbreaks in the Greater Yellowstone Area Originated from Elk. *Journal of Wildlife Diseases* 45:1174–1177

Bengis R, Leighton F, Fischer J, Artois M, Morner T, Tate C (2004) The role of wildlife in emerging and re-emerging zoonoses. *Revue Scientifique et Technique-Office International des Epizooties* 23:497–512

Callaby R, Toye P, Jennings A, Thumbi SM, Coetzer JAW, Van Wyk IC, Woolhouse MEJ (2016) Seroprevalence of respiratory viral pathogens of indigenous calves in Western Kenya. *Research in Veterinary Science* 108:120–124

Cleaveland S, Laurenson M, Taylor L (2001) Diseases of humans and their domestic mammals: pathogen characteristics, host range and the risk of emergence. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences* 356:991–999

Courcoul A, Ezanno P (2010) Modelling the spread of Bovine Viral Diarrhoea Virus (BVDV) in a managed metapopulation of cattle herds. *Veterinary Microbiology* 142:119–128

Daliner G, Staak C (1973) A case of orchitis caused by *Brucella abortus* in the African buffalo. *Journal of Wildlife Diseases* 9:251–253

Devleesschauwer, B., Torgerson, P., Charlier, J., Levecke, B., Praet, N., Roelandt, S. et al. (2014). prevalence: Tools for prevalence assessment studies.

de Vries SG, Visser BJ, Nagel IM, Goris MG, Hartskeerl RA, Grobusch MP (2014) Leptospirosis in Sub-Saharan Africa: a systematic review. *International Journal of Infectious Diseases* 28:47–64

Ducrotoy M, Bertu WJ, Matope G, Cadmus S, Conde-Álvarez R, Gusi AM, Moriyón I (2015) Brucellosis in Sub-Saharan Africa: Current challenges for management, diagnosis and control. *Acta Tropica* 165:179–193

Georgiadis NJ, Olwero J, Ojwang G, Romanch SS (2007) Savanna herbivore dynamics in a livestock-dominated landscape: I. Dependence on land use, rainfall, density, and time. *Biological Conservation* 137:461–472

Goe M, Alldredge J, Light D (2001) Use of heart girth to predict body weight of working oxen in the Ethiopian highlands. *Livestock Production Science* 69:187–195

Grace D, Himstedt H, Sidibe I, Randolph T, Clausen P-H (2007) Comparing FAMACHA eye color chart and Hemoglobin Color Scale tests for detecting anemia and improving treatment of bovine trypanosomosis in West Africa. *Veterinary Parasitology* 147:26–39

Gradwell DV, Schutte AP, Van Niekerk CAWJ, Roux DJ (1977) The isolation of *Brucella abortus* biotype I from African buffalo

in the Kruger National Park. *Journal of the South African Veterinary Association* 48:41–44

Groocock CM, Staak C (1969) The isolation of *Brucella abortus* from a waterbuck (*Kobus ellipsiprymnus*). *Veterinary Record* 85:318

Grooms DL (2006) Reproductive losses caused by bovine viral diarrhea virus and leptospirosis. *Theriogenology* 66:624–628

Kamath PL, Foster JT, Drees KP, Luikart G, Quance C, Anderson NJ, Clarke PR, Cole EK, Drew ML, Edwards WH, Rhyan JC, Treanor JJ, Wallen RL, White PJ, Robbe-Austerman S, Cross PC (2016) Genomics reveals historic and contemporary transmission dynamics of a bacterial disease among wildlife and livestock. *Nature Communications* 7:11448

Karesh WB, Dobson A, Lloyd-Smith JO, Lubroth J, Dixon MA, Bennett M, et al. (2012) Ecology of zoonoses: natural and unnatural histories. *The Lancet* 380:1936–1945

Kashoma, I., Luziga, C., Werema, C., Shirima, G. & Ndossi, D. (2011). Predicting body weight of Tanzania shorthorn zebu cattle using heart girth measurements. *Livestock Research for Rural Development*, 23.

Kinnaird MF, O'Brien TG (2012) Effects of Private, ÄêLand Use, Livestock Management, and Human Tolerance on Diversity, Distribution, and Abundance of Large African Mammals. *Conservation Biology* 26:1026–1039

Kock, M. (2005). The health paradigm and protected areas: linkages between people and their livelihoods, ecosystems and natural communities, and health and disease. *Conservation and development interventions at the wildlife/livestock interface: Implications for wildlife, livestock and human health. Gland, Switzerland and Cambridge, UK: IUCN, p. xxv. Available:* http://www.wcs-ahead.org/book/AHEADbook6.5MB.pdf.

Lanyon SR, Anderson ML, Bergman E, Reichel MP (2013) Validation and evaluation of a commercially available ELISA for the detection of antibodies specific to bovine viral diarrhoea virus (bovine pestivirus). *Australian Veterinary Journal* 91:52–56

Lindberg A, Houe H (2005) Characteristics in the epidemiology of bovine viral diarrhea virus (BVDV) of relevance to control. *Preventive Veterinary Medicine* 72:55–73

LoGiudice K, Duerr ST, Newhouse MJ, Schmidt KA, Killilea ME, Ostfeld RS (2008) Impact of host community composition on Lyme disease risk. *Ecology* 89:2841–2849

Mazeri S, Scolamacchia F, Handel IG, Morgan KL, Tanya VN, Barend M (2013) Risk factor analysis for antibodies to Brucella, Leptospira and C. burnetii among cattle in the Adamawa Region of Cameroon: a cross-sectional study. *Tropical Animal Health and Production* 45:617–623

McDermott J, Grace D, Zinsstag J (2013) Economics of brucellosis impact and control in low-income countries. *Revue Scientifique et Technique - Office International des Epizooties* 32:249–261

Molyneux D, Hallaj Z, Keusch GT, McManus DP, Ngowi H, Cleaveland S, et al. (2011) Zoonoses and marginalised infectious diseases of poverty: where do we stand. *Parasites & Vectors* 4:106

Moore SL, Wilson K (2002) Parasites as a viability cost of sexual selection in natural populations of mammals. *Science* 297:2015–2018

Myburgh J, Staley G, Van Der Merwe S (1989) Serological evidence of bovine leptospirosis in Malawi. *Onderstepoort Journal of Veterinary Research* 56:285

Niang M, Will LA, Kane M, Hussain M (1994) Seroprevalence of leptospiral antibodies among dairy cattle kept in communal corrals in periurban areas of Bamako, Mali, West Africa. *Preventive Veterinary Medicine* 18:259–265

Nelson DD, Duprau JL, Wolff PL, Evermann JF (2015) Persistent Bovine Viral Diarrhea Virus Infection in Domestic and Wild Small Ruminants and Camelids Including the Mountain Goat (*Oreamnos americanus*). *Frontiers in Microbiology* 6:1415

Njeru J, Wareth G, Melzer F, Henning K, Pletz MW, Heller R, Neubauer H (2016) Systematic review of brucellosis in Kenya: disease frequency in humans and animals and risk factors for human infection. *BMC Public Health* 16(1):853

O'Brien MP, Beja-Pereira A, Anderson N, Ceballos RM, Edwards WH, Harris B, Wallen RL, Costa V, Luikart G (2017) Brucellosis Transmission between Wildlife and Livestock in the Greater Yellowstone Ecosystem: Inferences from DNA Genotyping. *Journal of Wildlife Diseases* 53:339–343

Ogutu JO, Piepho H-P, Said MY, Ojwang GO, Njino LW, Kifugo SC, Wargute PW (2016) Extreme wildlife declines and concurrent increase in livestock numbers in Kenya: What are the causes? *PloS One* 11:e0163249

Schiemann B, Staak C (1971) *Brucella melitensis* in impala (*Aepyceros melampus*). *Veterinary Record* 88:344

Olsen S, Tatum F (2010) Bovine brucellosis. *The Veterinary Clinics of North America. Food Animal Practice* 26:15

Patz JA, Daszak P, Tabor GM, Aguirre AA, Pearl M, Epstein J, et al. (2004) Unhealthy landscapes: policy recommendations on land use change and infectious disease emergence. *Environmental Health Perspectives* 112:1092

Perry BD, Grace D, Sones K (2011) Current drivers and future directions of global livestock disease dynamics. *Proceedings of the National Academy of Sciences* 110:20871–20877

Pulliam JR, Epstein JH, Dushoff J, Rahman SA, Bunning M, Jamaluddin AA, et al. (2012) Agricultural intensification, priming for persistence and the emergence of Nipah virus: a lethal bat-borne zoonosis. *Journal of the Royal Society Interface* 9:89–101

Schoonman L, Swai ES (2010) Herd-and animal-level risk factors for bovine leptospirosis in Tanga region of Tanzania. *Tropical Animal Health and Production* 42:1565–1572

Schulte-Hostedde AI, Zinner B, Millar JS, Hickling GJ (2005) Restitution of mass-size residuals: validating body condition indices. *Ecology* 86:155–163

Siembieda JL, Kock RA, McCracken TA, Newman SH (2011) The role of wildlife in transboundary animal diseases. *Animal Health Research Reviews* 12:95–111

Sundaresan SR, Riginos C (2010) Lessons learned from biodiversity conservation in the private lands of Laikipia, Kenya. *Great Plains Research* 20:17–27

Tanner M, Inlameia O, Michel A, Maxlhuza G, Pondja A, Fafetine J, Macucule B, Zacarias M, Manguele J, Moiane IC, Marranangumbe AS, Mulandane F, Schönfeld C, Moser I, van Helden P, Machado A (2015) Bovine Tuberculosis and Brucellosis in Cattle and African Buffalo in the Limpopo National Park, Mozambique. *Transboundary and Emerging Diseases* 62:632–638

Vijayachari P, Sugunan A, Shriram A (2008) Leptospirosis: an emerging global public health problem. *Journal of Biosciences* 33:557–569

Vilcek S, Nettleton P (2006) Pestiviruses in wild animals. *Veterinary Microbiology* 116:1–12

Waghela S, Karstad L (1986) Antibodies to *Brucella* spp. among blue wildebeest and African buffalo in Kenya. *Journal of Wildlife Diseases* 22:189–192

Walz P, Grooms D, Passler T, Ridpath J, Tremblay R, Step D, et al. (2010) Control of bovine viral diarrhea virus in ruminants. *Journal of Veterinary Internal Medicine* 24:476–486