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Declines in large wildlife increase landscape-level prevalence of rodent-borne disease in Africa

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Populations of large wildlife are declining on local and global scales. The impacts of this pulse of size-selective defaunation include cascading changes to smaller animals, particularly rodents, and alteration of many ecosystem processes and services, potentially involving changes to prevalence and transmission of zoonotic disease. Understanding linkages between biodiversity loss and zoonotic disease is important for both public health and nature conservation programs, and has been a source of much recent scientific debate. In the case of rodent-borne zoonoses, there is strong conceptual support, but limited empirical evidence, for the hypothesis that defaunation, the loss of large wildlife, increases zoonotic disease risk by directly or indirectly releasing controls on rodent density. We tested this hypothesis by experimentally excluding large wildlife from a savanna ecosystem in East Africa, and examining changes in prevalence and abundance of *Bartonella* spp. infection in rodents and their flea vectors. We found no effect of wildlife removal on per capita prevalence of *Bartonella* infection in either rodents or fleas. However, because rodent and, consequently, flea abundance doubled following experimental defaunation, the density of infected hosts and infected fleas was roughly twofold higher in sites where large wildlife was absent. Thus, defaunation represents an elevated risk in *Bartonella* transmission to humans (bartonellosis). Our results (*i*) provide experimental evidence of large wildlife defaunation increasing landscape-level disease prevalence, (*ii*) highlight the importance of susceptible host regulation pathways and host/vector density responses in biodiversity–disease relationships, and (*iii*) suggest that rodent-borne disease responses to large wildlife loss may represent an important context where this relationship is largely negative.

Kenya | dilution effect

We are in the midst of a global extinction crisis (1). Among mammals, for which population trends are known, more than 50% of species are currently declining (2). The current pulse of global defaunation is widely recognized to be size-selective, with larger species exhibiting greater risks for population declines or extinctions (3–5). This bias derives from a variety of factors, including harvester preference for large-bodied species, expansive habitat requirements of these large species, and the fact that large animals typically have life history traits associated with slow population growth (e.g., low fertility, long generation time, later age of reproduction) (6, 7). Body size is also strongly correlated with functional roles (8), and large species thus often play functionally distinct and impactful roles in ecosystems (9). The systematic decline of large species, both herbivores and predators, is thus often associated with pronounced effects on other aspects of community composition and structure (5, 10, 11), ecosystem function (11), and even evolutionary trajectories (12).

Recently, there has been growing interest in understanding what the effects of wildlife declines of this type may be for the emergence and prevalence of infectious zoonotic diseases, with active debate on both the direction and generality of diversity–disease relationships (13–18) and on the likely implications for human health (19, 20). Characterizing the nature of this relationship has broad significance. Zoonotic disease agents present a growing threat to global health. At least 60% of all human disease agents are zoonotic in origin (21), and mammals are the primary reservoir hosts for most known zoonotic diseases in humans (22, 23). The suggestion that anthropogenically driven wildlife declines may lead to increased disease risk in a landscape has thus generated a great deal of interest because it raises the possibility that conservation of intact natural landscapes may be an effective intervention strategy for mediating emerging threats to public health. Much of the research and debate on this relationship thus far has focused on the mechanism of transmission interference, or the process by which systematic changes in community richness (number of species), composition (identity of species), and host competence (the proportion of individuals of a species that can maintain and transmit infections) affects community competence and, ultimately, prevalence of pathogens (proportion of hosts infected) in a community, without necessarily changing the absolute abundance of susceptible hosts (individuals that can be infected by a pathogen) (15, 24, 25). The present study focuses primarily instead on a second, hitherto less explored pathway, susceptible host regulation, or the process by which biodiversity loss changes the abundance of susceptible hosts (26).

Rodents are common reservoir hosts (long-term source hosts for a pathogen) for many human zoonotic pathogens, such as *Borrelia burgdorferi* (Lyme disease) (24), hantaviruses [hantavirus

Significance

Understanding the effects of biodiversity loss on zoonotic disease is of pressing importance to both conservation science and public health. This paper provides experimental evidence of increased landscape-level disease risk following declines in large wildlife, using the case study of the rodent-borne zoonosis, bartonellosis, in East Africa. This pattern is driven not by changes in community composition or diversity of hosts, as frequently proposed in other systems, but by increases in abundance of susceptible hosts following large mammal declines. Given that rodent increases following large wildlife declines appear to be a widespread pattern, we suggest this relationship is likely to be general.

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pulmonary syndrome (HPS)] (27), *Yersinia pestis* (plague) (28), and *Bartonella* (bartonellosis) (29). They are particularly important hosts for flea-borne diseases, which are absent or low in prevalence in most larger wild animals, many of which do not carry fleas (30). A great deal of work in both the Lyme disease and HPS systems has documented relationships between host species richness, community composition, and prevalence of these pathogens (31, 32). Changes in susceptible host abundance would appear to be a particularly likely pathway by which wildlife loss and disturbance could affect rodent-borne disease risk. By contrast to larger-bodied mammals, rodents, particularly small-sized species (i.e., <500 g), are often relatively robust to human disturbance and many species live commensally with humans (33, 34). Due to their generally rapid reproductive rates and small home range sizes, populations can fluctuate dramatically over both small spatial and temporal scales, and in response to declines or removals of either rodent predators or rodent competitors, including large herbivores (35–37). Because both large predators and herbivores face a high risk of decline from human disturbances (6, 10), susceptible host regulation may be a strong potential pathway by which wildlife loss can affect human disease risk.

There is theoretical support and empirical evidence of a negative correlation between large wildlife loss and increased rodent-borne disease risk (26, 27, 38). However, we largely lack experimental data that document if and how the cascading effects of large wildlife loss have an impact on rodent-borne zoonoses via susceptible host regulation. In this study, we look at the overall effects of declines in large wildlife, particularly large herbivores (>15 kg), on landscape-level risk for the rodent-borne pathogen *Bartonella* spp., known to affect human health negatively (bartonellosis) (29). *Bartonella* is a globally distributed, facultative intracellular bacterial parasite (Alphaproteobacteria) that is found in a wide variety of mammals, including humans and rodents. The identity and pathogenicity of many species of *Bartonella* to humans are unknown, particularly in Africa. However, many species, including several of the species detected in this study (e.g., *Bartonella rochalimae*, *Bartonella grahamii*, *Bartonella elizabethae*) are known or suspected to be human pathogens. *Bartonella* spp. infection is characterized by long-lasting intraerythrocytic, relapsing bacteremia that can induce pathology in multiple organ systems in humans (29). Although host specificity varies widely, many strains appear to have rodents as the primary reservoir hosts (39, 40). *Bartonella* also appears to be primarily vector-borne [although direct and vertical transmission, from parent to offspring, may also occur (40)], with fleas being confirmed vectors (41, 42). *Bartonella* is increasingly being recognized as a zoonotic disease with implications for human and animal health (29).

We conducted this work in a long-term and well-controlled, replicated large herbivore removal experiment based in central Kenya, the Kenya Long-Term Exclosure Experiment (KLEE) (43). Within this experimental setup, we examined the effects of large wildlife removal on four different metrics of *Bartonella* disease risk: (*i*) the abundance of infected hosts (rodents), (*ii*) the prevalence of infection in these hosts, (*iii*) the abundance of infected vectors (fleas), and (*iv*) the infection prevalence in vectors.

As research and debate continue to expand regarding the direction, magnitude, and generality of the relationship between biodiversity and disease, there is consensus that no single relationship is likely to be universal across pathogens and systems (15–18, 44, 45). Thus, to make knowledge on the diversity–disease relationship useful to managers, policy makers, and scientists, research needs to focus on the following: (*i*) understanding the context (e.g., types of hosts, vectors, pathogens, disturbance) in which either positive or negative relationships are most likely and (*ii*) understanding the mechanisms by which these relationships occur (19). Here, based on our results from infection prevalence of *Bartonella* spp. in rodents under experimental defaunation, we argue that the intersection of large wildlife decline and rodent-borne disease may provide a particularly likely context for negative diversity–disease relationships to be general. Additionally, we conclude that susceptible host regulation, rather than transmission interference, likely drives the relationship in this ecological context.

Results

Rodent and Flea Abundance. A total of 832 rodents, representing 11 species, were captured in five sampling periods in the KLEE (Fig. 1*A*). Treatments in which we simulated the loss of large wildlife demonstrated strong and consistent elevation (compared with control treatment) in total rodent density per unit area, despite pronounced seasonal variation in rodent abundance (Fig. S1; $F_{1,4} = 54.9$, $P < 0.01$). This pattern is quite robust across years and seasons, having been shown to persist in the KLEE for more than a decade despite large fluctuations in rainfall patterns (32, 35, 46). One species, *Saccostomus mearnsi* (Mearns's pouched mouse), dominated capture rates in both exclosures and open plots, accounting for 75% of all captures. The same pattern of roughly doubled abundance in exclosure plots across time was observed when considering just *S. mearnsi* (Fig. 1*B*). There was no significant change observed in rodent diversity between control and large mammal removal treatments (Fig. 2*A*; Shannon diversity index: $F_{1,4} = 0.3$, $P = 0.6$) and no significant change in overall community composition [analysis of similarity (ANOSIM): $R = 0.30$, $P = 0.20$]. There was also no significant difference in age or sex structure of populations between exclosure and control plots (data not shown). Thus, for the rodent community, only abundance varied significantly among plots.

Fleas comprised more than 95% of the total ectoparasites sampled from captured rodents, with only occasional lice or mites (*Androlaelaps* spp.) and no ticks observed. A total of 1,570 fleas were surveyed in this study. Flea diversity encompassed four genera. From the genus *Xenopsylla*, three species were identified: *Xenopsylla brasiliensis*, *Xenopsylla cheopis* (*aequisetosa*), and *Xenopsylla sarodes sarodes*. Other species found were *Dinopsyllus*

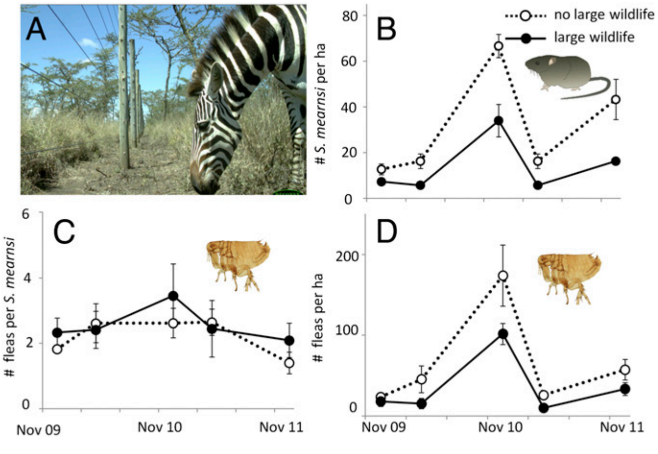

Fig. 1. Working in the KLEE, which examines the effect of large wildlife (e.g., zebra, giraffe, elephant, gazelle) removal (*A*), we found that *S. mearnsi* abundance was significantly higher (roughly double on average) in plots where large mammals had been removed compared with open plots (*B*) despite strong seasonal variability. (*C*) There was no significant difference in the intensity of infestation of fleas per rodent between treatments. (*D*) As a result, the density of fleas per hectare is significantly higher (roughly double) in plots without large wildlife. Error lines represent 1 SE, based on three replicate blocks. Data for all rodents are shown in Fig. S1. Photography credits: *A*, KLEE exclosure, D. Kimuyu (*Equus quagga*, Nanyuki, Kenya); *Inset* of flea in *C* and *D X. sarodes sarodes* (male), M. Hastriter and Michael Whiting, Monte L. Bean Museum, Brigham Young University, Provo, UT.

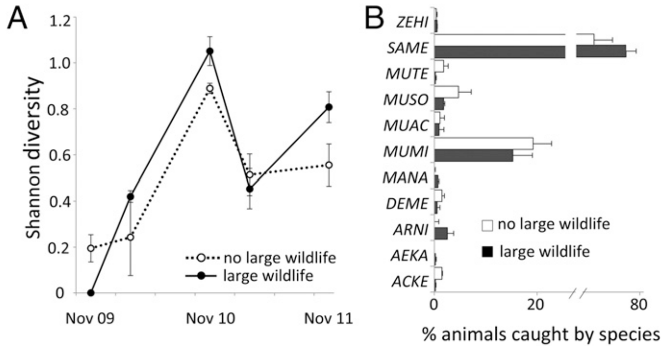

Fig. 2. (*A*) Rodent diversity (Shannon diversity index ± SE, across plots within a season) was not significantly different between treatments with and without large mammalian wildlife. (*B*) There were also no significant differences in community similarity between experimental plots (animals pooled across sampling seasons). Species codes are as follows: ACKE, *Acomys kempi*; AEKA, *Aethomys kaiseri Arvicanthis niloticus*; DEME, *Dendromus melanotis*; MANA, *Mastomys natalensis*; MUAC, *Mus cf. acholi*; MUMI, *Mus minutoides*; MUSO, *Mus sorella*; MUTE, *Mus tenellus*; SAME, *S. mearnsi*; ZEHI, *Zelotomys hildegardeae*.

lypusus, *Parapulex echinatus*, and *Ctenophthalmus calceatus cabirus*. More than 98% of all fleas were *Xenopsylla* spp. (Fig. S2), and there was no difference in flea diversity ($F_{1,4} = 0.7$, $P = 0.5$) or community composition between exclosure and control treatments (ANOSIM: $R = 0.15$, $P = 0.4$). Four of the six species of fleas found were present on *S. mearnsi.* Three of the species were found on multiple host species (all host flea associations are shown in Tables S1 and S2).

S. mearnsi had much higher flea density than any other species, and this single species thus accounted for >95% of all fleas in both exclosure and control plots. Given the very low number of fleas from other species, and the lack of any differences in diversity or composition of hosts among treatments, all subsequent analyses were conducted using only *S. mearnsi* (data with all rodents are shown in Figs. S1 and S3). There was no significant difference in average abundance of fleas per individual rodent across treatments (Fig. 1*C*; $F_{1,4} = 4.9$, $P = 0.09$). However, because of the increases in rodent abundance, there was more than a doubling (103 ± 23% increase) in total numbers of fleas per hectare (Fig. 1*D*; $F_{1,4} = 27.7$, $P < 0.01$). This abundance pattern was robust and persistent across years (47).

***Bartonella* Prevalence.** We measured *Bartonella* prevalence (proportion of individuals infected) in both the rodent host *S. mearnsi* and its fleas. A total of 157 *S. mearnsi* individuals were screened for *Bartonella* across the three time periods (94 in exclosures and 63 in open sites). BLAST analyses identified *Bartonella* genotypes related to the Sc-tr1 group (from *Saccostomus campestris*, South Africa) (48), the OY group (including *B. rochalimae*, *B. grahamii*, and *B. elizabethae*) (49), and several previously unidentified genotypes.

Bartonella prevalence in hosts did not differ significantly between treatments with and without wildlife (Fig. S3; $F_{1,4} = 0.4$, $P = 0.55$), although it did vary across sampling periods (Fig. S3; $F_{2,3} = 14.6$, $P = 0.03$). There was no significant treatment × time interaction ($F_{2,3} = 3.7$, $P = 0.16$). Likewise there was no significant difference in the prevalence of *Bartonella* in the fleas sampled from *S. mearnsi* in control plots compared with wildlife exclosures (Fig. 3*A*; $F_{1,4} = 0.6$, $P = 0.49$) or any evidence of a treatment × time interaction (Fig. 3*A*; $F_{2,3} = 2.2$, $P = 0.26$). There was again variation by sampling period ($F_{1,4} = 53.7$, $P = 0.01$). In contrast to these results, the abundance of *Bartonella*-infected hosts varied strongly by treatment (Fig. 3*B*; $F_{1,4} = 19.3$, $P = 0.01$), with roughly twice as many infected hosts in plots where wildlife had been removed, due entirely to the higher density of rodent populations. There was both a sampling period effect (Fig. 3*B*; $F_{2,3} = 283.2$, $P < 0.0001$) and a time × treatment interaction ($F_{2,3} = 55.3$, $P < 0.01$). The abundance of infected fleas also varied by treatment (Fig. 3*C*; $F_{1,4} = 10.7$, $P = 0.03$) and by sampling period ($F_{2,3} = 463.6$, $P < 0.001$). When a time × treatment interaction effect occurred, the effects of treatment were stronger in sampling periods where rodent or vector abundance was higher.

Discussion

As large wildlife continues to decline globally, ecologists, conservation scientists, and health practitioners are challenged to understand and interpret the implications of these changes for ecological communities and the people who inhabit these spaces. These results provide strong experimental evidence that the effects of differential loss of large wildlife can cascade to cause increases in the abundance of zoonotic pathogens across a landscape, via an increase in the abundance of hosts and vectors.

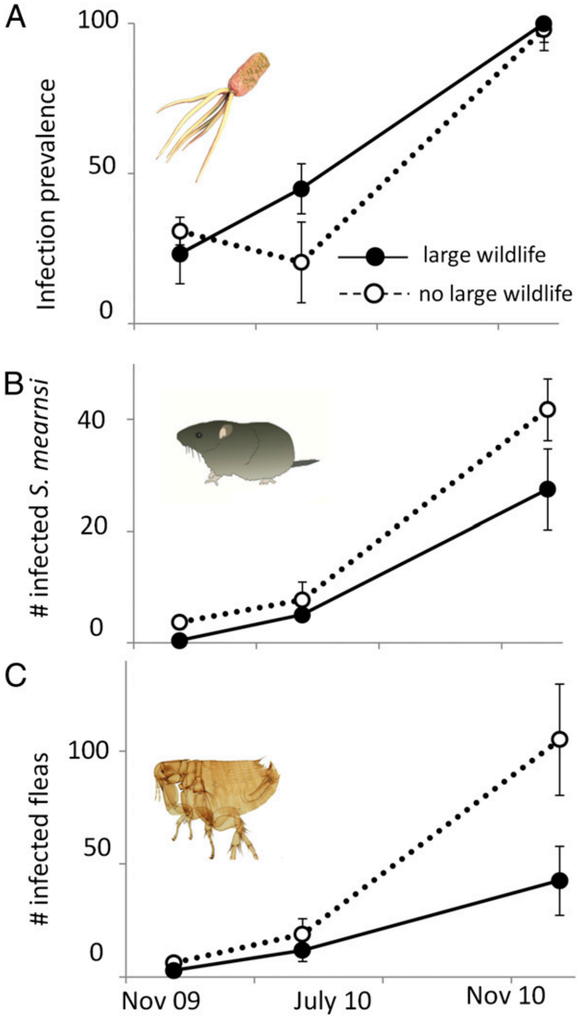

Fig. 3. There was no significant overall difference in the prevalence of *Bartonella* spp. infection either in the dominant rodent *S. mearnsi* or in the fleas of *S. mearnsi* (*A*) between control and large wildlife removal treatments. However, because of increased abundance of *S. mearnsi* in sites without large wildlife, there was a significant increase in the abundance of infected *S. mearnsi* (*B*) and infected vectors (*C*) in these simulated large wildlife loss treatments. Error lines represent 1 SE, based on three replicate blocks. Data is qualitatively similar when considering all rodents (Fig. S3).

Notably, the primary mechanism by which large wildlife loss appears to affect *Bartonella* risk in this system is through susceptible host regulation. Although the effects of susceptible host regulation have been experimentally documented in plant systems (50) and, for zoonotic diseases, have been suggested to result from fragmentation (24), this study is, to our knowledge, the first experimental demonstration of the phenomenon for a rodent-borne zoonosis. In this case, host regulation may be occurring due to both direct (competition) and indirect (vegetation structural changes) effects of changes in abundance of large vertebrates (35, 51) rather than via changes in rodent predators (47), the interacting species more often considered (14, 38).

Interestingly, we found no support that transmission interference, as documented in other systems (52, 53), operates in this system. There were no significant changes in the diversity or composition of the rodent host community (or of flea communities) between treatments. Given the much lower density of larger mammals relative to small mammals in this landscape, transmission interference is also not likely relevant when the whole mammal community is considered. There was also no change in the prevalence of *Bartonella* spp. in the dominant host, *S. mearnsi*, despite its much higher abundance in exclosures. Although increased prevalence is often seen when densities of competent hosts increase, this relationship is expected only when transmission is density-dependent. For pathogens with frequency-dependent transmission, effects of density of prevalence are not necessary unless contact rates change (54). Other studies of *Bartonella* have observed a similar lack of correlation between *Bartonella* prevalence and host population density (55), although positive and delayed relationships have also been observed (56, 57).

Although it is theoretically possible that the increased number of infected fleas on rodents in defaunated landscapes could be offset by fewer fleas from the (absent) large wildlife, such an effect is highly unlikely. Most of the animals excluded are large ungulates, which, for ecological and evolutionary reasons, almost entirely lack fleas, particularly in the tropics of Africa [the currently known flea fauna on ungulates has a decidedly Holarctic or South Asian distribution (Vermipsyllidae and Ancistropsyllidae, respectively)] (30, 58, 59). Although large carnivores do carry *Pulex* and *Ctenocephalides* fleas (not *Xenoypsylla* fleas), these large carnivores are at extremely low densities in the landscape and are just one of many host groups for these fleas (58). From a human risk perspective, the wild ungulates and large carnivores excluded in this study are also unlikely to interact closely with humans, making transmission less likely than rodent–human transmission. However, we expect that diseases transmitted by ectoparasites more common on ungulates than on rodents (e.g., predominantly tick-borne pathogens) will show very different, even inverted, responses (60, 61).

The conclusion that wildlife decline causes a substantial increase in *Bartonella* risk assumes that abundance of infected hosts and vectors is the best metric of disease risk. Using per capita prevalence responses would have led to a conclusion of no effect. Clearly, the choice of prevalence metric can produce qualitatively different outcomes and emphasizes the importance of including infected host and vector abundance as a response metric (62). We suggest that per capita prevalence, sometimes used in the discussion of negative biodiversity relationships through transmission interference (24), may not be the most relevant metric to many real world disturbance–disease relationships. In this case, for example, there would need to be a nearly 50% decrease in per capita prevalence to counter the amplifying effects of changes in host and vector abundance following defaunation.

Although abundance of infected vectors is likely a better response metric than per capita prevalence, there are still many questions that need to be answered to assess if this change in abundance of infected vectors will translate to a proportionate increase in human disease in those anthropogenic settings. One set of questions revolves around patterns of human contact with these dominant *S. mearnsi* hosts. Although *Bartonella* spp. infected *S. mearnsi* were found in human habitations and in more disturbed agricultural or pastoral settings (*SI Text*), the presence of infected rodents is not itself sufficient for transmission. If humans do not interact closely with these rodents, disease transmission may be inconsequential regardless of changes in abundance. Likewise, if human behavior in defaunated habitats changes human–*S. mearnsi* contact patterns, the relationship between number of infected vectors and human risk will be nonlinear. However, because defaunation seems most likely to be associated with increased human contact rates (e.g., human presence is a cause of defaunation, humans are more willing to spend time in safer defaunated landscapes), we expect such changes would only exacerbate the effects of defaunation on transmission, rendering our conclusions of increased risk conservative. There are also outstanding questions about human vector contact. The most common flea in this study, *X. cheopis*, is a known generalist that can feed on humans and effectively transfer other pathogens (58). Because fleas are also known to transmit *Bartonella* spp. to humans, it seems likely that fleas in this system will be able to transmit *Bartonella* spp. to humans. However, the effectiveness and frequency of *X. cheopis* or *X. sarodes* transmission of *Bartonella* spp. to humans remain undetermined. As with the transmission of most zoonotic pathogens to humans, the relationship is likely to be complex and multifactorial. The density of infected vectors, although an important and commonly used indicator of risk, remains an imperfect metric for assessing true risk to humans. Explicit links to human health outcomes will be needed to demonstrate this connection fully.

More generally, however, given the ubiquity of large wildlife loss; the pervasive and well-substantiated observations of rodent increases following such defaunation globally (35, 58, 63, 64); and the fact that rodents are one of the most frequent hosts of zoonotic diseases (65), particularly flea-borne diseases (58), these results suggest that size-selective animal loss may have a major impact on global risk of rodent-borne diseases. This study also adds to a growing body of evidence from both the Lyme and hantavirus systems that pathogen regulation through susceptible host regulation of rodent-borne diseases may be equally or more important than transmission interference in some cases (31, 38). We suggest susceptible host regulation may be an underappreciated ecological function of the preservation of intact mammalian communities. Further research on other rodent-borne pathogens, particularly with alternative transmission pathways (e.g., direct transmission) or with higher host specificity (66), will be critical to assessing the generality of our findings.

Methods

Study Site. This work was conducted between November 2009 and November 2011 in the Laikipia County of Kenya, at the Mpala Research Centre. To determine how the loss of large native wildlife influences disease risk, we conducted all sampling in the KLEE (0°17′ N, 36°52′ E) (43). Established in 1995, the KLEE uses a block design that includes three replicates of different types of wildlife exclosures. In this experiment, we used only the total exclosure sites, which effectively exclude all animals larger than 15 kg, and the control sites, which allow free access to all wildlife. Each experimental plot in the KLEE is 4 ha in size, but we sampled only the central hectare to avoid confounding edge effects.

The KLEE is located on "black cotton" soil (nutrient-rich but poorly drained vertisols with high clay content) and set within an *Acacia drepanolobium* savanna-woodland. Mean annual rainfall at the KLEE site is ~630 mm and is weakly trimodal. Resident large wildlife in the area includes elephants (*Loxodonta africana*), giraffes (*Giraffa camelopardalis*), zebras [*Equus quagga*

(*Equus burchellii*) *and Equus grevyi*], and lions (*Panthera leo*), among others (43).

Rodent and Ectoparasite Sampling. Rodent trapping followed methodologies previously used in long-term monitoring of rodents in these plots. Briefly, in the inner hectare in each of the total exclosure ($n = 3$) and control ($n = 3$) plots, we set 100 Sherman traps, placed on a 10 × 10-m grid with 10-m spacing. Traps were baited every evening with peanut butter and oats for three consecutive nights. Trapping occurred in five sampling periods over a 2-y period: November 2009, March 2010, November 2010, March 2011, and November 2011. All captured rodents were identified to species (using morphological and genetic techniques), sexed, weighed, and marked. Rodent blood was collected onto Whatman paper. Animals were released at their capture sites. Due to low capture rates of some species, we assessed abundance of rodents per site per capture period simply as the minimum number of animals known to be alive, based on the number of unique individuals captured per site.

Ectoparasites were sampled using a modification of protocols described by McCauley et al. (46). Essentially, the animal was held over a tub of ethanol and then combed for 10 strokes to provide an index of infestation intensity. All ectoparasites that fell into the tub or could be recovered from the flea comb were collected and counted. If an animal was subsequently captured again in the same 3-d trapping session, it was not sampled for fleas a second time. Ectoparasites were morphologically identified to species level.

***Bartonella* Surveillance.** *Bartonella* screening was conducted only for rodents captured in the first three trapping periods ($n = 168$ hosts screened). This screening included all rodents captured in November 2009 and March 2010; given the very high capture rate in November 2010, only a random subsampling (10%) of those rodents was screened. For each selected rodent host, we (*i*) screened the host blood for *Bartonella* and (*ii*) screened at least one flea from each animal if it had fleas. For the subset of hosts with multiple fleas ($n = 131$), we also screened all remaining fleas individually from a randomly selected 30% of individuals ($n = 373$ fleas screened). Extraction, PCR screening, and analysis were conducted independently at two sites, the Centers for Disease Control and Prevention and the University at Buffalo, The State University of New York. Protocols followed those detailed by Billeter et al. (67) for fleas and by Kosoy et al. (68) for rodent hosts. Individual fleas were titrated using a sterile needle, and DNA was extracted using a Qiagen QIAamp tissue kit. Fleas were screened for the presence of *Bartonella* DNA using protocols described by Billeter et al. (67), with primers targeting 767-bp and 357-bp fragments of the citrate synthase gene, *gltA*. PCR products were purified using the QIAquick PCR purification kit (Qiagen). DNA sequences were analyzed using Geneious R6-1 (Biomatters) and Lasergene version 8 (DNASTAR) software.

Host prevalence of *Bartonella* was defined as the number of *Bartonella*-positive rodents (all *Bartonella* strains pooled) over the number of tested rodents. Vector prevalence of *Bartonella* was defined as the number of positive fleas over the total number of fleas screened per host, considering only the subset of data taken from hosts that both had fleas and where all fleas were screened. Abundance of infected hosts was defined as the product of total host abundance (minimum number known alive) and host prevalence of *Bartonella.* Abundance of infected vectors was defined as the product of host abundance, mean vector abundance per host, and vector prevalence of *Bartonella*. All metrics were calculated per site (three exclosures, three control sites) per sampling interval.

For all rodent and flea analyses, we used factorial, repeated measures ANOVA. To compare species similarity across host and flea communities, we conducted ANOSIMs among treatments pooled across sampling periods. Reported values are mean ± SE unless otherwise noted. All analyses were conducted in R 2.12.1 (R Development Core Team, 2010).

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1. Barnosky AD, et al. (2011) Has the Earth's sixth mass extinction already arrived? *Nature* 471(7336):51–57.
2. Schipper J, et al. (2008) The status of the world's land and marine mammals: Diversity, threat, and knowledge. *Science* 322(5899):225–230.
3. Dirzo R, Mendoza E, Ortíz P (2007) Size-related differential seed predation in a heavily defaunated neotropical rain forest. *Biotropica* 39(3):355–362.
4. Fritz SA, Bininda-Emonds OR, Purvis A (2009) Geographical variation in predictors of mammalian extinction risk: Big is bad, but only in the tropics. *Ecol Lett* 12(6):538–549.
5. Trebilco R, Baum JK, Salomon AK, Dulvy NK (2013) Ecosystem ecology: Size-based constraints on the pyramids of life. *Trends Ecol Evol* 28(7):423–431.
6. Cardillo M, et al. (2005) Multiple causes of high extinction risk in large mammal species. *Science* 309(5738):1239–1241.
7. Fritz SA, Purvis A (2010) Phylogenetic diversity does not capture body size variation at risk in the world's mammals. *Proc Biol Sci* 277(1693):2435–2441.
8. Petchey OL, Gaston KJ (2006) Functional diversity: Back to basics and looking forward. *Ecol Lett* 9(6):741–758.
9. Bellwood DR, Hoey AS, Choat JH (2003) Limited functional redundancy in high diversity systems: Resilience and ecosystem function on coral reefs. *Ecol Lett* 6(4): 281–285.
10. Estes JA, et al. (2011) Trophic downgrading of planet Earth. *Science* 333(6040): 301–306.
11. Pringle RM, Young TP, Rubenstein DI, McCauley DJ (2007) Herbivore-initiated interaction cascades and their modulation by productivity in an African savanna. *Proc Natl Acad Sci USA* 104(1):193–197.
12. Galetti M, et al. (2013) Functional extinction of birds drives rapid evolutionary changes in seed size. *Science* 340(6136):1086–1090.
13. Johnson PT, Preston DL, Hoverman JT, LaFonte BE (2013) Host and parasite diversity jointly control disease risk in complex communities. *Proc Natl Acad Sci USA* 110(42): 16916–16921.
14. Ostfeld RS, Holt RD (2004) Are predators good for your health? Evaluating evidence for top-down regulation of zoonotic disease reservoirs. *Front Ecol Environ* 2(1):13–20.
15. Ostfeld RS, Keesing F (2012) Effects of host diversity on infectious disease. *Annu Rev Ecol Evol Syst* 43(1):157–182.
16. Salkeld DJ, Padgett KA, Jones JH (2013) A meta-analysis suggesting that the relationship between biodiversity and risk of zoonotic pathogen transmission is idiosyncratic. *Ecol Lett* 16(5):679–686.
17. Young H, Griffin RH, Wood CL, Nunn CL (2013) Does habitat disturbance increase infectious disease risk for primates? *Ecol Lett* 16(5):656–663.
18. Wood CL, Lafferty KD, DeLeo G, Young H, Kuris AM (2014) Does biodiversity protect against infectious disease? *Ecology* 95(4):817–832.
19. Myers SS, et al. (2013) Human health impacts of ecosystem alteration. *Proc Natl Acad Sci USA* 110(47):18753–18760.
20. Hough RL (2014) Biodiversity and human health: Evidence for causality? *Biodivers Conserv* 23(2):267–288.
21. Taylor LH, Latham SM, Woolhouse ME (2001) Risk factors for human disease emergence. *Philos Trans R Soc Lond B Biol Sci* 356(1411):983–989.
22. Cleaveland S, Laurenson MK, Taylor LH (2001) Diseases of humans and their domestic mammals: Pathogen characteristics, host range and the risk of emergence. *Philos Trans R Soc Lond B Biol Sci* 356(1411):991–999.
23. Wolfe ND, Dunavan CP, Diamond J (2007) Origins of major human infectious diseases. *Nature* 447(7142):279–283.
24. LoGiudice K, Ostfeld RS, Schmidt KA, Keesing F (2003) The ecology of infectious disease: Effects of host diversity and community composition on Lyme disease risk. *Proc Natl Acad Sci USA* 100(2):567–571.
25. Lacroix C, et al. (2014) Non-random biodiversity loss underlies predictable increases in viral disease prevalence. *J R Soc Interface* 11(92):20130947.
26. Keesing F, Holt RD, Ostfeld RS (2006) Effects of species diversity on disease risk. *Ecol Lett* 9(4):485–498.
27. Kallio ER, et al. (2009) Cyclic hantavirus epidemics in humans—Predicted by rodent host dynamics. *Epidemics* 1(2):101–107.
28. Gage KL, Kosoy MY (2005) Natural history of plague: Perspectives from more than a century of research. *Annu Rev Entomol* 50:505–528.
29. Chomel BB, Kasten RW (2010) Bartonellosis, an increasingly recognized zoonosis. *J Appl Microbiol* 109(3):743–750.
30. Whiting MF, Whiting AS, Hastriter MW, Dittmar K (2008) A molecular phylogeny of fleas (Insecta: Siphonaptera): Origins and host associations. *Cladistics* 24(5):677–707.
31. Suzán G, et al. (2009) Experimental evidence for reduced rodent diversity causing increased hantavirus prevalence. *PLoS ONE* 4(5):e5461.
32. LoGiudice K, et al. (2008) Impact of host community composition on Lyme disease risk. *Ecology* 89(10):2841–2849.
33. Mills JN (2006) Biodiversity loss and emerging infectious disease: An example from the rodent-borne hemorrhagic fevers. *Biodiversity* 7(1):9–17.
34. Stenseth NC, et al. (2003) Mice, rats, and people: the bio-economics of agricultural rodent pests. *Front Ecol Environ* 1(7):367–375.
35. Keesing F (2000) Cryptic consumers and the ecology of an African savanna. *Bioscience* 50(3):205–215.

36. Leirs H, et al. (1997) Stochastic seasonality and nonlinear density-dependent factors regulate population size in an African rodent. *Nature* 389(6647):176–180.
37. Previtali MA, Lima M, Meserve PL, Kelt DA, Gutiérrez JR (2009) Population dynamics of two sympatric rodents in a variable environment: Rainfall, resource availability, and predation. *Ecology* 90(7):1996–2006.
38. Levi T, Kilpatrick AM, Mangel M, Wilmers CC (2012) Deer, predators, and the emergence of Lyme disease. *Proc Natl Acad Sci USA* 109(27):10942–10947.
39. Hayman DT, McDonald KD, Kosoy MY (2013) Evolutionary history of rat-borne Bartonella: The importance of commensal rats in the dissemination of bacterial infections globally. *Ecol Evol* 3(10):3195–3203.
40. Kosoy MY, et al. (1998) Isolation of *Bartonella* spp. from embryos and neonates of naturally infected rodents. *J Wildl Dis* 34(2):305–309.
41. Stevenson HL, et al. (2003) Detection of novel *Bartonella* strains and *Yersinia pestis* in prairie dogs and their fleas (Siphonaptera: Ceratophyllidae and Pulicidae) using multiplex polymerase chain reaction. *J Med Entomol* 40(3):329–337.
42. Billeter SA, Levy MG, Chomel BB, Breitschwerdt EB (2008) Vector transmission of Bartonella species with emphasis on the potential for tick transmission. *Med Vet Entomol* 22(1):1–15.
43. Young TP, Okello B, Kinyua D, Palmer TM (1997) KLEE: A long-term multispecies herbivore exclusion experiment in Laikipia, Kenya. *African Journal of Range and Forage Science* 14(3):94–102.
44. Randolph SE, Dobson AD (2012) Pangloss revisited: A critique of the dilution effect and the biodiversity-buffers-disease paradigm. *Parasitology* 139(7):847–863.
45. Venesky MD, Liu X, Sauer EL, Rohr JR (2013) Linking manipulative experiments to field data to test the dilution effect. *J Anim Ecol*, 10.1111/1365-2656.1259.
46. McCauley DJ, Keesing F, Young T, Dittmar K (2008) Effects of the removal of large herbivores on fleas of small mammals. *J Vector Ecol* 33(2):263–268.
47. McCauley DJ, Keesing F, Young TP, Allan BF, Pringle RM (2006) Indirect effects of large herbivores on snakes in an African savanna. *Ecology* 87(10):2657–2663.
48. Pretorius AM, Beati L, Birtles RJ (2004) Diversity of *Bartonellae* associated with small mammals inhabiting Free State province, South Africa. *Int J Syst Evol Microbiol* 54: 1959–1967.
49. Inoue K, et al. (2009) Exotic small mammals as potential reservoirs of zoonotic *Bartonella* spp. *Emerg Infect Dis* 15(4):526–532.
50. Mitchell CE, Tilman D, Groth JV (2002) Effects of grassland plant species diversity, abundance, and composition on foliar fungal disease. *Ecology* 83(6):1713–1726.
51. Young HS, et al. (2013) Effects of mammalian herbivore declines on plant communities: Observations and experiments in an African savanna. *J Ecol* 101(4):1030–1041.
52. Clay CA, Lehmer EM, Jeor SS, Dearing MD (2009) Sin nombre virus and rodent species diversity: A test of the dilution and amplification hypotheses. *PLoS ONE* 4(7):e6467.
53. Ezenwa VO, Godsey MS, King RJ, Guptill SC (2006) Avian diversity and West Nile virus: Testing associations between biodiversity and infectious disease risk. *Proc Biol Sci* 273(1582):109–117.
54. Weber N, et al. (2013) Badger social networks correlate with tuberculosis infection. *Curr Biol* 23(20):R915–R916.
55. Bai Y, Kosoy MY, Ray C, Brinkerhoff RJ, Collinge SK (2008) Temporal and spatial patterns of *Bartonella* infection in black-tailed prairie dogs (*Cynomys ludovicianus*). *Microb Ecol* 56(2):373–382.
56. Telfer S, et al. (2007) Contrasting dynamics of *Bartonella* spp. in cyclic field vole populations: The impact of vector and host dynamics. *Parasitology* 134(Pt 3):413–425.
57. Telfer S, et al. (2007) Ecological differences and coexistence in a guild of microparasites: *Bartonella* in wild rodents. *Ecology* 88(7):1841–1849.
58. Bitam I, Dittmar K, Parola P, Whiting MF, Raoult D (2010) Fleas and flea-borne diseases. *Int J Infect Dis* 14(8):e667–e676.
59. Wall R, Shearer D (1997) *Veterinary Entomology: Arthropod Ectoparasites of Veterinary Importance* (Chapman and Hall, London).
60. Keesing F, Allan BF, Young TP, Ostfeld RS (2013) Effects of wildlife and cattle on tick abundance in central Kenya. *Ecol Appl* 23(6):1410–1418.
61. Perkins SE, Cattadori IM, Tagliapietra V, Rizzoli AP, Hudson PJ (2006) Localized deer absence leads to tick amplification. *Ecology* 87(8):1981–1986.
62. Salkeld DJ, Lane RS (2010) Community ecology and disease risk: Lizards, squirrels, and the Lyme disease spirochete in California, USA. *Ecology* 91(1):293–298.
63. Caro T (2001) Species richness and abundance of small mammals inside and outside an African national park. *Biol Conserv* 98(3):251–257.
64. Goheen JR, et al. (2013) Piecewise disassembly of a large-herbivore community across a rainfall gradient: The UHURU experiment. *PLoS ONE* 8(2):e55192.
65. Luis AD, et al. (2013) A comparison of bats and rodents as reservoirs of zoonotic viruses: Are bats special? *Proc Biol Sci* 280(1756):20122753.
66. Kedem H, et al. (2013) Multiple effects of host species diversity on co-existing host-specific and host-opportunistic microbes. *Ecology*, 10.1890/13-0678.1.
67. Billeter SA, Gundi VA, Rood MP, Kosoy MY (2011) Molecular detection and identification of *Bartonella* species in *Xenopsylla cheopis* fleas (Siphonaptera: Pulicidae) collected from *Rattus norvegicus* rats in Los Angeles, California. *Appl Environ Microbiol* 77(21):7850–7852.
68. Kosoy MY, et al. (1997) Distribution, diversity, and host specificity of *Bartonella* in rodents from the Southeastern United States. *Am J Trop Med Hyg* 57(5):578–588.

Supporting Information

Young et al. 10.1073/pnas.1404958111

SI Text

The applications of this study for human health depend on the assumptions that *Saccostomus mearnsi* individuals that interact closely with humans will also carry infected fleas capable of biting humans. Additional trapping conducted in human-dominated landscapes (homes, agricultural fields, and pastoral landscapes) in nearby areas of Laikipia district during the time period of this study supports this conclusion. Of 25 *S. mearnsi* sampled in these anthropogenic landscapes, the mean flea density was 12.0 ± 10.4 fleas, which is well above the levels observed in the Kenya Long-Term Exclosure Experiment (KLEE). As in the KLEE sites, these fleas were primarily composed of *Xenopsylla cheopis* and *Xenopsylla sarodes* (94% of fleas); *X. cheopis* in particular are known to be generalists that bite humans and transmit pathogens. The fleas of *S. mearnsi* in human-dominated areas were also dominated by *Xenopsylla* spp. Of the 21 *S. mearnsi* from anthropogenic landscapes that were screened for *Bartonella* spp., 16 (76%) were positive for *Bartonella* spp., predominantly of *Bartonella elizabethae,* a suspected human pathogen. Of the 97 fleas from *S. mearnsi* from anthropogenic landscapes that were screened for *Bartonella*, 56% were positive for at least one strain of *Bartonella*. For the 18 fleas from *S. mearnsi* found specifically in human dwellings, 79% were positive for at least one strain of *Bartonella*. Although detailed surveillance of *Bartonella* prevalence in human-dominated landscapes will be needed to assess direct human health outcomes, all initial surveillance suggests that the relevant host–vector pathogen relationships exist to make human transmission likely.

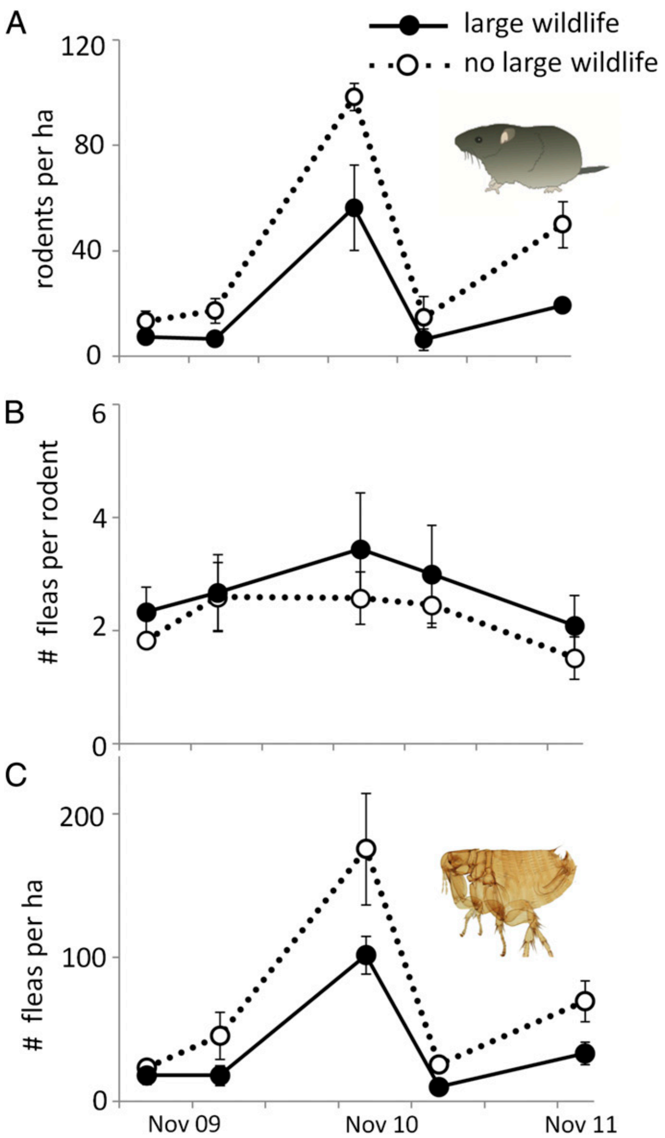

Fig. S1. Examining all rodents and their fleas instead of only *S. mearnsi* does not qualitatively change the interpretations of effects of defaunation on rodent or flea abundance. (*A*) Abundance of all rodents was approximately twice as high in large wildlife exclosures compared with open plots, which is a significant difference. (*B*) Density of fleas per rodent did not vary among treatments. (*C*) Thus, the density of fleas per hectare is also significantly higher (approximately doubled) in plots where large wildlife has been removed. Error lines represent 1 SE, based on three replicate blocks.

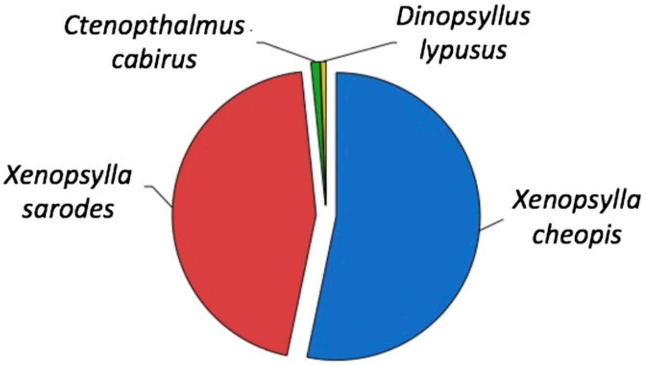

Fig. S2. Flea communities of *S. mearnsi* in both large wildlife exclosures and control sites (pooled) are dominated by two *Xenopsylla* species. Fleas in the *Xenopsylla* genus are competent vectors of *Bartonella* as well as other pathogens of relevance to human health (e.g., *Yersinia pestis*).

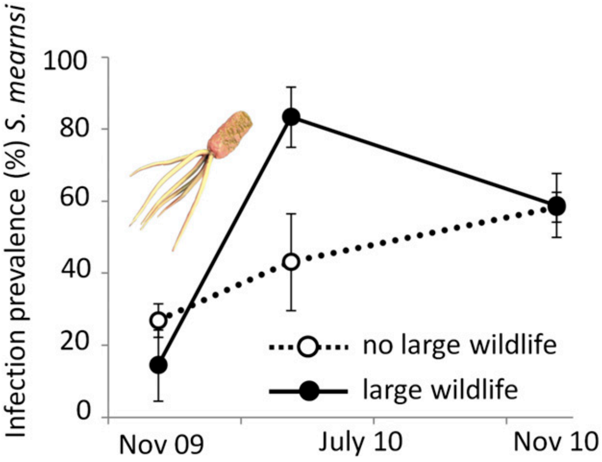

Fig. S3. Prevalence of infection in *S. mearnsi* was not significantly different among treatments across seasons; however, there were significant differences in prevalence over time (as also observed in prevalence among fleas). The interaction effect was not significant. Error lines represent 1 SE based on three replicate blocks.

Table S1. Average (±SD) number of hosts and fleas/host per site, pooled across all five sampling periods and three experimental blocks

	Individuals per hectare		Fleas/host	
Species	Exclosure	Open	Exclosure	Open
Acomys kempi	0.3 ± 0.6	1.3 ± 2.3	0	0
Aethomys kaiseri	0.3 ± 0.6	0	1.0	0
Arvicanthis niloticus	4.7 ± 3.5	0	1.9 ± 1.9	0
Dendromus melanotis	1.0 ± 1.7	1.3 ± 1.5	0	0
Mastomys natalensis	1.3 ± 0.6	0	0.3 ± 0.6	0
Mus minutoides	28.3 ± 16.3	17.7 ± 8.1	0	0
Mus cf. acholi	1.7 ± 2.9	1.0 ± 1.0	0	0
Mus sorella	3.3 ± 0.6	4.3 ± 3.8	0	0
Mus tenellus	0.3 ± 0.6	1.7 ± 0.6	0	0
Saccostomus mearnsi	143 ± 15.5	64.3 ± 17.4	2.2 ± 0.3	2.8 ± 0.9
Zelotomys hildegardeae	1.0 ± 0	0.33 ± 0.58	8	0

Table S2. Matrix of the presence of flea species (columns) found on each rodent host species (rows)

Species	*Ctenophthalmus cabirus*	*Parapulex echinatus*	*Xenopsylla brasiliensis*	*Xenopsylla cheopis*	*Xenopsylla sarodes*	*Dinopsyllus lypusus*
Acomys kempi		X				
Aethomys kaiseri			X			
Arvicanthis niloticus				X		X
Mastomys natalensis						X
Saccostomus mearnsi	X			X	X	X
Zelotomys hildegardeae						X

None of the species of *Mus* (*Mus minutoides, Mus cf. acholi, Mus sorella, and Mus tenellus*) or *Dendromus melanotis* had fleas; therefore, they are not listed.