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ABSTRACT: *Rattus rattus* was first reported from the West Nile Region of Uganda in 1961, an event that preceded the appearance of the first documented human plague outbreak in 1970. We investigated how invasive *R. rattus* and native small mammal populations, as well as their fleas, have changed in recent decades. Over an 18-month period, a total of 2,959 small mammals were captured, sampled, and examined for fleas, resulting in the identification of 20 small mammal taxa that were hosts to 5,109 fleas (nine species). Over three-fourths (75.8%) of captured mammals belonged to four taxa: *R. rattus*, which predominated inside huts, and *Arvicanthis niloticus*, *Mastomys* sp., and *Crocidura* sp., which were more common outside huts. These mammals were hosts for 85.8% of fleas collected, including the efficient plague vectors *Xenopsylla cheopis* and *X. brasiliensis*, as well as likely enzootic vectors, *Dinopsyllus lypusus* and *Ctenophthalmus bacopus.* Flea loads on small mammals were higher in certain environments in villages with a recent history of plague compared to those that lacked such a history. The significance of these results is discussed in relation to historical data, the initial spread of plague in the WNR and the continuing threat posed by the disease. ***Journal of Vector Ecology* 45 (2): 333-355. 2020.**

Keyword Index: Plague, Uganda, Africa, invasive rats, fleas, *Rattus rattus.*

INTRODUCTION

Plague is a highly pathogenic and frequently fatal disease of many mammals, including humans, caused by *Yersinia pestis*, a gram-negative bacterium maintained in nature through transmission between susceptible rodent hosts and their fleas. Humans do not play a role in maintaining *Y. pestis* in nature but can acquire the disease through exposure to infectious flea bites, handling infected animals, or inhaling infectious materials (Gage and Kosoy 2005, Gage 2012). Bites from infectious fleas are the most common and most epidemiologically important mode of transmission to humans (Gage 1999, Eisen and Gage 2012). Risk of exposure increases when persons are exposed to infectious rodent fleas that abandon their animal hosts in the wake of plague epizootics that cause widespread mortality among infected rodent populations (Gage 1998). Exposure risk also can be affected by human behaviors, types of home construction, food storage methods, and agricultural practices that increase rodent densities and the likelihood of human exposures to infectious fleas (Gould et al. 2012, Eisen et al. 2014). Areas of greatest risk often occur where plague is enzootic, poverty rates are high and thus housing construction is poorly suited for rodent exclusion, and subsistence farming creates an abundant food supply for rodents inside housing (Gage 1999). Worldwide, the highest risk areas are found in the African region, which accounted for 97% of human cases reported to the World Health Organization (WHO) (11,247 of 11,598 cases) over the last decade (2006-2015). Most of these cases were reported from Madagascar and the east African countries of Democratic Republic of the Congo, Tanzania, and Uganda where plague has a long history (Neerinckx et al. 2010, Bertherat 2016, Forrester et al. 2017).

Outbreaks of disease in humans are typically defined as occurrence above baseline data for a given location and/or time period and may include qualifiers such as one standard deviation above a baseline mean or median (Teutsch and Churchill 2000). Plague typically occurs as sporadic individual, or very small clusters of, human cases in endemic regions. There is no published number of cases that quantifies a plague outbreak, nor an epidemic. Due to the potential for rapid spread and high mortality rates, single cases are reportable in most jurisdictions, and to the World Health Organization (WHO), and WHO recommends investigations and control measures be initiated for single cases (Gage 1999). For the purposes of this paper, we define in general terms that a human outbreak represents a case count clearly above baseline for a given place and time period. Epidemics and pandemics are cited from the literature and are based on the respective author's usage of the terms.

Unlike some parts of Africa, which are thought to have first experienced plague during the most recent pandemic (late 19th and early 20th centuries), molecular phylogeny studies suggest that *Y. pestis* has existed in east Africa for many hundreds of years and isolates from much of east Africa (including Uganda) belong to the Antiqua biotype (Dennis

and Gage 2003, Morelli et al. 2010). Roberts (1935a) noted that local traditions in Uganda suggested the disease had been present in the country for "ages," but actual documentation of human cases in modern records did not occur until British missionaries reported the disease in 1877 (Schwetz 1929). This was more than a decade prior to the most recent (third) pandemic that originated in China and was caused by Orientalis biotype strains (Dennis and Gage 2003, Neerinckx et al. 2010). Roberts (1935a) also reported that several outbreaks of one to four years duration occurred between 1883 and 1932 in southern Uganda, with the last of these reportedly causing 10,777 deaths. Numerous plague outbreaks occurred in five southern districts after 1877, with more than 61,000 cases being noted from 1910-1946 (Hopkins 1949). These reports, from the first half of the 20th century, were confined to the southern portions of the country, particularly those areas around Lake Edward and, to a lesser extent, along southern trade routes that passed through Kampala and Jinja on their way east to the seaport of Mombasa, Kenya (Roberts 1935a, 1935b). These large outbreaks, in spatial and temporal terms, likely had devastating effects on local small mammal populations up and down the food chain (Biggins and Kosoy 2001), but longitudinal small mammal studies in this region and time period are scant and insufficient for statistical correlation. By the 1940s, southern Uganda still reported occasional human plague cases but no additional outbreaks (Davis 1953, Pollitzer 1954), although outbreaks were still noted in DRC along its border with Uganda (Davis 1953).

The means by which plague first became established in the West Nile Region (WNR) remain uncertain, but the molecular evidence suggests that plague is likely to have been present in the region's native rodent populations for many centuries. If this is true, these animals and their fleas could have been responsible for sporadic cases of human plague prior to the 1970s that simply went unnoticed or were misdiagnosed. It is also possible that *Y. pestis* was first introduced to the WNR from elsewhere in Uganda as a result of transportation by rail or road following completion of the Pakwach railway bridge that spanned the Albert Nile River and lead to Arua, in 1964, or a roadway bridge near the same site in 1969. A third possibility is that plague invaded from the adjoining plague endemic Ituri District of the DRC's Orientale Province, which has experienced human plague since at least the 1920s (Abedi et al. 2018). Regardless of its origin, it is highly unlikely that large outbreaks of human plague went completely unnoticed in the WNR prior to the 1970s because officials recognized and frequently reported numerous other outbreaks in southern Uganda during the late 19th and first half of the 20th centuries (Neerinckx 2010).

The 1970 outbreak in the WNR was followed over the next four decades by several others (Winters et al. 2009), including epidemics that were recorded at Nyapea Hospital, a large health facility in Okoro County, where 2,591 suspect cases of bubonic plague (fever and bubo) were identified from 1989-1995. Another examination of selected health center records for 1989-2003 in Okoro and neighboring Vurra counties by the United States Centers for Disease Control and Prevention (CDC) and the Uganda Virus Research Institute (UVRI) identified 1,610 clinically diagnosed cases of plague (Winters et al. 2009). From 1999-2008, the WNR experienced repeated outbreaks with a mean of 206 plague cases per year (range 11-462) (Winters et al. 2009, Eisen et al. 2010, Borchert et al. 2012). Over the interval from 2008-2016, a total of 255 suspected cases were reported in the WNR, of which about one-third were later laboratory-confirmed by bacterial culture with temperature specific bacteriophage lysis or by paired serology, with the remainder testing negative or not confirmed (i.e., no convalescent follow-up serology obtained) (Forrester et al. 2017). The number of reported human cases has dropped noticeably in recent years, presumably reflecting cyclic fluctuations or extended periods of localized quiescence within the human population that are typical of many plague foci (Pollitzer 1954, Pollitzer 1966, Dennis 1998, Carniel 2008, Zeppelini et al. 2018).

The apparent absence of reported human plague outbreaks in the WNR prior to the early 1970s and its sudden emergence thereafter suggest that local ecological or epidemiological conditions changed in ways that favored the spread of the disease. In other tropical countries the spread of plague typically has been associated with the occurrence of the disease among commensal rats (*R. rattus* or other commensal *Rattus*) (Davis 1953, Gage 2012), making it logical to examine how invasive *R. rattus* (commonly referred to as "black rats" or "roof rats") might have contributed to the emergence of human plague and whether their interactions with native small mammals and their fleas continue to affect human plague risk in the WNR.

Although Delany and Neal (1966) reported capturing *R. rattus* in the WNR during 1961, it remains uncertain exactly when *R. rattus* first appeared in the region. *Rattus rattus* was reported from the adjoining Ituri District of DRC in 1958 (Misonne 1969), but evidence for its absence during the mid-20th century in the WNR of Uganda includes a survey by Hopkins (1949) which was conducted in 1937-1938. Although Hopkins' WNR survey captured a single black rat aboard a steamship docked at the port of Rhino Camp on the Albert Nile River, no *R. rattus* were captured at the 11 sites sampled on land. Nevertheless, the presence of this single ship-borne rat represents the first documented evidence of *R. rattus* along the west bank of the river and suggests a possible means by which these animals could have first entered the WNR. The presence of black rats in DRC's Ituri District in the 1950s (Misonne 1969) suggests another likely point of entry. The observation that the first recorded human plague outbreaks in the WNR occurred about a decade after the first reports of *R. rattus* in both the WNR and in neighboring parts of the DRC suggests that black rats played a crucial role in the regional emergence of the disease.

For the past decade and a half, the CDC and UVRI staff have conducted collaborative studies on the control, ecology, and epidemiology of plague in the WNR (Eisen et al. 2008, Winters et al. 2009, Eisen et al. 2012, Borchert et al. 2012, Graham et al. 2013, Boegler et al. 2014, Moore et al. 2015). Eisen et al. (2012) examined how the abundance and diversity of hosts and fleas varied over an elevation gradient that extended from apparently plague-free low elevation

(~725-1,160 m) sites west of the Albert Nile River to higher elevation (~1,380-1,630 m) sites found within the plague-endemic zone. Although host diversity was similar in both elevation ranges, flea diversity was significantly higher at high elevation sites that lay within the zone at greatest risk for plague. Moore et al. (2015) reported that the abundance of certain small mammals was positively correlated with local rainfall patterns, a general observation that is consistent with previously published models relating precipitation to the occurrence of human plague cases elsewhere (Parmenter et al. 1999, Enscore et al. 2002). Moore et al. (2015) also noted that flea species richness and diversity on small mammals did not vary with climatic seasons, but flea species richness was higher on small mammals collected outside of huts during the plague season (September through December) than at other times of the year. Pollitzer (1954) indicated that human plague risk is increased when the rat flea index reaches 1.0 or higher, and this has been used as a standard threshold for many years (Gage 1999).

A subset of the host-flea data from the first three, of nine, sampling periods described in our study were preliminarily reported by some of the co-authors of the current manuscript (Amatre et al. 2009). That preliminary report detailed flea loads found on rodents within domestic, peridomestic and sylvatic sites but did not closely examine the distribution of hosts in sylvatic sites, the distribution or abundance of *Crocidura* shrews and their fleas, nor did that report examine the current observations with respect to historical data.

In this paper, we present the results of rodent and flea collections for all nine sampling periods completed during our study and compare the results with those observed prior to the introduction of *R. rattus* into the WNR. We also discuss the significance of these results for the epidemiology and ecology of plague in this region, and we examine the distributions of the most epidemiologically important fleas. In addition, we identified the environments where *R. rattus* and native small mammal ranges most frequently overlap, possibly leading to exchanges of *Y. pestis* infected fleas between those hosts. Finally, we examine whether the occurrences of recent cases of human plague were associated with levels of flea infestations on different hosts.

MATERIALS AND METHODS

Study area

Our study occurred in two adjoining counties located within the Arua (Vurra County) and Zombo (Okoro County) Districts of the WNR, Uganda. The primary occupation in both districts is subsistence farming. Common subsistence crops included maize, cassava, millet, sugar cane, bananas, onions, melons, and squash. Coffee and tobacco are grown as cash crops on a very limited scale due to the lack of reliable roads to reach markets and are more commonly found at the higher elevations of Okoro County. Goats and chickens are commonly raised for meat; pigs and cattle somewhat less so (Emwanu et al. 2007, Eisen et al. 2014).

Plague cases

In 1999, a standardized clinic logbook was adopted for use in all the rural clinics found in the study area. A review of these clinic log books in 2005-2006, identified 1,570 suspect cases of plague. For this purpose, a suspect case was defined as a patient who presented at a clinic for which a clinical diagnosis of plague was recorded by the health care provider based on a presentation of acute painful lymphadenopathy of the axillary or inguinal region, sudden onset of fever combined with headache or prostration, and no other apparent cause of illness, such as a wound or parasitic infection of the leg or arm. Patients that chose not to or were unable to present at a clinic, expired prior to clinic presentation, presented with primary cervical lymphadenopathy, or presented with primary pneumonic plague, would not have been counted using this case definition. Hence, this assessment provided a conservative estimate of the suspected human disease burden and allowed us to initially characterize the spatio-temporal aspects of human disease within the study area.

These logbooks also provided information on the village of residence for each suspect case, which in all instances were located above 1,067 m. This observation agrees with a previous one indicating that plague rarely occurs at lower elevations in Uganda (Hopkins 1949), and with other studies, subsequent to ours, reporting that human plague is uncommon in the WNR at elevations lower than 1,300 m (Winters et al. 2009, Eisen et al. 2010).

Study villages

As noted in Table 1, each of the ten villages selected for our study, except one, was located above the 1,300 m elevation previously cited as being at increased risk for human plague and the elevation of the remaining village (at 1,293 m) was nearly so. Five villages had experienced multiple clinically diagnosed cases of human plague over the six-year interval from 1999-2005 and were considered to have recently (trapping began in January, 2006) experienced plague, while the remaining five had no such diagnoses of plague over this same period and were considered to have had no recent history of plague (Table 1). Four of the villages were located within Arua District (two with a plague history and two without such history) and six were located within Zombo District (three with a plague history and three without such history) (Table 1). In order to help ensure adequate trapping opportunity and rodent populations for study, each of the ten villages were furthermore selected to contain a minimum of 300 huts which was also subjectively deemed to be an average size village for this region. Each village typically consisted of identifiable family compounds consisting of three to eight huts, inhabited by members of a multi-generational, extended family. Conventional huts were single room round or square structures built either of locally fired mud bricks or on a frame of tree branches covered with mud to a thickness of about 25 cm and then plastered with a mixture of mud and animal manure. Other features often included packed dirt floors, lack of windows, door entrances often covered only by a hanging cloth or poorly fitting wood door, and a thatch roof that typically overhung the hut walls by approximately

Table 1. Numbers of *Xenopsylla cheopis* and *Xenopsylla brasiliensis* collected from hosts trapped in villages located at different elevations.

Village[history] (District)		**All mammals***		***Rattus rattus***		***Arvicanthis niloticus***		***Mastomys sp.***		***Crocidura sp.***	
	Elevation (m)	*Xenopsylla cheopis*	*Xenopsylla brasiliensis*	*Xenopsylla cheopis*	*Xenopsylla brasiliensis*	*Xenopsylla cheopis*	*Xenopsylla brasiliensis*	*Xenopsylla cheopis*	*Xenopsylla brasiliensis*	*Xenopsylla cheopis*	*Xenopsylla brasiliensis*
Pembeleku[neg] (Arua)	1,293	128	16	26	4	19	3	63	6	9	2
Olii[POS] (Arua)	1,372	200	31	78	11	25	2	41	9	34	3
Kaza[neg] (Arua)	1,376	109	12	31	7	36	2	17	3	10	0
Pomosi[POS] (Arua)	1,445	190	0	48	0	32	0	54	0	43	0
TOTAL ARUA		**627**	**59**	**183**	**22**	**112**	**7**	**175**	**18**	**96**	**5**
Monkwerocoo[neg] (Zombo)	1,440	0	193	0	64	0	129	0	0	0	0
Sokonzi[POS] (Zombo)	1,491	12	138	0	67	12	57	0	5	0	1
Agore[POS] (Zombo)	1,554	15	112	15	69	0	20	0	17	0	0
Anyiku[neg] (Zombo)	1,554	1	193	1	113	0	51	0	24	0	0
Gbalia[neg] (Zombo)	1,624	0	318	0	227	0	87	0	0	0	0
Uyaru-Agada[POS] (Zombo)	1,638	1	320	0	132	1	174	0	7	0	7
TOTAL ZOMBO		**29**	**1,274**	**16**	**672**	**13**	**518**	**0**	**53**	**0**	**8**

* In addition to the four mammalian taxa listed in the table, the "All mammals" category also includes *X. cheopis* and *X. brasiliensis* collected from all other small mammal species.

"History" denotes human plague case history for this village (POS = positive, neg = negative) during the six years preceding this study.

one meter to provide limited shade and water diversion. Most huts were located within walking distance of a drinking water source and the family's subsistence agricultural plots.

Small mammal trapping and flea collections

The geographical environment of the village and surrounding area was divided into three categories for small mammal trapping. The domestic environment was defined as being inside the hut and included only traps placed inside the hut structure. The peridomestic environment was defined as being that area extending from the exterior hut structure outward in all directions for a distance of five m. This distinction allowed us to best define the observed ranges of certain small mammals. The sylvatic environment was defined as extending from the village perimeter outward to 300 m.

Rodents and shrews were live-trapped during nine time points, approximately every other month, during the 18-month period from January, 2006 through June, 2007 using either Tomahawk (Model TLT102, Tomahawk Live Trap Company, Tomahawk, WI) or Sherman live catch traps (Model 3310A, H.B. Sherman Trap Company, Tallahassee, FL). Tomahawk traps were constructed of wire mesh and measured 13x13x41 cm. Sherman traps were constructed of galvanized sheet metal and measured 6.4x6.4x25 cm. Trapping was conducted inside and around ten huts in each of the ten selected villages. The criterion for hut selection included one or more persons routinely sleeping in the hut. Selected huts were dispersed throughout the village with no more than one hut chosen within each family compound. For each sampling session, traps were set using a standardized bait (even proportions of ground nuts, maize, and dried fish), on two consecutive afternoons and checked the following morning. Four traps were placed inside each hut (two Tomahawk and two Sherman) (n = 40 traps per day per village) to sample the domestic environment. Another six traps (three Tomahawk and three Sherman) were placed at sites of likely rodent activity in the peridomestic environment, which included exterior areas up to five m from the previously selected hut (n = 60 traps per day per village). Samples from sylvatic sites were collected along four transect lines that radiated 300 m in each cardinal direction (one transect line per direction) from the edge of each village perimeter. One Sherman and one Tomahawk trap were both placed every 20 m along each transect line, resulting in 15 trapping stations per 300 m transect line (n = 120 traps per day for all four transect lines per village). Sylvatic trap stations were marked with an iron bar driven into the ground and remained constant throughout the study. Native vegetation at sylvatic sites varied among the ten study villages, with elevation and slope noted as the key drivers. Within the relatively short 300 m transect lines, flora were comprised largely of native vegetation that was often highly disturbed by agricultural practices, wood harvesting/gathering, animal rearing, and thatch collection.

Small mammals were anesthetized using the inhalation anesthesia halothane prior to handling, measurement, and blood collection; after which, mammals were euthanized using an overdose of halothane confirmed by either cervical dislocation or bilateral thoracotomy. Small mammals were identified to genus or species in the field using morphological measurements and characteristics and the regional taxonomic keys of Delany and Neal (1966), Delany (1975), and Nowak (1999). Selected voucher specimens were sent to the Department of Zoology Museum at Makerere University in Kampala, Uganda for confirmation of field identifications and archiving. Because of the well-known difficulties involved in identifying African *Mastomys* (Roberts 1944, Green et al. 1980, Lecompte et al. 2005) and *Crocidura* (Jacquet et al. 2015) to species in the field, these specimens were identified to genus only. A recent study by Moore et al. (2015) provided cytochrome B (*cytB*) sequencing results for small numbers of individuals of these two genera that were collected in the same area where our study took place. Among the 24 specimens they identified as belonging to the genus *Mastomys* by *cytB* sequencing, 20 sequences were most similar to *M. erythroleucus* and the remaining four most closely resembled those for *M. natalensis*. The *cytB* sequences for all 31 specimens of *Crocidura* analyzed most closely resembled those previously published for *C. olivieri.*

Fleas were removed from anesthetized small mammals by vigerously combing over plastic collection pans, using the technique described by Gage (1999). Fleas were then collected from the pan using entomological forceps, as well as from the anesthesia bags originally used to anesthetize the mammals (bags were not reused). Finally, the mammals were thoroughly examined and any remaining fleas picked directly from the bodies/fur, using entomological forceps. The halothane used to anesthetize the small mammals also served to effectively, and simultaneously, anesthetize their fleas. All flea specimens were collected in glass vials (one vial per mammal) containing 2% saline with Tween-80® surfactant (two drops per liter), held at ambient temperature, and sent to the Entomology and Ecology Team, Bacterial Diseases Branch, Division of Vector-Borne Diseases, Centers for Disease Control and Prevention, Fort Collins, CO, where they were enumerated and identified using standard taxonomic keys appropriate for East Africa (Hopkins 1947a, Hopkins 1947b, Hopkins and Rothschild 1953, Hopkins and Rothschild 1962) and selected voucher specimens were retained.

Small mammal serology and bacterial culture

Approximately 1.0 ml of whole blood was obtained from each terminally anesthetized mammal by cardiac puncture and then placed on Nobuto blood absorption strips (Advantec MFS, Inc., Dublin, CA). The strips were air dried before being placed into collection envelopes and sent to the Entomology and Ecology Team, Bacterial Diseases Branch, Division of Vector-Borne Diseases, Centers for Disease Control and Prevention, Fort Collins, CO, where they were analyzed for *Y. pestis* antibodies according to previously published procedures (Wolff and Hudson 1974, Chu 2000). A reciprocal titer of $\geq$1:32 was considered positive. Data on *Y. pestis* infections in small mammal carcasses were also obtained from routine plague surveillance activities that were part of simultaneous efforts conducted within the plague risk area by CDC and UVRI. Carcasses from this surveillance

program were identified to species and necropsied to obtain liver and spleen tissues that could be tested for *Y. pestis* infection by direct fluorescent antibody (DFA) staining (Chu 2000). If found to be DFA-positive, tissues were further evaluated by direct culture (Chu 2000). Cultured bacterial colonies that were morphologically consistent with *Y. pestis* were picked from culture plates, placed into vials containing a HIB/glycerol mixture, frozen at -80° C, and later shipped on dry ice to the Diagnostics and Reference Team, Division of Vector Borne Diseases, Bacterial Disease Branch, Centers for Disease Control and Prevention, Fort Collins, CO, for confirmation by temperature specific bacteriophage lysis and biovar typing (Chu 2000).

Data and statistical analysis

Data from host and flea identification and enumeration of samples taken from the four traps placed inside each hut were aggregated with counts from samples taken inside the other huts sampled within the same village in order to provide total host and flea counts inside huts (domestic environment) for each village sampled in our study. Samples collected from peridomestic and sylvatic environments were similarly aggregated. Mean flea loads per host were compared between species, districts, and sampling locations (domestic, peridomestic, sylvatic environments in each village) using a generalized linear model with a negative binomial error structure and log link. Analyses were conducted in Matlab (Version 2018a, Mathworks, Inc., Natick, MA). Differences in the proportions of hosts infested with given fleas in different environments were analyzed by contingency table analyses and chi-square tests for significance.

We tested the hypothesis that rodent diversity increases with distance from homes using a linear mixed model with distance at 0, 3, 60, 160, and 260 m from homes as a continuous covariate and village as a random effect. The latter three distances were averages for rodents collected at 20-100, 120-200, and 220-300 m. We hypothesized that diversity would increase rapidly outside homes and thus tested whether quadratic terms on distance might provide a better explanation of the data. Rodent diversity estimates (*H*) for small mammal taxa were calculated by distance (five categories as above) and village (N = 10) using the Shannon-Wiener index (Magurran 1988) that accounts for both number of taxa identified and their abundances (Ludwig and Reynolds 1988). Analyses were conducted in Matlab (Version 2018a, Mathworks, Inc., Natick, MA). We evaluated models with and without a non-linear relationship for the effects of distance on diversity and used Akaike's Information Criterion (AIC) for model selection (Akaike 1974).

To test the second hypothesis, that rodent diversity, rodent abundance, and flea abundance would predict the probability of villages reporting concurrent plague cases, we fit case surveillance data from January, 2016 through June, 2017 (corresponding to the time frame of village small mammal/flea sampling) using a generalized linear mixed model with a binomial distribution and logit link. We aggregated both the response and the covariate data to seasons corresponding to established plague seasons (February-July for the off-season and August-January for the plague season). Thus, there were three sampling periods for each village (27 data points in total; Monkwerocoo was dropped because sampling of covariates was much less than the other villages due to a failed bridge that limited access to the village later in the study). Villages that reported at least one plague case during a given time period were assigned a 1 and villages with no reports were assigned a 0 (response data). Because the data were small we examined the fixed effects of each of the following 12 covariates independently: diversity of rodents in homes, diversity of rodents within 3 m of homes, diversity of rodents beyond 20 m of homes, total rodents captured in homes, total rodents captured within 3 m of homes, total number of fleas captured off rodents in homes, and total number of the following flea species captured on rodents within 300 m of homes (*Dinopsyllus* sp., *X. brasiliensis*, *Ctenophtalmus* sp., *Ctenocephalides* sp., *X. cheopis*, other flea species). Variables were examined separately because we did not have enough statistical power to put multiple covariates together. However, we were interested in understanding if any of the covariates predicted the cases better than stochastic (or unobserved) local conditions, and if so, which ones. AIC was used to reveal which covariates did the best job of predicting the data relative to one another.

Diversity calculations employed the Shannon-Wiener diversity index, as above. Each model also included a random effect of village to account for error correlation among samples from the same village. We tested the effect of each covariate on plague case reports both within the same time step and lagged by one season to examine if conditions during the previous season were predictive of conditions during the current season. We used AIC of each one-covariate model relative to AIC of the model with only random effects to evaluate whether predictors were important for explaining the data.

RESULTS

Small mammal and flea collections

A total of 20,211 trap nights were conducted in the ten villages resulting in the capture of 2,959 small mammals (14.6% overall trap success) that were hosts to 5,109 fleas. Twenty small mammal taxa were identified with 1,516 mammals trapped in Arua District and 1,443 captured in Zombo District. Twenty-two of the small mammals captured (0.7%) were juveniles lacking necessary taxonomic characteristics and therefore could not be reliably identified in the field. Three rodents: black rats (*Rattus rattus*-27.7% of all small mammals captured), Nile grass rats (*Arvicanthis niloticus*-22.1%), multimammate mice (*Mastomys* sp.-11.1%), and shrews of the insectivore genus *Crocidura* (14.9%) represented 2,242 of the 2,959 small mammals captured (75.8%).

R. rattus comprised a smaller than expected proportion of the taxonomically identifiable small mammal captures in Arua District (294 of 1,504 (19.6%)) compared to what would have been expected based on analogous results from Zombo District (525 of 1,433 (36.6%)) ($\chi^2 = 107.54$ $p < 0.0001$). Five

of the six villages in Zombo District were found at higher elevations than those in Arua District and the single Arua District village that occurred at a higher elevation than one of the Zombo villages exceeded the latter's elevation by only five m (Table 1).

The proportion of *A. niloticus* observed among the captures from Arua District (323 of 1,504 (21.5%)) did not differ significantly from what would have been expected based on the Zombo District results (332 of 1,433 (23.2%)). By contrast, *Mastomys* sp. comprised a greater than expected proportion of the identifiable mammals captured in Arua District villages (210 of 1,504 (14.0%)) compared to captures of this same genus in Zombo District villages (117 of 1,433 (8.2%)) ($\chi^2 = 25.29$; $p < 0.0001$). *Crocidura* sp. also accounted for a greater than expected proportion in Arua District villages (287 of 1,504 (19.1%)) compared to what was observed in Zombo District villages (154 of 1,433 (10.8%)) ($\chi^2 = 40.57$; $p < 0.0001$).

The small mammals captured in our study were hosts for 5,109 fleas belonging to nine taxa. Fleas of the genus *Xenopsylla* were abundant and represented by two efficient plague vectors, *X. cheopis* and *X. brasiliensis* (12.7% and 25.9% of all fleas collected, respectively) (Politzer 1954). *Dinopsyllus lypusus* and *Ctenophthalmus bacopus* were also abundant, accounting for 35.0% and 21.8% of all fleas collected, respectively. The combined totals for the above four flea species accounted for 95.4% (4,875 of 5,109) of all fleas collected from all hosts. Small numbers of *Echidnophaga gallinaceae* (142), *Stivalius torvus* (48), *Ctenocephalides felis* (23), *Leptopsyllus sp.* (4), and *Nosopsyllus sp.* (1) also were collected from the small mammals captured. The sum of these latter fleas, as well as a few others that were too damaged to be identified (16 of 5,109 = 0.3%), accounted for the remaining 4.6% (234 of 5,109 fleas) of all fleas collected.

Because of their dominance, the remaining analyses will focus primarily on the four major host taxa and the four fleas that predominated on these hosts. As indicated in Table 2, the proportions of *R. rattus, A. niloticus,* and *Mastomys* sp. found infested with *X. cheopis* were significantly higher in Arua District than in the villages within Zombo District, which lie at higher elevation (Table 1). By contrast, the proportions of *R. rattus*, *A. niloticus,* and *Mastomys* sp. infested with *X. brasiliensis* in Arua District were significantly lower than that observed in Zombo District (Table 2). The proportions of *R. rattus*, *A. niloticus,* and *Mastomys* sp. found infested with *D. lypusus* were also significantly lower in Arua District than in Zombo District, whereas the proportion of *A. niloticus* infested with *C. bacopus* in Arua District was significantly higher than in Zombo District. All remaining differences in the proportions of hosts infested with the four main flea species in Arua or Zombo Districts were statistically insignificant (Table 2).

Table 3 presents the means for the four major flea species collected from each of the four dominant host taxa, which were modeled using a generalized linear model with a negative binomial error structure and log link. The response variable was the count of fleas on a given individual small mammal. The fixed effects were a three-way interaction of the factors: district (two levels: Zombo and Arua), major small rodent genera (four levels: *Arvicanthis*, *Mastomys*, *Rattus,* and *Crocidura*), and flea taxa (four levels: *X. cheopis*, *X. brasiliensis*, *Dinopsyllus*, *Ctenopthalmus*). Three-way interactions were significant; thus, we present the means for each district/rodent/flea combination in Table 3 and in Figure 1.

Within Zombo District, *Arvicanthis* was clearly the most parasitized small mammal with means of 1.97 *Dinopsyllus* (CI [1.553, 2.392]), 1.56 *X. brasiliensis* (CI [1.223, 1.898]), 0.84 *Ctenophtalmus* (CI [0.649, 1.038]), and 0.04 *X. cheopis* (CI [0.016, 0.062]). *Mastomys* was primarily infested with *Dinopsyllus* fleas (mean 1.27, CI [0.801, 1.746]) and *Rattus rattus* was almost exclusively parasitized by *X. brasiliensis* (mean 1.28, CI [1.056, 1.504]). *Crocidura* in Zombo District were infested with few fleas, of which *Dinopsyllus* (mean 0.23, CI [0.131, 0.336]) and *Ctenophtalmus* (mean 0.19, CI [0.101, 0.276]) were most abundant.

Arvicanthis was again heavily parasitized in Arua District with means of 1.42 *Ctenophtalmus* (CI [1.110, 1.739]), 1.11 *Dinopsyllus* (CI [0.863, 1.367]), 0.35 *X. cheopis* (CI [0.252, 0.442]), and 0.02 *X. brasiliensis* (CI [0.005, 0.038]). *Mastomys* was primarily infested with *X. cheopis* (mean 0.83, CI [0.592, 1.075]) and *Dinopsyllus* (mean 0.57, CI [0.398, 0.751]). *Rattus rattus* was almost exclusively parasitized by *X. cheopis* (mean 0.62, CI [0.463, 0.782]). *Crocidura* in Arua District were infested with few fleas, of which *X. cheopis* (mean 0.33, CI [0.237, 0.432]) was most abundant.

Comparing mean flea loads on like small mammal taxa between Zombo and Arua Districts shows several significant differences, most notably in the distribution of *Xenopsylla* fleas. The mean number of *X. cheopis* on individual *R. rattus*, *A. niloticus*, *Mastomys* sp., and *Crocidura* sp. were all significantly higher in Arua District villages than in Zombo District villages. By contrast, the mean numbers of *X. brasiliensis* per *R. rattus, A. niloticus*, and *Mastomys* sp. were significantly lower in Arua District than in Zombo District, clearly demonstrating far greater prevalence of *X. cheopis* in Arua District and *X. brasiliensis* in Zombo District which is generally at higher elevation (see below). The mean numbers of *D. lypusus* on *R. rattus*, *A. niloticus*, and *Mastomys* sp. in Arua District, were also significantly lower than in Zombo District, but the numbers of *C. bacopus* found on these hosts did not differ significantly between districts.

Distributions of two of the four major flea species collected per host in our study also differed by the elevations of the villages sampled. The numbers of *X. cheopis* collected were negatively correlated with village elevations (Spearman's Correlation Coefficient = -0.6453, $p = 0.04$), but the numbers of *X. brasiliensis* were positively correlated with this factor (Spearman's Correlation Coefficient = 0.7348, $p = 0.02$). The numbers of the other two dominant flea species (*D. lypusus* and *C. bacopus*) collected in each village were not correlated with village elevations (Spearman's Correlation Coefficient = 0.0304, $p = 0.9336$; Spearman's Correlation Coefficient = -0.4073, $p = 0.2427$, respectively) (Table 1).

Our study also examined the distribution of small mammals and fleas in domestic, peridomestic, and sylvatic environments (Table 4). A total of 3,768 trap nights were

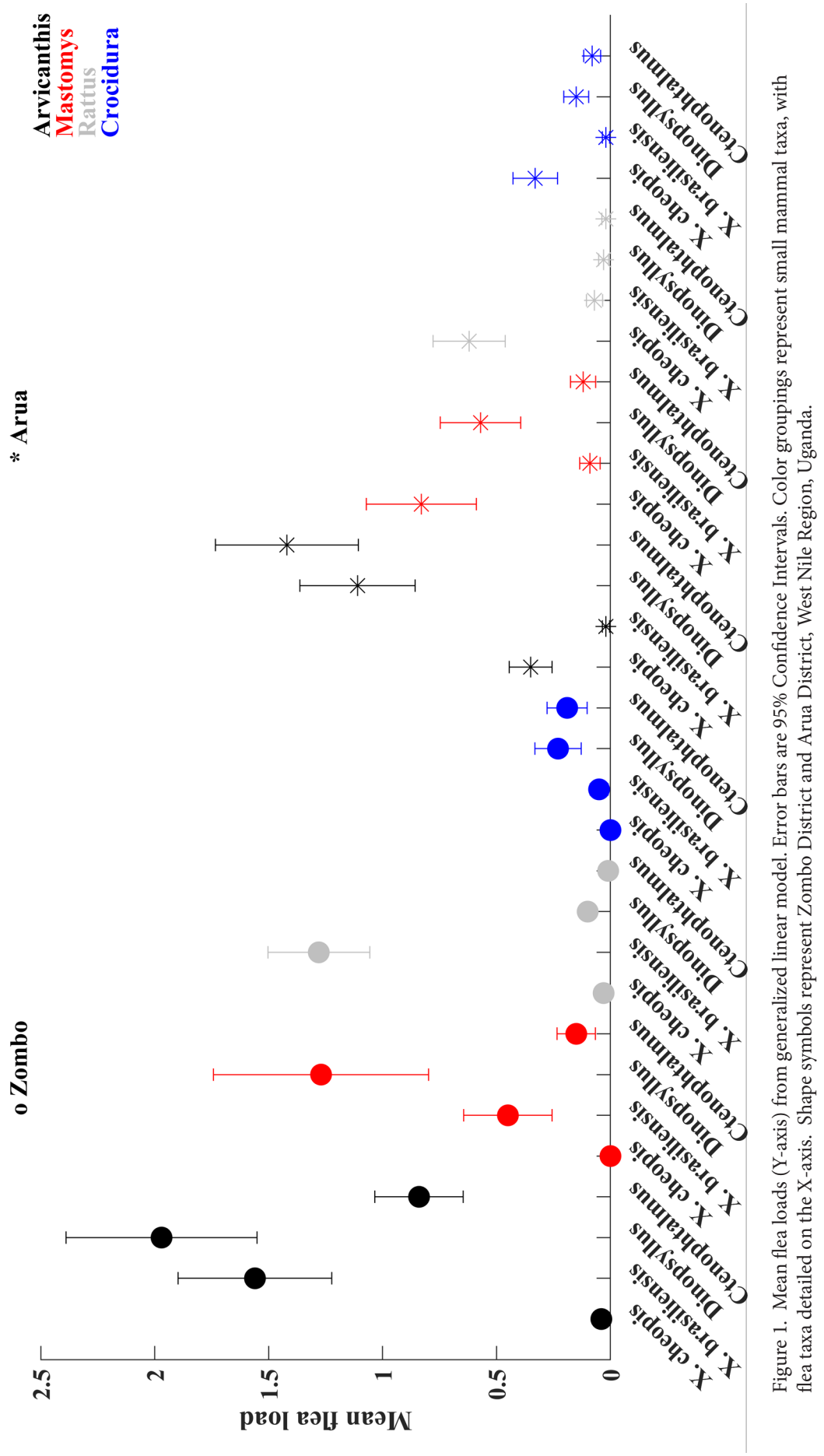

Figure 1. Mean flea loads (Y-axis) from generalized linear model. Error bars are 95% Confidence Intervals. Color groupings represent small mammal taxa, with flea taxa detailed on the X-axis. Shape symbols represent Zombo District and Arua District, West Nile Region, Uganda.

Table 2. Proportion of the four dominant small mammal hosts infested with predominant flea species, by district.

Arua District	**Type of Host**											
	Rattus rattus			*Arvicanthis niloticus*			*Mastomys* sp.			*Crocidura* sp.		
Flea Species	No. Hosts	No. Infested	Proportion of Hosts Infested	No. Hosts	No. Infested	Proportion of Hosts Infested	No. Hosts	No. Infested	Proportion of Hosts Infested	No. Hosts	No. Infested	Proportion of Hosts Infested
Xenopsylla cheopis	294	74	0.2517[A]	323	48	0.1486[A]	210	46	0.2286[B]	287	42	0.1463[B]
Xenopsylla brasiliensis	294	10	0.0340	323	6	0.0186	210	10	0.0476	287	4	0.0139
Dinopsyllus lypusus	294	8	0.0272	323	148	0.4582	210	66	0.3143	287	29	0.1010
Ctneophthalmus bacopus	294	6	0.0204	323	170	0.5263[A]	210	20	0.0952	287	18	0.0627
Zombo District	**Type of Host**											
	Rattus rattus			*Arvicanthis niloticus*			*Mastomys* sp.			*Crocidura* sp.		
Flea Species	No. Hosts	No. Infested	Proportion of Hosts Infested	No. Hosts	No. Infested	Proportion of Hosts Infested	No. Hosts	No. Infested	Proportion of Hosts Infested	No. Hosts	No. Infested	Proportion of Hosts Infested
Xenopsylla cheopis	525	6	0.0114	332	3	0.0090	117	0	0.0000	154	0	0.0000
Xenopsylla brasiliensis	525	228	0.4343[A]	332	98	0.2952[A]	117	21	0.1795[B]	154	3	0.0195
Dinopsyllus lypusus	525	36	0.0686[B]	332	198	0.5964[B]	117	62	0.5299[B]	154	23	0.1494
Ctneophthalmus bacopus	525	5	0.0095	332	220	0.3373	117	16	0.1368	154	15	0.0974

Capital letter superscripts denote significantly higher proportions of infestation for a given host with a particular flea species in the indicated district compared to the proportion of the same hosts infested in the corresponding district. The levels of statistical significance are as follows: [A] = $p < 0.0001$, [B] = $p < 0.001$, [C] = $p < 0.01$, and [D] = $p < 0.05$, respectively.

Table 3. Mean flea loads from linear model showing all district/rodent/flea model combinations.

District	Rodent	Flea	Mean	Standard Error	95% Confidence Interval - lower	95% Confidence Interval - upper
Zombo	*Arvicanthis*	*X. cheopis*	0.04	0.012	0.016	0.062
Zombo	*Arvicanthis*	*X. brasiliensis*	1.56	0.172	1.223	1.898
Zombo	*Arvicanthis*	*Dinopsyllus*	1.97	0.214	1.553	2.392
Zombo	*Arvicanthis*	*Ctenophtalmus*	0.84	0.099	0.649	1.038
Zombo	*Mastomys*	*X. cheopis*	0.00	0.000	0.000	0.000
Zombo	*Mastomys*	*X. brasiliensis*	0.45	0.099	0.259	0.647
Zombo	*Mastomys*	*Dinopsyllus*	1.27	0.241	0.801	1.746
Zombo	*Mastomys*	*Ctenophtalmus*	0.15	0.043	0.061	0.230
Zombo	*Rattus*	*X. cheopis*	0.03	0.008	0.015	0.046
Zombo	*Rattus*	*X. brasiliensis*	1.28	0.114	1.056	1.504
Zombo	*Rattus*	*Dinopsyllus*	0.10	0.016	0.069	0.132
Zombo	*Rattus*	*Ctenophtalmus*	0.01	0.005	0.003	0.023
Zombo	*Crocidura*	*X. cheopis*	0.00	0.000	0.000	0.000
Zombo	*Crocidura*	*X. brasiliensis*	0.05	0.020	0.013	0.091
Zombo	*Crocidura*	*Dinopsyllus*	0.23	0.052	0.131	0.336
Zombo	*Crocidura*	*Ctenophtalmus*	0.19	0.045	0.101	0.276
Arua	*Arvicanthis*	*X. cheopis*	0.35	0.048	0.252	0.442
Arua	*Arvicanthis*	*X. brasiliensis*	0.02	0.008	0.005	0.038
Arua	*Arvicanthis*	*Dinopsyllus*	1.11	0.129	0.863	1.367
Arua	*Arvicanthis*	*Ctenophtalmus*	1.42	0.160	1.110	1.739
Arua	*Mastomys*	*X. cheopis*	0.83	0.123	0.592	1.075
Arua	*Mastomys*	*X. brasiliensis*	0.09	0.023	0.041	0.131
Arua	*Mastomys*	*Dinopsyllus*	0.57	0.090	0.398	0.751
Arua	*Mastomys*	*Ctenophtalmus*	0.12	0.028	0.064	0.174
Arua	*Rattus*	*X. cheopis*	0.62	0.081	0.463	0.782
Arua	*Rattus*	*X. brasiliensis*	0.07	0.018	0.040	0.110
Arua	*Rattus*	*Dinopsyllus*	0.03	0.011	0.012	0.056
Arua	*Rattus*	*Ctenophtalmus*	0.02	0.009	0.004	0.037
Arua	*Crocidura*	*X. cheopis*	0.33	0.050	0.237	0.432
Arua	*Crocidura*	*X. brasiliensis*	0.02	0.008	0.002	0.033
Arua	*Crocidura*	*Dinopsyllus*	0.15	0.028	0.092	0.201
Arua	*Crocidura*	*Ctenophtalmus*	0.08	0.019	0.043	0.117

Table 4. Flea species infesting dominant small mammals by environment type.

Small Mammals by Environment *Ctenophthalmus bacopus*				Flea Species														
				Dinopsyllus lypusus			*Xenopsylla cheopis*			*Xenopsylla brasiliensis*			All others*					
Domestic Environment	**No. Hosts**	**No. per 100 trap nights**	**Percent infested**	**No. Fleas**	**Mean**	**Median**	**No. Fleas**	**Mean**	**Median**	**No. Fleas**	**Mean**	**Median**	**No. Fleas**	**Mean**	**Median**	**No. Fleas**	**Mean**	**Median**
Arvicanthis niloticus	17	0.45	94.1%	27	1.59	0.00	29	1.71	0.00	17	1.00	0.00	45	2.65	1.00	1	0.06	0.00
Crocidura sp.	21	0.56	33.3%	1	0.05	0.00	3	0.14	0.00	1	0.05	0.00	5	0.24	0.00	2	0.10	0.00
Mastomys sp.	16	0.42	81.3%	3	0.19	0.00	13	0.81	0.50	17	1.06	0.00	35	2.19	0.50	0	0.00	0.00
Rattus rattus	649	17.22	42.2%	6	0.01	0.00	45	0.07	0.00	114	0.18	0.00	579	0.89	0.00	114	0.18	0.00
Other small mammals	19	0.50	42.1%	3	0.16	0.00	7	0.37	0.00	10	0.53	0.00	5	0.26	0.00	1	0.05	0.00
TOTAL	722	19.16	44.5%	40	0.06	0.00	97	0.13	0.00	159	0.22	0.00	669	0.93	0.00	118	0.16	0.00
Peridomestic Environment																		
Arvicanthis niloticus	249	4.78	71.5%	311	1.25	0.00	367	1.47	0.00	83	0.33	0.00	319	1.28	0.00	0	0.00	0.00
Crocidura sp.	141	2.71	35.5%	22	0.16	0.00	40	0.28	0.00	55	0.39	0.00	4	0.03	0.00	10	0.07	0.00
Mastomys sp.	40	0.77	67.5%	9	0.23	0.00	19	0.48	0.00	74	1.85	0.00	13	0.33	0.00	1	0.03	0.00
Rattus rattus	110	2.11	41.8%	5	0.05	0.00	10	0.09	0.00	47	0.43	0.00	78	0.71	0.00	33	0.30	0.00
Other small mammals	92	1.77	21.7%	16	0.17	0.00	36	0.39	0.00	9	0.10	0.00	5	0.05	0.00	0	0.00	0.00
TOTAL	632	12.14	50.6%	363	0.57	0.00	472	0.75	0.00	268	0.42	0.00	419	0.66	0.00	44	0.07	0.00
Sylvatic Environment																		
Arvicanthis niloticus	389	3.46	73.0%	402	1.03	0.00	619	1.59	1.00	25	0.06	0.00	161	0.41	0.00	16	0.04	0.00
Crocidura sp.	279	2.41	27.2%	29	0.10	0.00	35	0.13	0.00	40	0.14	0.00	4	0.01	0.00	19	0.07	0.00
Mastomys sp.	271	0.53	53.5%	30	0.11	0.00	237	0.87	0.00	84	0.31	0.00	23	0.08	0.00	18	0.07	0.00
Rattus rattus	60	2.48	43.3%	2	0.03	0.00	8	0.13	0.00	38	0.63	0.00	37	0.62	0.00	2	0.03	0.00
Other small mammals	606	5.39	41.6%	246	0.41	0.00	320	0.53	0.00	36	0.06	0.00	12	0.02	0.00	17	0.03	0.00
TOTAL	1605	14.29	48.8%	709	0.44	0.00	1219	0.76	0.00	223	0.14	0.00	237	0.15	0.00	72	0.04	0.00

*Includes the 16 fleas unidentifiable to species level due to damage during collection.

completed inside residents' huts (domestic environment) in Arua District and Zombo District villages compared to 5,208 and 11,235 trap nights completed in peridomestic and sylvatic environments in these villages, respectively. The (sylvatic) transect lines also crossed multiple distinct agricultural crops and native vegetation types, offering a variety of habitats for small mammals. This was reflected by host species diversity in each of the three habitats, as measured by the corrected Shannon-Wiener index. Host diversity was lowest in the domestic environment (H = 0.495), somewhat higher in the peridomestic environment (H = 1.636), and greatest along each of the three distinct transect (sylvatic environment) intervals analyzed (H = 2.113, H = 2.206, and H = 2.256 at 20-100 m, 120-200 m, and 220-300 m intervals, respectively). Although small mammal diversity along the transects increased with distance from the domestic and peridomestic sites, these differences were slight and not statistically significant (Figure 2).

The predominant species captured inside huts was *R. rattus* (89.9% of total captures inside huts); *A. niloticus* (2.4%), *Mastomys* sp. (2.2%), and *Crocidura* sp. (2.9%) were rarely captured in the domestic environment. *A. niloticus* accounted for 39.6% of all captures in peridomestic environments, while *Crocidura* sp., Mastomys sp., and *R. rattus* represented 22.3%, 17.4%, and 6.3% of the total peridomestic captures, respectively. The most abundant small mammal in sylvatic locations was *A. niloticus* (24.2% of total captures), followed by *Crocidura* sp. and *Mastomys* sp. (17.4% and 16.9% of total captures, respectively). Very few *R. rattus* were collected from transects in sylvatic environments (only 3.6% of total captures) (Table 4) and those black rats were spread somewhat evenly at low numbers along the transects (Figure 3). *Arvicanthis niloticus, Mastomys* sp., and *Crocidura* sp. were also spread relatively evenly along the transects but at higher numbers. Figure 3 also shows that *A. niloticus* was the dominant small mammal in the peridomestic environment and at 14 of the 15 transect trapping stations (distances) along the transects (26 *Mastomys* sp. were captured at the 260 m trap stations compared to 24 *A. niloticus*). Other small mammal taxa were rare in domestic and peridomestic environments but accounted for 39.1% of the total animals captured in the sylvatic habitat (1,605), including *Praomys* sp. (7.1%), *Mus* sp. (6.2%), *Tatera* sp. (6.0%), *Lophuromys sikapusi* (4.5%), *L. flavopunctatus* (4.4%), *Lemniscomys striatus* (3.4%), *Aethomys kaiseri* (2.5%), *Cricetomys gambianus* (0.9%), *Dasymys* sp. (0.6%), *Gerbillus* sp. (0.3%), *Thamnomys* sp. (0.2%), *Otomys* sp. (0.1%), *Oenomys* sp. (0.1%), *Dendromus mystacalis* (0.1%), *Taterillus emini* (0.04%), and 18 unidentified specimens representing 1.1%.

One thousand eighty-three total fleas were collected from all animals captured inside huts (21.2% of total fleas collected during the study). The rat fleas *X. brasiliensis* (61.8%) and *X. cheopis* (14.7%), represented 76.5% of all fleas captured on all hosts inside huts (Table 2). Less frequently encountered fleas included *D. lypusus* (9.0%) and *C. bacopus* (3.7%). Other flea species combined accounted for only 10.9% of all fleas collected from all hosts inside huts. The fleas most commonly collected from *R. rattus* in huts were *X. brasiliensis* (67.5% of total fleas on *R. rattus*) and *X. cheopis* (13.3%). Only 5.2% of all fleas on *R. rattus* captured inside huts were *D. lypusus* and <1% were *C. bacopus* (Table 4).

Small mammals captured in peridomestic environments were hosts for 1,566 total fleas reported in Table 4, including *X. cheopis* (17.1% of total fleas found on all hosts in the peridomestic environment), *X. brasiliensis* (26.8%), *D. lypusus* (30.1%), and *C. bacopus* (23.2%). *R. rattus* were hosts for 17.5% of all *X. cheopis* removed from all hosts in the peridomestic environment, 18.6% of *X. brasiliensis*, 2.1% of *D. lypusus*, and 1.4% of *C. bacopus. Arvicanthis niloticus* were hosts for 31% of all *X. cheopis,* 76.1% of *X. brasiliensis*, 77.8% of *D. lypusus*, and 85.7% of *C. bacopus* in peridomestic habitats. Fleas recovered from *Mastomys* sp. accounted for 27.6% of all *X. cheopis* recovered from all hosts in the periodomestic environment, 3.1% of *X. brasiliensis*, 4.0% of *D. lypusus*, and 2.5% of *C. bacopus* fleas collected in peridomestic environments. *Crodicura* sp. were hosts for 20.5% of *X. cheopis*, 0.1% of *X. brasiliensis*, 8.5% of *D. lypusus*, and 6.1% of *C. bacopus* fleas recovered in these environments.

Small mammals captured in sylvatic environments were hosts to 2,460 fleas (48.2% of all fleas collected for the study). The dominant flea in these sites was *D. lypusus* (50.0% of total fleas from all hosts in sylvatic habitats), over half (50.8%) of which were collected from *A. niloticus*. *D. lypusus* was also commonly encountered on *Mastomys* sp. in sylvatic habitats (19.5% of all *D. lypusus* collected). Only 0.7% and 2.9% of the *D. lypusus* collected in sylvatic environments were found on *R. rattus* and *Crocidura* sp., respectively. The remaining *D. lypusus* (26.1% of total) in this environment were distributed on a variety of other small mammals. *C. bacopus* was also common in sylvatic environments (28.8% of total), occurring most often on *A. niloticus* (56.7% of all *C. bacopus* collected). *Crocidura* sp., *Mastomys* sp., and *R. rattus* were hosts to less than 5% of the *C. bacopus* collected in sylvatic environments. More than one-third (34.7%) of *C. bacopus* were recovered from other less frequently captured small mammals. In the sylvatic environment, *R. rattus* were hosts for 17.0% and 15.6% of the *X. cheopis* and *X. brasiliensis* collected, respectively. Also, in sylvatic locations, the primary host of *X. brasiliensis* was *A. niloticus* (67.9% of total) and *Mastomys* sp. was the major host of *X. cheopis* (37.7% of total) (Table 4).

Comparison of flea abundance among villages with and without a prior history of human plague

In some instances, the proportion of hosts infested with certain flea species differed significantly among villages that had previously experienced human plague (Olli and Pomosi in Arua District and Sokonzi, Agore, and Uyaru-Agada in Zombo District) and those that had not (Pembeleku and Kaza in Arua District and Monkwerocoo, Anyiku, and Gbalia in Zombo District) (Table 1). When infestations of black rats with *X. cheopis* or *X. brasiliensis* combined were analyzed, it was found that the proportion of rats infested with either, or both, of these vector-competent fleas was significantly higher in villages with a history of plague (χ^2 = 24.87; $p < 0.0001$). When these rat fleas were analyzed separately, it was found that the proportion of *R. rattus* infested with *X. cheopis,*

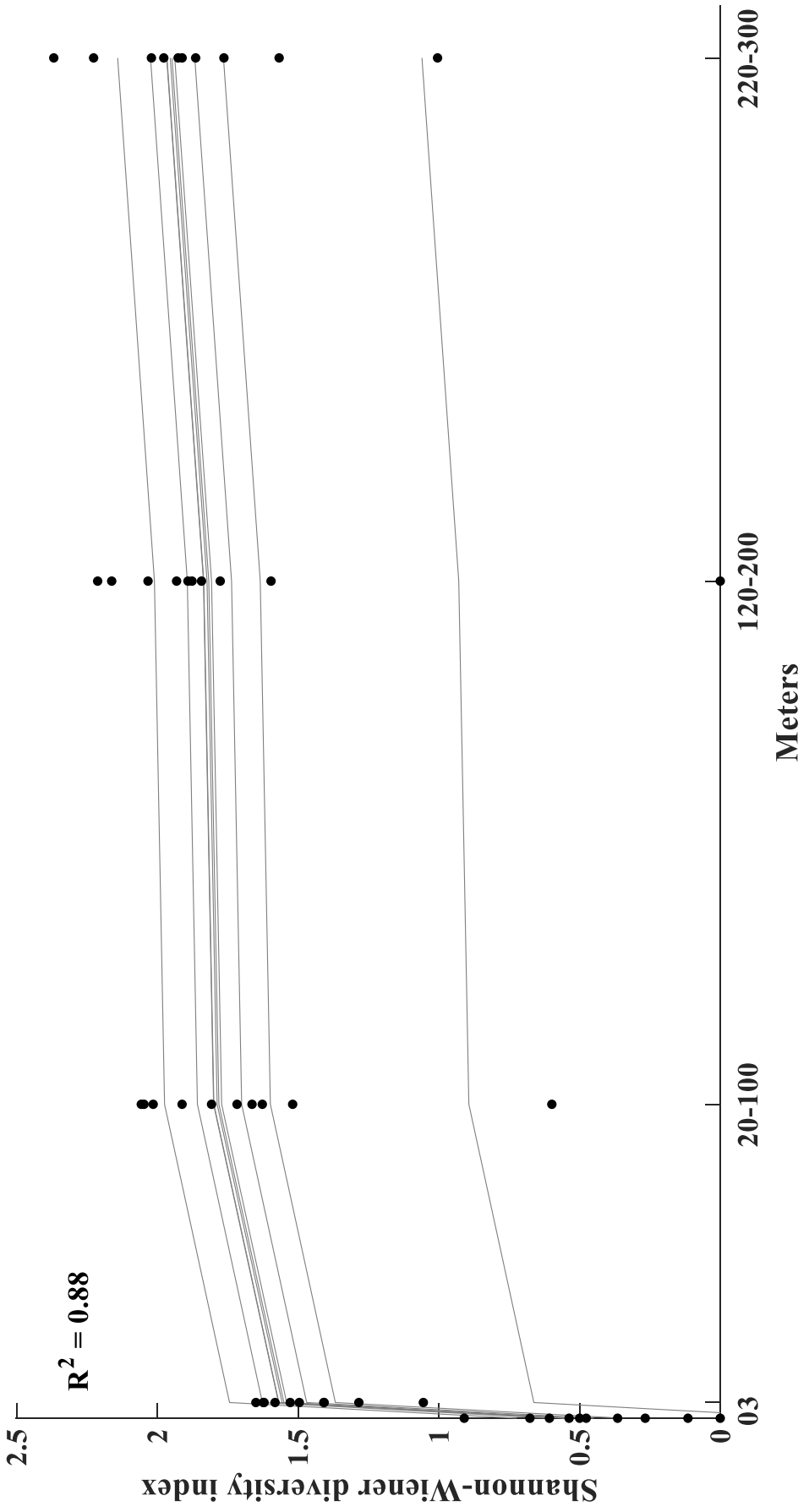

Figure 2. Relationship of small mammal diversity by distance from homes. Location for sampled mammals relative to homes are shown on the X-axis in meters, where distance 0 indicates the rodent was trapped inside the home (domestic environment), and distance 3 indicates the rodent was trapped in the peridomestic environment (up to 5 meters from a home). Distances 20-100 m, 120-200 m and 220-300 m are grouped data for those trap stations (located at 20 m intervals). Points represent the observed diversity indices for each village at each distance sampled. Grey lines show the model fit.

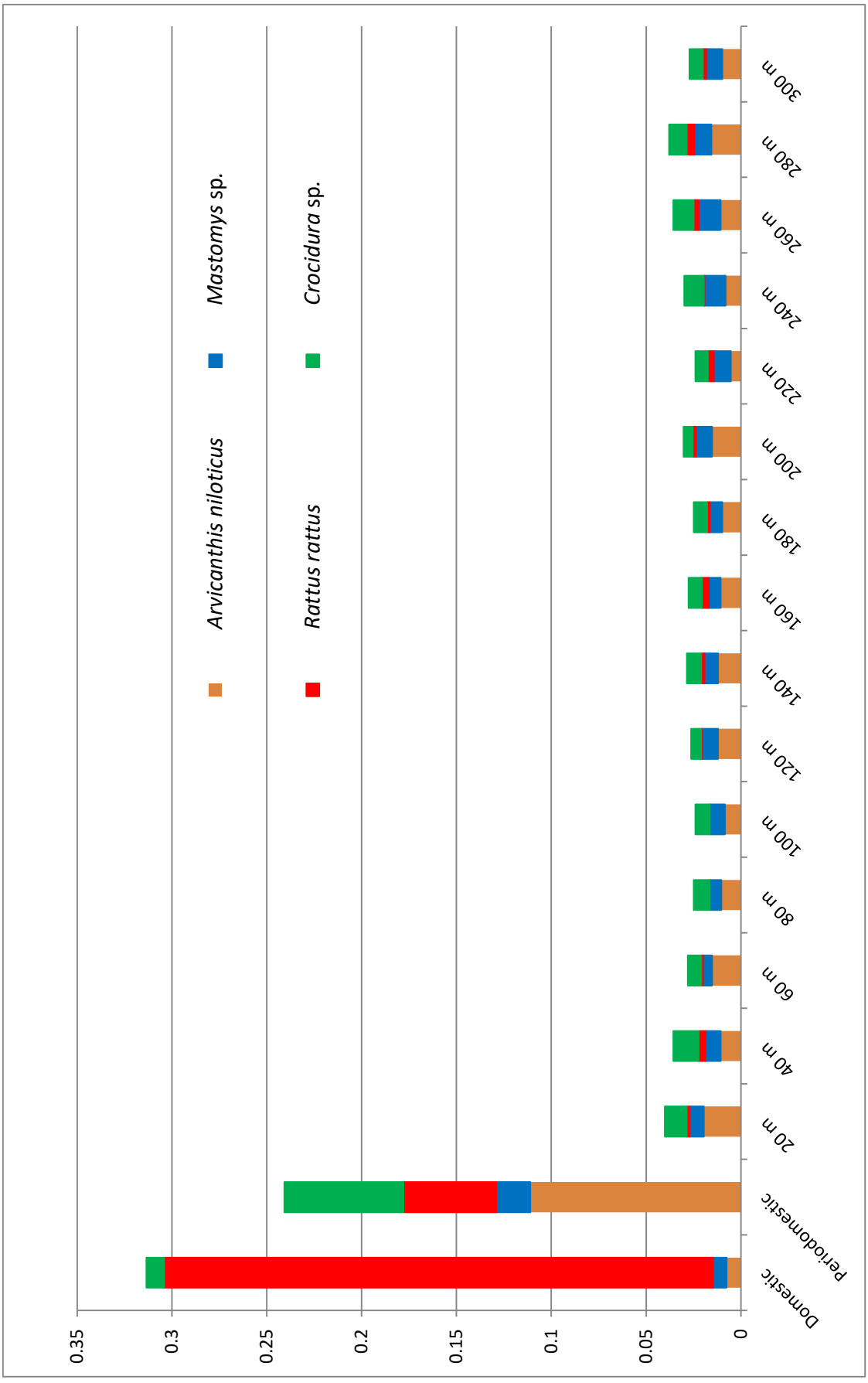

Figure 3. Distributions of key small mammal taxa trapped, as a proportion of all key mammal taxa trapped (Y-axis), in the domestic, peridomestic and sylvatic environments (X-axis). Distances 20 m – 300 m represent trap stations along transect lines (sylvatic environment) measured outward from the village perimeter.

regardless of environment type, also was higher in the plague-affected villages (χ^2 = 18.81; p < 0.0001). Surprisingly, the proportion of *R. rattus* infested with *X. brasiliensis* was lower in plague-affected villages (χ^2 = 7.48; p = 0.0062). No other comparisons of the proportions of hosts infested by specific flea species in villages with or without a history of plague were found to be statistically significant.

The proportions of hosts infested with certain flea species in different environments also differed significantly among villages with and without a recent history of human plague. The proportion of *R. rattus* infested within homes (domestic environment) by *X. cheopis* was higher in villages that had reported recent plague cases compared to those that had not reported cases (χ^2 = 26.12; p <0.0001). No significant differences were found between *C. bacopus* infestations on *A. niloticus* in villages with a plague history and those that had not experienced plague (x^2 = 3.06; p = 0.0804). Comparisons of proportions of hosts infested with a given flea species in other environments did not differ significantly among those villages with a history of plague and those without such a history.

The total numbers of fleas recovered from small mammals in villages with a recent history of plague were higher than those observed on small mammals from villages without reported cases (χ^2 = 10.76, df = 1, p = 0.0010). When flea loads were compared among villages with and without a history of human plague cases for specific environments, no significant differences in flea loads were observed on small mammals among these villages within the domestic environment for any combination of fleas and hosts. However, within the peridomestic environment, numbers of *X. cheopis, X. brasiliensis, D. lypysus,* and *C. bacopus* were higher on *Crocidura* sp. in villages with a history of human plague (median = 0, range = 0-17) compared with those that had not experienced human plague (median = 0, range = 0-3) (χ^2 = 4.44, df = 1, p = 0.0351) (Table 1), which is somewhat surprising as *Crocidura* sp. generally harbored fewer fleas than the other three dominant hosts (χ^2 = 4.44; p = 0.0351). This observed difference in flea loads between shrews in villages with and without prior histories of human plague was the result of higher numbers of *C. bacopus* being found on *Crocidura* sp. collected in peridomestic environments in villages with a history of human plague (median 0, range 0-3) than in villages without human cases (median:0, range 0-1; χ^2 = 4.57, df = 1, p = 0.0326).

The combined total of the four dominant flea species collected from the four dominant small mammals (*R. rattus*, *A. niloticus, Mastomys* sp., and *Crocidura* sp.) captured in sylvatic environments was greater in villages with a history of plague (median = 1, range = 0-25) than in villages lacking such a history (median = 0, range 0-19) ; χ^2 = 10.93, df = 1, p = 0.0009), and this difference held across all key flea species: *C. bacopus* (χ^2 = 12.18, p = 0.0005), *D. lypusus* (χ^2 = 11.17, p = 0.0008), *X. cheopis* (χ^2 = 6.23, p = 0.01), and *X. brasiliensis* (χ^2 = 5.48, p = 0.02). Specifically, *D. lypusus* fleas in the sylvatic habitat were more abundant on *A. niloticus* in villages with a history of plague (χ^2 = 8.98, p = 0.0027). The abundances of *X. cheopis* and *X. brasiliensis* did not differ significantly among villages with and without a recent history of human plague (χ^2 = 2.79, p = 0.1000 and χ^2 = 2.50, p = 0.1100) but the combined abundance of both *Xenopsylla* fleas (*X. cheopis* and *X. brasiliensis*) collected from *Crocidura* sp. captured in the sylvatic environment were greater in villages that had experienced human plague (χ^2 = 4.55, df = 1, p = 0.0329). Other combinations of key small mammals and fleas in villages with differing plague histories failed to produce statistically significant differences in flea infestations.

Model of small mammal diversity by distance from dwellings

Using AIC, we found that the best model included distance to the fourth power (AIC = 36.4; AICs for distance alone, squared or cubed were 86.5, 81.5, 77.2, respectively). The model demonstrates a large increase (approximately doubled on average) between the domestic (0 m) and peridomestic (3 m) distances, with a much smaller increase in distance at 20-100 and similar levels of diversity at 120-200 and 220-300 m (Figure 2).

Model of flea abundance and small mammal diversity as a predictor of concurrent human plague cases by village

None of the fixed effects produced a significantly better fit to the data relative to the model with random effects of village alone according to AIC scores. This suggests that local conditions within or among villages explained more of the variation in plague case reports than the individual covariates we examined. We did not have information on movement patterns of humans among these villages to examine effects of connectivity. None of the individual covariates were significant as the dataset was small and did not allow us to examine combinations of variables, but we noted some trends that might merit further attention. First, in the models with lagged effects, there were trends of positive relationships between plague reports and four covariates in the previous time step: diversity of rodents beyond 20 m, total rodents within 3 m of homes, abundance of *Dinosyllus* sp. on rodents in all locations, and abundance of *X. cheopis* on rodents in all locations. For these covariates, upper and lower prediction intervals were wide but trended in a positive direction for all villages. For covariates within the same time step, the only consistent trend was a positive relationship between the diversity of rodents in the peridomestic environment and the status of plague case reports. On average, the probability of at least one plague case report increased from 0 to 0.6 when peridomestic diversity increased from 1 to 1.7.

Laboratory-based evidence of recent plague activity

Eight of 2,959 (0.0027%) small mammal blood samples collected on Nobuto strips and tested for antibodies to *Y. pestis* by passive hemagglutination (Chu 2000) were found to be positive (reciprocal titer $\geq$ 1:32). These seropositive animals included four *Crocidura* sp., three *A. niloticus,* and a single *R. rattus.* One seropositive animal was collected from Sokonzi (Zombo District, *Crocidura* sp., titer 1:64) and another from Olli (Arua District, *Crocidura* sp., titer 1:32) villages, both of which had a recent history of plague cases per our study

criteria. The remaining six seropositive small mammals were collected in Kaza (Arua District), a village without a recent history of recent plague cases prior to the beginning of our study. These six specimens included three *A. niloticus* (titers 1:128, 1:256, 1:1024), two *Crocidura* sp. (titers 1:32, 1:64) and a single *R. rattus* (titer 1:1024). As noted below, a case of human plague was reported from the previously plague-free (1999-2005) village of Kaza during our study.

In November, 2006, during our study, a plague outbreak occurred in the WNR that resulted in at least 127 clinically suspect cases of human plague (Ogen-Odoi, et al. 2009). In addition to these human cases, eight rodent carcasses were collected in December, 2006 as part of the ongoing plague outbreak investigation. Seventeen of the human cases occurred in our study villages (16 in Olli and one in Kaza). Four of the carcasses collected (50%) yielded isolates of *Y. pestis* (biovar Antiqua) that were confirmed by CDC (Diagnostic and Reference Team, Bacterial Diseases Branch, Division of Vector-Borne Diseases, Centers for Disease Control and Prevention). Three of the positive carcasses were *R. rattus*, one each from the villages of Olli, Andossi, and Nave. The fourth carcass, identified as *Lophuromys* sp., was collected in the village of Cirifi. All four carcasses were collected from villages where human plague cases had just occurred. Among the villages where plague-positive rat carcasses were identified, only Olli was included in our study but the other three were located nearby (12-18 km). Additionally, *Y. pestis* (biovar Antiqua) was also isolated from a single *X. cheopis* flea removed from a *R. rattus* carcass collected in Odrani village during a case investigation two years later (October, 2008).

DISCUSSION

Although it is uncertain when *R. rattus* first appeared in the currently plague endemic WNR, it was clearly present in the adjoining Ituri District of the DRC by 1958 (Misonne 1969). The fact that the WNR's first reported outbreak of human plague did not occur until 1970 strongly suggests that this outbreak was linked to the invasion of *R. rattus.* The establishment of *R. rattus* in this region also undoubtedly increased human plague risk and played a significant role in the occurrence of subsequent outbreaks over the past few decades. Along with other recent studies from this geographic region (Amatre et al. 2009, Eisen et al. 2013, Eisen et al. 2014), our study confirmed that *R. rattus* is common in and near homes in the WNR, where it is frequently infested with two highly competent plague vectors (*X. cheopis* and *X. brasiliensis*). The distributions and abundances of hosts and fleas in key habitats, as well as serological analyses and local plague surveillance data, also identified *A. niloticus, Mastomys* sp.*,* and *Crocidura* sp. as the native hosts most likely to be ecologically significant in the WNR and interact with invasive *R. rattus* in ways that could influence human plague risk. This conclusion agrees with Amatre et al. (2009) who also identified *A. niloticus* and *Mastomys* sp. as important native flea hosts. Their study, however, involved only rodents and, therefore, did not investigate *Crocidura* sp., although the potential importance of shrews as plague hosts was addressed in a subsequent study by our group (Moore et. al. 2015).

Among the 20 taxa of small mammals identified in our study, *R. rattus, Mastomys* sp.*, A. niloticus,* and *Crocidura* sp. were clearly dominant, accounting for more than three-fourths of all mammals captured. The predominance of these four types of small mammals was even more striking in domestic and peridomestic environments where they comprised 97.4% and 85.4% of all captures, respectively. The identification of *Y. pestis* infections in these small mammals, either as a result of our study or the activities of the local plague surveillance program, suggests that these four small mammal taxa were the ones most likely to play roles in the ecology and epidemiology of plague within the WNR. Although seropositive small mammals were scant in our study (4/441 *Crocidura* sp., 3/655 *A. niloticus*, 1/819 *R. rattus,* and 0/1,044 others), the presence of at least some positive specimens demonstrates these species do become infected with plague and underscores the potential importance of these small mammals to enzootic plague maintenance and possible spread to humans. Moore et al. (2015) reported that abundances of *A. niloticus* and *Crocidura* sp. increased during the plague season within the plague risk area, as defined by Eisen et al. (2010), and were, therefore, considered potentially important plague hosts. The fact that seropositive small mammals were rare in our study might be an indication that populations of these animals in the WNR are highly susceptible to plague and seldom survive *Y. pestis* infections to later seroconvert. Such extreme susceptibility, might suggest that plague is a relatively recent (since 1970) introduction into the WNR, which could be epidemiologically important because rodents that succumb to plague are the ones most likely to develop the high-level bacteremias required to infect fleas (Eisen and Gage 2012). As these highly susceptible rodents die of plague, their host-seeking fleas can also serve as sources of *Y. pestis* infection in local human populations. This notion is supported by data from the local plague surveillance program which confirmed the presence of *Y. pestis* in carcasses of *R. rattus* collected within our study area during a human plague outbreak that occurred in the WNR in 2006 (Ogen-Odoi et al. 2009). Additional surveillance data gathered after the completion of our study identified other *Y. pestis*-positive carcasses of *R. rattus*, as well as those of *Mastomys* sp., *A. niloticus,* and *Crocidura* sp. (Boegler et al. 2018).

The peridomestic environment was commonly shared by *R. rattus* and the three major native hosts (*A. niloticus, Mastomys* sp., and *Crocidura* sp.) most dominant in our study (Figure 3), making it a probable site for flea exchanges between these hosts. Sylvatic sites are less likely to be significant sites of flea exchange between black rats and native small mammals. The relatively few *R. rattus* captured in sylvatic sites were found scattered along the lengths of the transects rather than clustered near the boundaries of the peridomestic and sylvatic habitats, suggesting that their presence did not result simply from limited spillover from peridomestic habitats, but rather represented the dispersion of some individuals into sylvatic sites, perhaps in attempts to find mates, establish new territories, or locate new food sources in nearby villages.

The dominant fleas in our study were *X. cheopis*, *X.*

brasiliensis, *D. lypusus,* and *C. bacopus*. The first two of these fleas are known to be excellent vectors of *Y. pestis* (Pollitzer 1954) and were common on *R. rattus*, *A. niloticus*, and *Mastomys* sp. Although *X. cheopis* was common on *Crocidura* sp., *X. brasiliensis* was rarely found on these animals. Populations of both species of *Xenopsylla* were highest in domestic environments and lowest in sylvatic environments. A third flea, *D. lypusus,* is not known to readily feed on humans but has been reported elsewhere in eastern Africa to be a competent plague vector (Devignat 1949, Pollitzer 1954) and important in enzootic maintenance of *Y. pestis* (Devignat 1949, Kilonzo 1992). Although we found this flea only occasionally on *R. rattus* or *Crocidura* sp., it was common on *A. niloticus* and *Mastomys* sp., as indicated by the fact that 25.1% of all fleas collected during our study were *D. lypusus* taken from these last two rodents. Unlike the two species of *Xenopsylla, D. lypusus* was most abundant in sylvatic environments and least so in domestic ones. In addition to its probable role as an enzootic vector in the WNR, *D. lypusus* could act as a bridging vector to spread plague bacteria from *A. niloticus* or *Mastomys* sp. living in sylvatic or peridomestic environments to *R. rattus* found in peridomestic environments. Because *X. cheopis* and *X. brasiliensis* are also common on *A. niloticus* and *Mastomys* sp. in peridomestic environments and not infrequently observed in sylvatic environments, they could likewise act as additional bridging vectors to spread plague bacteria to *R. rattus* in peridomestic environments. Once infested with *Y. pestis*-infected fleas, black rats could carry infected fleas into huts where we believe most human plague exposures occur. The *R. rattus* themselves could become infected, thus resulting in the infection of still more vector fleas and further increasing human plague risks.

The importance of peridomestic environments as sites where flea exchanges might occur between *R. rattus* and *A. niloticus, Mastomys* sp., or *Crocidura* sp. is suggested by the fact that, although most *R. rattus* were captured in residents' huts where *A. niloticus, Mastomys* sp., and *Crocidura* sp. are rarely encountered, 17.1% of all captures in peridomestic environments were black rats. *A. niloticus* and *Crocidura* sp. also occurred at relatively high frequencies in peridomestic environments (39.6% and 22.3% of captures in this environment, respectively) and *Mastomys* sp., albeit not as abundant as the previous two species, accounted for 6.4% of captures in periodomestic locations, an amount that was almost three times the percentage of captures represented by this rodent inside huts (Table 4). The importance of *C. bacopus* in plague transmission in the WNR remains unknown, but its potential role as an enzootic vector is suggested by reports that other *Ctenophtlamus* fleas (*C. calceatus cabirus* and *C. calceatus phyris*) have been found infected with *Y. pestis* in the DRC, as noted by Gratz (1999a).

Abundances on hosts of the four dominant flea species are likely to affect enzootic transmission and human plague risk (Krasnov 2006, Gage 2012). Unlike Amatre et al. (2009), whose analyses used a subset of the results presented in the present paper, analyses of the complete dataset revealed differences in local plague history and the environments where small mammals were captured correlated significantly with the flea loads observed on the dominant small mammal hosts. The numbers of *X. cheopis*, *X. brasiliensis*, *D. lypusus*, and *C. bacopus* were higher on *Crocidura* sp. in villages with a history of human plague than in those villages that had not reported human cases. *R. rattus, A. niloticus*, *Mastomys* sp., and *Crocidura* sp. collected in sylvatic environments surrounding villages that had experienced human plague cases also carried significantly more fleas of the above four species than were found on these same hosts in the sylvatic habitats associated with villages that had not reported human plague. The reasons for these differences remain unknown and suggest topics for further study. All villages sampled in this study, regardless of whether they had experienced cases within recent years, were located within the high-risk area for plague as defined by our study criteria, as well as those described by Eisen et al. (2010). Following the conclusion of our study, a human case occurred in one of the villages (Kaza) we classified as having no recent (six years) history of plague. One possible explanation for this observation is that all the villages we examined could be at risk for plague over longer periods (>six years) and the risk for each village changes depending on current local environmental conditions that temporarily favor increased flea survival and abundance. These conditions would not necessarily persist over time and could occur later in other villages that had not recently (1999-2005) experienced human plague at the time our study began. As these villages experienced environmental conditions that were more favorable for the flea vectors and small mammal hosts of plague, human risks could be expected to increase. If this is true, our negative control villages may be overmatched and carry similar long-term risk compared to our villages selected because they had a history of plague cases (within six years). Hence, the results we obtained regarding flea infestations on small mammals in villages with or without plague could simply reflect current local environmental conditions and epizootic activity rather than actual differences in the likelihood of epizootic activity and plague transmission to humans in these villages over longer periods of time.

Although plague epizootics are likely to begin among native rodent species in sylvatic habitats and then spread to *R. rattus* active in peridomestic sites, human exposures to plague in the WNR and other plague-endemic regions of eastern Africa are thought to occur most frequently in domestic sites where black rats predominate. The fleas most likely to transmit *Y. pestis* to humans during *R. rattus*-associated plague outbreaks are either *X. cheopis* or *X. brasiliensis* (Pollitzer 1954, Gage and Kosoy 2006). In most instances, the flea of greatest concern in tropical plague foci is *X. cheopis*, which is widespread on rats and was the fourth most commonly collected flea in our study. *X. brasiliensis*, the third most abundant flea in our study, has a more restricted distribution but is recognized as an important vector of human plague in sub-Saharan Africa and reportedly rivals *X. cheopis* in its ability to transmit *Y. pestis* efficiently (Roberts 1936, Vincke and Devignat 1937, Riel and Mol 1939, Davis 1946, Hopkins 1949, Roberts 1950). The distribution of these two flea species differed markedly in our study. *Xenopsylla cheopis* occurred primarily at lower elevation sites in the Arua District while

X. brasiliensis predominated in the higher elevation villages of Zombo District. Although some studies reported *X. brasiliensis* from a few low elevation sites in east Africa, such as the port of Mombasa in Kenya (Roberts 1935a), elsewhere in this part of Africa it is most abundant at relatively high elevation sites, including mountainous regions of Tanzania and DRC where severe plague outbreaks have occurred over the past three decades (Neerinckx et al. 2008, Bertherat 2016).

How elevation influences the abundances of *X. cheopis* and *X. brasiliensis* in the WNR remains to be determined, but the relative distributions of these fleas could be important in the epidemiology of plague in Uganda and elsewhere in sub-Saharan Africa. Studies conducted in Kenya during the mid-20th century suggest various factors that might influence the distributions of *X. cheopis* and *X. brasiliensis* (Symes and Hopkins 1932, Roberts 1935a, Roberts 1935b, Roberts 1936, Roberts 1950). Roberts (1950) reported that *X. brasiliensis* was the predominant vector during the initial phases of plague outbreaks in Kenya and *X. cheopis* predominated during later phases. He attributed this shift in dominance to structural differences in the homes affected in different localities as the disease progressed from highly rural areas where homes were covered by thatched roofs to other homes, including those in urban areas, which were covered by metal roofs. Roberts (1935b) reported, in other Kenyan studies, that *X. cheopis* was found most frequently on rats living underground, while *X. brasiliensis* occurred most often on rats nesting in thatched roofs resembling those found in the WNR. *X. brasiliensis* was almost the only flea observed on rural rat populations where these rodents lived in thatched roofs. *X. cheopis* was most frequently encountered in more urbanized areas and occurred in conjunction with other flea species. Roberts (1950) speculated that rats in Pumwani, Kenya were more heavily infested with *X. cheopis* than *X. brasiliensis* because homes in this region were covered with iron roofs that precluded rats from building aboveground nests and forced them to live underground in sites that were more favorable for *X. cheopis.* Historically, Hopkins (1949) agreed that *X. cheopis* was more numerous than *X. brasiliensis* in burrows in Uganda but noted that the latter flea was often found in burrows as well as aboveground sites. He further states that *X. brasiliensis* was the only flea typically found on rats in rural plague-endemic areas of Uganda and that *X. cheopis* was rare or absent from such areas, an observation that led him to believe that *X. cheopis* was "unsuited for life on roof-dwelling rats." The burrow nesting habits of *Mastomys* sp. could perhaps explain why numbers of *X. cheopis* in the peridomestic environments in our study were higher on these mice than on thatch-dwelling *R. rattus*.

Hopkins (1949) also proposed that *X. brasiliensis* was the "normal initiator of all plague outbreaks" in Uganda and many areas of Kenya. He additionally believed that *X. cheopis* was of little importance in spreading plague in Uganda because it was confined to large townships and rarely encountered in rural areas where plague outbreaks typically began. This report differs from our observations because we found *X. cheopis* in rural areas in the WNR, particularly in the lower elevation portions of the plague risk area in Arua District. Interestingly, Hopkins (1949) did not report the presence of *X. brasiliensis* at any of the 11 sites he studied in the WNR, most likely because he chose not to sample habitats farther from the river above the approximately 1,400 m elevation where *X. brasiliensis* was found to be most abundant in our study. His observation that *X. brasiliensis* was the initiator of human outbreaks remains intriguing, however, particularly if one considers that certain relatively high elevation areas of Uganda, Tanzania, and the DRC represent east Africa's long-term endemic foci of plague and are sites where human plague outbreaks continue to occur (Neerinckx et al. 2008, Bertherat 2016).

If *X. brasiliensis* fleas found at relatively high elevations are indeed critical for the spread of human plague in east Africa, the disease could spread from native rodent reservoirs to commensal black rats through the bites of infectious *D. lypysus* or *X. brasiliensis*, both of which are abundant on native rodents in high elevation sites in the WNR. As noted previously, *D. lypusus* is considered an important enzootic vector among rodents in DRC and Tanzania, and *X. brasiliensis* is a primary vector of human plague in both these countries (Devignat 1949, Kilonzo 1992). In addition to supporting abundant populations of *X. brasiliensis,* high elevation sites in the WNR have greater flea diversity than those at lower elevations where *X. cheopis* occurs more abundantly (Eisen et al. 2012). Flea diversity within the villages we studied, all of which were located within the plague risk zone as defined by Eisen et al. (2010), were also high, a factor that could be important for maintaining enzootic circulation of *Y. pestis* in sylvatic sites (Wimsatt and Biggins 2009) and spreading plague to black rats found in peridomestic or domestic environments during epizootics that originated among native small mammals, such as *A. niloticus* or *Mastomys* sp. and their fleas. Once *R. rattus* becomes infected with *Y. pestis,* or infested with infectious fleas*,* they can carry plague into huts, placing the human occupants of these structures at risk for acquiring the disease.

In the upland Lushoto District of neighboring Tanzania, Laudisoit et al. (2007) reported a possible role for the human flea (*Pulex irritans*) in *Y. pestis* transmission to humans, noting that *P. irritans* was the predominate flea species collected inside huts (72.4%). In sharp contrast, our studies have found a complete absence of *P. irritans* inside huts from the WNR of Uganda (Amatre et al. 2009) where *Ctenocephalides felis* accounts for 94.0% of off-host fleas collected (Eisen et al. 2008). Eisen et al. (2008) also reported that *C. felis* is a relatively inefficient plague vector, rarely becomes blocked, and transmits *Y. pestis* by early phase transmission at much lower rates than for other flea species tested to date. Bland and Hinnebusch (2016) suggest that the feeding patterns of *C. felis* and the frequency of midgut clearance may hinder biofilm accumulation in the proventriculus and account for the observed poor vector competence.

Today black rats are nearly ubiquitous in domestic environments within the WNR, comprising 89.9% of all small mammals captured within huts during our study. Hopkins' 1937-1938 survey of 11 sites in the WNR failed to capture any *R. rattus*, which differs greatly with what he

observed elsewhere in Uganda (Hopkins 1949, Delany and Neal 1966). Recent studies have indicated that *R. rattus* are major members of the small mammal communities found in the WNR (Amatre et al. 2009, Moore et al. 2015). The small mammals most commonly captured in WNR huts during Hopkins' survey were multi-mammate mice, which he referred to as *Rattus coucha* but are now considered to belong to one or more species of the genus *Mastomys* (Roberts 1944, Hopkins 1949, Green et al. 1980). All but five of the 62 (80.6%) multi-mammate mice captured by Hopkins (1949) in the WNR were trapped inside huts, suggesting a marked preference for domestic environments in the absence of *R. rattus*. *Arvicanthis* sp. (presumably *A. niloticus*) also were abundant in huts in Hopkins's survey and comprised 11.0% of total captures of this species. In our study, *Mastomys* sp. and *A. niloticus* accounted for only 2.2% and 2.4%, respectively, of within-hut captures, undoubtedly because of displacement by *R. rattus*. This agrees with the observation of Hopkins (1949) that when *R. rattus* was present in huts elsewhere in Uganda, *Mastomys* sp. were "forced to become a field-rat."

Shrews were not investigated by Hopkins, nor in the later study of Amatre et al. (2009), but *Crocidura* and *Suncus* genera have been suggested by some to be involved in transmission of plague to humans in Southeast Asia, India, Africa, and Madagascar (Wu 1936, Rao 1941, Marshall et al. 1967, Andrianaivoarimanana 2013). Moore et al. (2015) suggested shrews (*Crocidura*) might play an important role in natural plague cycles in the WNR of Uganda. Our study occasionally encountered shrews in huts (about 3% of within home captures) but found them to be much less common in this habitat than in peridomestic and sylvatic environments. Although a small number of shrews captured during our study were found to be seropositive for *Y. pestis* and these mammals were hosts for 14.8% of all *X. cheopis* recovered, only 13 of the 1,325 *X. brasiliensis* (1.0%) collected were taken from shrews, suggesting their role in plague transmission to humans might be limited in the higher elevation sites where *X. brasiliensis* dominates on *R. rattus*. However, if the *C. bacopus* fleas frequently found on these animals are reasonably efficient plague vectors, shrews could play an important role as enzootic hosts.

The impact of invasive *R. rattus* on human plague risks in the WNR might be due in part to differences in its nesting behaviors and utilization of hut habitats compared to *Mastomys* sp. or other native small mammals. As noted previously, we believe that most persons in this region acquire plague in home environments following exposure to infectious rat fleas, either *X. cheopis or X. brasiliensis.* This agrees well with our observation that 79.2% of all *R. rattus* were captured within huts as compared to 13.4% in peridomestic and 7.3% in sylvatic environments (Table 4). Captures of *Mastomys* sp., *A. niloticus,* or *Crocidura* sp. within huts were much lower. *Mastomys* sp., which was the dominant hut rat prior to the invasion of *R. rattus,* typically nests at ground level in burrows or under clumps of grass or tree roots (Coetzee 1975, Isaacson 1986). Although *R. rattus* can use burrows or other ground level nesting sites, they are much more likely to nest in above-ground sites, hence this rat's other common name, the roof rat (Corrigan 2001). In villages, these above-ground nesting sites are most frequently located in the thatched roofs of huts (Monadjem et al. 2015), a fact that could increase the risk of plague exposures for residents of *R. rattus*-infested huts, especially when *Y. pestis*-infected fleas or dead rats fall from the thatch roofs into huts during plague epizootics.

In addition to the obvious risk posed by the presence of infectious rat fleas, various human behaviors and environmental factors associated with home sites also affect human plague risk in the WNR, including those that favor rat infestations. Eisen et al. (2014) reported that persons living in villages in the WNR that had experienced human plague were more likely to sleep on reed or straw mats, store crops in huts, and have experienced rat bites (usually while sleeping). Residents of these same villages were also less likely to change the thatch on their roofs than those from other villages that had not reported human cases. Changing roof thatch bundles can be expected to result in the removal of rat nests as well as the fleas found in these nests or elsewhere in the thatch. Based on our observations, residents often burn thatch bundles following removal, which could further reduce flea bite risks near homes. Armed conflicts in the WNR and neighboring DRC during the last half of the 20th century probably further enhanced the spread of plague by *R. rattus* and its fleas because residents were compelled to move food supplies traditionally stored in outside granaries, or other structures apart from the home, into their huts in attempts to reduce the risk of theft. Although the threat of guerrilla warfare has lessened recently in the WNR, food theft remains a concern (Eisen et al. 2013) and most residents still prefer to store foods within their homes. Unfortunately, unprotected food storage in homes with thatched roofs can be expected to increase human plague risk because these dwellings provide not only food sources for *R. rattus* but also suitable shelter and nesting sites for these animals.

In conclusion, the invasion of the WNR by *R. rattus* and displacement of *Mastomys* sp. as the dominant small mammal in local huts in the late 1950s or early 1960s undoubtedly played a critical role in the appearance of the region's first reported outbreak of human plague in 1970 and the subsequent outbreaks that continue to occur in the region. In recent years, CDC and UVRI have worked to identify ways to improve surveillance, prevention, control, diagnosis, and treatment measures for plague in the WNR (Borchert et al. 2012, Boegler et al. 2014, Forrester et al. 2017). These efforts included development of a surveillance system to detect die-offs among *R. rattus* populations in villages so that emergency flea control measures can be applied in a timely manner to reduce residents' risk of exposure to infectious flea bites (Boegler et al. 2018). In this system, whenever plague-positive rat carcasses are detected, vector control staff are promptly dispatched to affected villages to conduct indoor residual spraying, which Borchert et al. (2012) demonstrated to be effective for flea control in the WNR. Other flea control methods evaluated and available for plague prevention include bait boxes treated with systemic insecticides (Borchert et al. 2010) and insecticide-treated tubes (Boegler et al. 2014). Achieving long-term reductions of human plague risk in

the WNR, however, will require not only these steps but also strategies for effectively managing and reducing local *R. rattus* populations and their interaction with humans. Sustained flea control, rodenticide applications, or the continuous trapping and removal of rats could conceivably reduce human plague risk. These approaches, however, are difficult to sustain because of the high costs and labor required for them to remain effective (Singleton et al. 1999, Borchert et al. 2010), and rodent control is not recommended during periods of plague occurrence (Gratz 1999b). The development of resistance of hosts and vectors to the rodenticides or insecticides used, respectively, also presents a problem. An alternative approach might involve modifying homes in ways that reduce their suitability for *R. rattus* and their fleas. Potential modifications could include replacement of thatched roofs with ones made of metal or other materials, use of concrete instead of dirt floors to reduce off-host flea survival, constructing walls from fired bricks rather than mud plastered over a stick frame that is susceptible to rodent burrowing, the provision of built-in food storage spaces protected by solid rat-proof doors, and the storage of certain foods in rodent- and theft-proof storage bins located outside of homes. Future research might reasonably evaluate the effectiveness of such methods, or combinations thereof, through local demonstration projects. If such efforts prove effective in reducing rat populations in plague-endemic areas, residents should experience reduced plague risk and other potential benefits, including reduced risks of other rodent-borne diseases, decreased frequency of rat bites, less damage to personal property from gnawing, and enhanced food security because of reduced consumption or destruction of stored foods by rats.

Acknowledgments

Participation of each village required the verbal consent of the village chairman and households studied required the permission of each participating head of household, as specified by the approved protocols. All activities associated with this study were detailed in specific protocols and approved by the Centers for Disease Control and Prevention (Human Subjects) Institutional Review Board (approval 4696), the Uganda Virus Research Institute's Research Ethics Committee (approval GC/127), and the Uganda National Council for Science and Technology (approval HS-73). The CDC Institutional Animal Care and Use Committee reviewed the protocol and delegated oversight authority to the UVRI Research Ethics Committee, hence the animal care and use approval number is also GC/127. We acknowledge the UVRI field team (Simon Wakaalo, David Drajole, Andabati, Jackson Olweny, the late Wilfred Cwinyaai, and co-authors NB and GA) for outstanding work on this project. Key field assistance was also provided by Ali Sebbi, Environmental Health Officer (EHO) for Zombo District, Santos Angualia, Assistant EHO for Vurra Sub-County, and Appolo Midra, Assistant EHO for Logiri Sub-County. We also acknowledge and express our appreciation for the contribution of John Montenieri, Biologist, Entomology and Ecology Team, Bacterial Diseases Branch, Division of Vector-Borne Diseases, Centers for Disease Control and Prevention, Fort Collins, CO, who spent many hours in the laboratory assisting with the identification of flea specimens. We thank Professor Bukheti Kilonzo, Sokoine University, Morogoro, Tanzania, for training our field staff in rodent identification, for many helpful discussions on plague ecology, and provision of regional taxonomic keys for flea identification. We thank Dr. Robert Kityo, Curator, Department of Zoology Museum, Makerere University, Kampala, for providing additional training to our field team in rodent identifications, for expert identification of mammal voucher specimens, and for long-term storage and preservation of mammal voucher specimens. We also thank Dr. Edward Katongole-Mbidde, (then) Director, UVRI, Entebbe, for his continued support and administration of the plague program. Finally, we recognize the lifetime contributions of Asaph Ogen-Odoi, who dedicated his life to improving the health of his fellow Ugandans and who died in December, 2006 while working to control a large plague outbreak in the WNR. His contributions to this study, to the control and understanding of plague ecology in Uganda, and to his fellow citizens can never be overstated. This study was supported, in part, by funds made available from the Centers for Disease Control and Prevention, Coordinating Office for Terrorism Preparedness and Emergency Response.

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