An Evaluation of the Flea Index as a Predictor of Plague Epizootics in the West Nile Region of Uganda

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Abstract

Plague is a low incidence flea-borne zoonosis that is often fatal if treatment is delayed or inadequate. Outbreaks occur sporadically and human cases are often preceded by epizootics among rodents. Early recognition of epizootics coupled with appropriate prevention measures should reduce plague morbidity and mortality. For nearly a century, the flea index (a measure of fleas per host) has been used as a measure of risk for epizootic spread and human plague case occurrence, yet the practicality and effectiveness of its use in surveillance programs has not been evaluated rigorously. We sought to determine whether long-term monitoring of the *Xenopsylla* flea index on hut-dwelling rats in sentinel villages in the plague-endemic West Nile region of Uganda accurately predicted plague occurrence in the surrounding parish. Based on observations spanning ~6 yr, we showed that on average, the *Xenopsylla* flea index increased prior to the start of the annual plague season and tended to be higher in years when plague activity was reported in humans or rodents compared with years when it was not. However, this labor-intensive effort had limited spatial coverage and was a poor predictor of plague activity within sentinel parishes.

Keywords

flea index; plague surveillance; *Xenopsylla cheopis*; *Rattus rattus*; *Yersinia pestis*

Plague is an often-fatal flea-borne, rodent-associated bacterial illness caused by *Yersinia pestis*. Although it has a nearly global distribution, in recent decades the majority of plague cases have been reported from east and central Africa and Madagascar (Bertherat 2016). In these regions and others, plague cases are reported sporadically with sometimes long periods of apparent quiescence between outbreaks (Stenseth et al. 2008, Eisen and Gage 2009). The timing and intensity of outbreaks are often associated with weather conditions that favor increasing rodent and flea abundance, which facilitate increased rates of *Y. pestis* transmission among hosts and vectors (Gage et al. 2008). Human plague cases are

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commonly linked with epizootics when large numbers of rodents die from infection forcing their potentially infectious fleas to seek alternative hosts, including humans. When epizootics are recognized, human risk of exposure to plague bacteria can be mitigated through environmental modification or behavioral changes (Gratz 1999). In addition, raising awareness within communities of on-going plague risk can aid in early recognition and treatment of infection, resulting in reduced case fatality (Crook and Tempest 1992).

In the plague endemic West Nile region of Uganda, several strategies consistent with World Health Organization recommendations (Gage 1999) have been employed to detect plague epizootics in rodents. These include collecting and testing dead rodents, and monitoring abundance of plague-susceptible hosts and infestation rates of vector fleas on rodents. In recent years, a community surveillance program was implemented in the region that focused on the recognition, reporting, and testing of dead rats for *Y. pestis*. In response to plague positive submissions, indoor residual spraying of insecticides is implemented in affected villages to reduce flea abundance in homes; community education focuses on raising awareness of ongoing risk, prevention, and stressing the importance of seeking care rapidly if villagers experience signs or symptoms consistent with plague (Borchert et al. 2012, Boegler et al. 2018). During approximately the same time period, in 10 sentinel villages, a concurrent study surveyed rodent abundance and flea loads on hut-dwelling rodents (Eisen et al. 2014). Throughout the duration of these programs, the local public health community continued to detect and report human plague cases (Forrester et al. 2017).

Although recommendations to monitor rodent dies-offs, changes in rodent abundances and vector flea indices to detect epizootic activity are consistent with theory on how *Y. pestis* spreads during epizootic periods when humans are most at risk (Hirst 1953, Pollitzer 1954, Eisen and Gage 2009), the practicality and effectiveness of surveillance programs utilizing these metrics have not, to the best of our knowledge, been evaluated. The existence of these simultaneous, multi-year programs afforded us the opportunity to evaluate the utility of monitoring the flea index as a predictor of plague epizootics in the West Nile region of Uganda. Specifically, we aimed to 1) describe temporal changes in the abundance of the primary hut-dwelling, plague-susceptible rodent (*Rattus rattus*) and changes in the *Y. pestis* vector (*Xenopsylla cheopis* Rothschild [Siphonaptera: Pulicidae] and *X. brasiliensis* Baker [Siphonaptera: Pulicidae]) flea index on rats collected inside homes, and 2) assess the sensitivity and positive predictive value (PPV) of the flea index measured in sentinel villages as a means of predicting plague activity in rodents or humans within the parish in which sentinel villages are located.

Methods

Description of Small Mammal and Flea Index Monitoring Within 10 Sentinel Villages

Previous studies from the West Nile region implicated the roof rat, *Rattus rattus*, as the primary hut-dwelling rodent in homes and *Xenopsylla cheopis* and *Xenopsylla brasiliensis* as the primary bridging vectors of *Y. pestis* to humans (Amatre et al. 2009, Graham et al. 2013, Eisen et al. 2014). We monitored small mammal abundance and changes in the *Xenopsylla* spp. (*X. cheopis* and *X. brasiliensis*) flea index on hut-dwelling *R. rattus* in 10 rural subsistence farming villages situated in eight parishes in Arua and Zombo districts

(Fig. 1). The ten sentinel villages represent those with a perceived elevated risk for plague activity and were described previously (Eisen et al. 2014). Briefly, sentinel villages were selected from areas characterized by a previous geographical information system-based statistical model as posing an elevated risk for plague occurrence (Eisen et al. 2010). Nine of the ten villages reported at least one laboratory-confirmed human plague case in 2008 when laboratory confirmation of human plague cases was initiated as a routine activity, and at least four probable or suspect cases were reported from these villages during 1999–2007. Although no laboratory-confirmed cases were reported from the remaining village, 73 suspect or probable cases were reported from 1999 to 2007 (Eisen et al. 2014). Villages were representative of others in the region, such that residents typically resided in earthen structures with thatch roofing (huts) that were spatially clustered in variable numbers of huts to accommodate differences in the number of family members. These clusters of huts, referred to as homesteads, typically have huts designated for cooking, others for sleeping, and some for both purposes. Homesteads are commonly surrounded by fields used for subsistence agriculture or are comprised of native vegetation.

In order to monitor changes in flea loads on hut-dwelling rodents, we trapped small mammals inside of sleeping and cooking huts four times per year from late June 2012 through late January 2018. Trapping was timed to represent four phases of the regional plague year: preplague season (June–August), plague season (September–November), late-plague season (December–February), and postplague season (March–May). Herein, a plague year is defined as June of calendar year 1 through May of calendar year 2. Climatically, these seasons represent the cool interval rainy season, the cool primary rainy season, the warm dry season, and warm secondary rainy season, respectively (Monaghan et al. 2012, Moore et al. 2012). Trapping methods were described previously (Eisen et al. 2014). Briefly, within each of the ten villages for each trapping session we randomly selected ten homesteads. Within each homestead, we placed two Sherman (model 3310A; H.B. Sherman Trap Company, Tallahassee, FL) and two Tomahawk traps (model TLT102; Tomahawk Live Trap Company, Tomahawk, WI) in a single sleeping hut and single cooking hut. This yielded a total of 80 trap nights inside huts per village per session. Traps were baited with equal portions of maize, ground nuts, and dried fish and all traps were operable from shortly before dusk to shortly after dawn for a single night per session. Upon collection, small mammals were anesthetized using halothane, combed for ectoparasites, and identified to genus or species based on morphologic features (e.g., length of body, tail, ear, and hind foot, and weight). Collected fleas were stored at ambient temperature in 70% ethanol and later shipped to the Centers for Disease Control and Prevention Division of Vector-Borne Diseases, Fort Collins, CO, for identification to species according to published taxonomic keys (Hopkins 1947, Haselbarth 1966, Hopkins and Rothschild 1966). All protocols were approved by the Science and Ethics Committee of the Uganda Virus Research Institute (UVRI), the Uganda National Council for Science and Technology, and the Institutional Animal Care and Use Committee (Protocol no. 12–008, 15–002, and 17–010) of the Division of Vector-Borne Diseases of the U.S. Centers for Disease Control and Prevention). The study was determined to be exempt from human studies research by the U.S. Centers for Disease Control and Prevention.

Monitoring Plague Activity in Small Mammal Carcasses Submitted Through the Rat Fall Surveillance Program

In this study, as evidence of plague activity at the parish level, we included *Y. pestis* positive small mammal carcasses submitted as part of an ongoing rat fall surveillance (RFS) program (Boegler et al. 2018). The RFS program, which began 1 July 2013, recruited a total of 83 villages with a history of human plague cases and represented a population of ~37,000 persons. Four of the sentinel villages included in this study were among the first villages participating in the RFS program. Over time, additional villages self-selected to participate in the RFS program and submitted carcasses. In September 2014, the remaining six sentinel villages in this study were included in the RFS program. As part of the RFS community engagement program, dead rodents are reported and tested at the UVRI plague laboratory in Arua Town for evidence of *Y. pestis*; positive results prompt indoor residual spraying of insecticides in affected villages to reduce flea abundance in huts and mitigate human disease risk. For the purposes of this study, we created a list of parishes with evidence of plague activity in small mammals from 1 July 2013 through 31 January 2018. Any carcass that was classified as *Y. pestis* positive based on results from the direct fluorescent antibody assay (Chu 2000) were included.

Human Plague Surveillance

Routine surveillance for human plague in the West Nile region was described previously (Winters et al. 2009, Eisen et al. 2010, Forrester et al. 2017). Briefly, we conducted active surveillance for human plague cases in 10 clinics and 2 hospitals in Arua and Zombo Districts. Suspected plague cases were also identified through active community engagement with village health teams and traditional healers. Clinical specimens from suspect plague patients were tested for evidence of plague at the UVRI Field Station, based in Arua Town. For each patient, the following information was recorded: age, sex, location of residence, clinical form of plague, date of illness onset, outcome, and whether a rat die-off had been noted in the village of residence prior to onset of illness. For this study, we included only probable and confirmed cases acquired in the West Nile region from June 2012 through January 2018. A confirmed case was defined as clinically compatible illness with isolation of *Y. pestis* from a clinical specimen or a fourfold rise in antibody titer against the F1 antigen of *Y. pestis*. A probable case was defined as clinically compatible illness with other suggestive laboratory evidence, such as a single positive serological titer, or those with clinically compatible illness who were epidemiologically linked to a confirmed case. Clinical signs of plague include sudden onset of fever with painful regional lymphadenopathy (bubonic), hematemesis or hematochezia (septicemic), or cough or chest pain with hemoptysis (pneumonic).

Statistical Analyses

Recognizing the importance of considering only known *Y. pestis* vectors in the estimate of the flea index (Pollitzer 1954); herein, the *Xenopsylla* spp. flea index was calculated per village per sampling period by totaling the number of *X. cheopis* and *X. brasilienses* collected from each hut-dwelling *R. rattus* collected and dividing that sum by the total number of *R. rattus* collected per village per session. Using data from each village and each

sampling session (10 villages followed over 23 sessions, yielding a total of 230 observations), we used logistic regression to determine whether there was a significant association between the observed flea index within sentinel villages and the probability of plague occurrence within the parish where the village was located (hereafter 'sentinel parish'). We then classified the flea index as a binary response of either greater than or equal to one, or less than one, based on published definitions of increased risk (Pollitzer 1954, Gage 1999). The flea index was compared with the occurrence of plague in humans or animals in sentinel parishes during the same 3-mo period or the 3-mo period immediately following; each 3-mo period corresponded with the seasons of the plague year. Sensitivity was calculated as the number of observations where plague activity in humans or rats is observed and the flea index was at least one, divided by the total number of observations where plague activity was observed. The PPV was calculated as the number of observations where the flea index was at least one and plague activity was observed in humans or rats divided by the total number of observations when the flea index was at least one. The flea index and rodent abundances were compared among or between time periods using Kruskal–Wallis or Wilcoxon rank sums tests, respectively. All analyses were performed in JMP Pro 13 (SAS Institute, Cary, NC) and considered significant when $P \leq 0.05$.

Results

Summary of Rodent Abundance and Flea Index Within 10 Sentinel Villages Over 18,400 trap nights, we captured 2,747 small mammals (14.9% trap success) comprised primarily of *R. rattus* ($n = 2,555$; 93.0%). Incidental captures included: *Crocidura* spp. ($n = 126$), *Arvicanthis niloticus* ($n = 16$), *Mastomys* spp. ($n = 15$), *Zelotomys hildegardae* ($n = 12$), *Mus* spp. ($n = 11$), unidentified ($n = 7$), *Cricetomys gambianus* ($n = 2$), *Thamnomys* spp. ($n = 1$), and *Praomys jacksoni* ($n = 1$). All subsequent analyses focus exclusively on *R. rattus*.

Among all trapping sessions per village, the number of *R. rattus* captured inside huts per 100 trap nights ranged from 1.25 to 31.25 (median 13.75 *R. rattus* per 100 trap nights). Seasonal abundance of *R. rattus* did not differ significantly (Table 1; Kruskal–Wallis, $\chi^2 = 0.26$; d.f. = 3; $P = 0.96$). However, *R. rattus* abundance was significantly lower in the 2017–2018 plague year compared with other plague years (Table 2; Wilcoxon tests, $P \leq 0.05$).

Of the 2,555 *R. rattus* examined for fleas, 589 (23.1%) were infested with at least one flea of any species. Although nine species were recovered from *R. rattus*, collectively non-*Xenopsylla* spp. represented only 8% of all fleas collected from *R. rattus*; the majority were either *X. cheopis* ($n = 1,032$) or *X. brasiliensis* ($n = 89$). Other species encountered included the following: *Ctenocephalides felis* Bouché (Siphonaptera: Pulicidae) ($n = 11$), *Ctenophthalmus cabirus* Jordan & Rothschild (Siphonaptera: Hystrichopsyllidae) ($n = 17$), *Cteonphthalmus bacopus* Jordan (Siphonaptera: Hystrichopsyllidae) ($n = 12$), *Dinopsyllus lypusus* Jordan & Rothschild (Siphonaptera: Hystrichopsyllidae) ($n = 48$), *Echidnophaga gallinacea* Westwood (Siphonaptera: Pulicidae) ($n = 4$), *Stivalius torvus* Rothschild (Siphonaptera: Pygiopsyllidae) ($n = 4$), and *Tunga penetrans* L. (Siphonaptera: Tungidae) ($n = 1$). Subsequent analyses focus exclusively on *Xenopsylla* spp. per *R. rattus*.

The *Xenopsylla* flea index was similar during the preplague and plague seasons (Table 1), and it was similar between the late- and post- plague seasons. However, the late- and postplague season flea indices were significantly lower compared with those observed during the preplague season (Wilcoxon pairwise comparisons, P 0.03). Throughout the observation period, the flea index declined steadily over time (Table 2). The *Xenopsylla* flea index was significantly higher in the earlier years of observation when plague was detected in rats or humans [2012–2013 through 2015–2016 (Table 3); median: 0.29, range: 0.0–2.9 fleas per rat] compared with subsequent years when it was not detected (median: 0.13, range: 0.0–2.5 fleas per rat; $\chi^2 = 5.33$, d.f. = 1, $P = 0.02$). *Yersinia pestis* infected rat carcasses were submitted to the RFS program from three separate villages and in response, affected villages were treated with insecticides in April and October of 2013 and December of 2015. When these villages on these sampling sessions were excluded from the analysis, the statistical significance of the comparison did not change.

Summary of RFS Submissions

Of 732 carcasses submitted for *Y. pestis* testing from 170 villages spanning 51 parishes (Fig. 1); only four were not tested owing to poor condition of the sample. Nearly three-quarters of all submissions were *R. rattus* ($n = 543$, 74.2%). *Arvicanthus niloticus* ($n = 107$, 14.6%), *Mastomys* spp. ($n = 32$; 4.4%), and *Crocidura* spp. ($n = 10$, 1.4%) were the next most common species submitted. The remaining 10 species each represented less than 1% of all submissions. In total, 24 *Y. pestis* positive small mammal carcasses were submitted to the RFS program from 16 villages spanning 9 parishes. Positive submissions were comprised of *R. rattus* ($n = 19$, 79%), *Arvicanthis niloticus* ($n = 4$, 17%), and *Mastomys* spp. ($n = 1$, 4%). Of all submissions, 408 (56%) were submitted from parishes in which a sentinel village was present. In total, 15 *Y. pestis*-positive carcasses (62.5%; $n = 13$ *R. rattus* and 2 *A. niloticus*) were submitted from parishes in which sentinel villages were located (Tables 3 and 4).

Trends for all RFS submissions were mirrored by those in sentinel parishes (Tables 3 and 4). Overall, the numbers of submissions were highest during the plague season and the greatest proportions of plague positive submissions were observed during the plague- and late-plague seasons (Table 4). From the 2013–2014 through 2017–2018 plague years, total submissions declined annually. The most substantial decline occurred between plague years 2015–2016 and 2016–2017 (Table 3), when notably, *R. rattus* abundance also declined in sentinel villages (Table 2) and after which no subsequent human plague cases were reported (Table 3). Although RFS submission continued through the observation period (January 2018), the last plague positive rat was submitted in early December 2015.

Summary of Reported Probable and Confirmed Human Plague Cases

In total, 16 probable or confirmed human plague cases were reported in the Arua and Zombo districts; 5 (31%) of those were reported from sentinel parishes (Tables 3 and 4). As expected, the majority of cases were reported during the plague- or late-plague seasons (Table 4). Cases were most abundant during the 2012–2013 plague year (Table 3). The last human plague case reported during the observation period was reported in late September of 2015.

Evaluation of the Flea Index as a Predictor of Plague Activity

Among 230 observations (10 villages monitored over 23 seasons), the *Xenopsylla* flea index exceeded one on 31 occasions (13.5% of observations). Among 23 seasonal observation periods within the 8 sentinel parishes where we monitored the flea index (Fig. 1), plague activity was detected through the RFS or human surveillance systems on 15 occasions (Table 5).

The *Xenopsylla* flea index measured in sentinel villages was significantly and positively associated with plague occurrence in the associated parish one season after the flea index was measured (effect likelihood ratio d.f. =1, χ^2=6.31 P = 0.01, unit odds ratio 2.8, 95% CI: 1.32–5.93). Among the 15 instances where plague activity was reported through the human surveillance or the RFS program within sentinel parishes one season after the flea index was monitored, the *Xenopsylla* flea index was elevated (above one) on seven occasions yielding a sensitivity of 46.7%. Among the 31 observations when the flea index was elevated, plague activity was detected in the sentinel parish one season later on only 7 occasions yielding a PPV of 22.6% (Table 5). When the three sentinel villages were excluded during the session when IRS was implemented in response to plague-infected rats, sensitivity was modestly improved (50%; 7 of 14 observations when plague was observed one season after the flea index was elevated), but the PPV was unchanged.

We sought to determine whether sensitivity and the PPV could be improved by dichotomizing the flea index at different values by incrementally increasing or decreasing it by 0.25 fleas per rat. Overall, increases in the flea index threshold reduced sensitivity and the PPV because for each plague occurrence, there were fewer observations when the flea index exceeded the threshold. Specifically, when we set the flea index threshold to 1.25 or 1.5 *Xenopsylla* per *R. rattus*, sensitivity decreased to 26.7 and 13.3%, respectively; the PPV decreased to 19.1 and 15.4%, respectively. Decreasing the threshold resulted in higher measures of sensitivity because there were more instances when plague occurred in the season after the threshold was exceeded. Specifically, when we decreased the flea index to 0.75 or 0.50 *Xenopsylla* per *R. rattus*, sensitivity increased to 53.3 and 60.0%, respectively. At these threshold values, measures of the PPV were 17.4 and 12.5%, respectively.

We did not find a statistically significant association between the flea index within sentinel villages and plague occurrence at the parish level within the same observation period (i.e., without imposing a time lag). Moreover, evaluation of the *Xenopsylla* flea index as a predictor of plague activity within the same observation period yielded a sensitivity of 13.3% (2 occurrences of the flea index exceeding 1 of 15 occurrences when plague activity was detected in the sentinel parish) and PPV of 6.5% (31 occurrences when the flea index exceeded 1, plague was observed in that parish only twice).

Among the eight sentinel parishes in which the flea index was monitored, plague was detected in two through both human and rat fall surveillance and in an additional three through RFS, yielding a total of five (62.5%) sentinel parishes from which plague was detected by either surveillance program. Six additional parishes that lacked sentinel villages reported plague in either humans (n = 2), rodents (n = 2), or both rodents and humans (n = 2). Thus, among the eleven parishes from which plague was detected as part of RFS or

human surveillance, flea indices were not measured in six (54.5%) because sentinel villages were not selected in those parishes (Fig. 1).

Discussion

For nearly a century, plague researchers have cataloged variability in the flea index as a measure of risk for epizootic spread and human plague case occurrence (Hirst 1926, Grubbs 1927, Eskey and Haas 1940, Hirst 1953, Pollitzer 1954, Seal 1960, Olson 1969, Njunwa et al. 1989, Chanteau et al. 1998, Pham et al. 2009, Jones et al. 2019). Although based on limited evidence, in areas where *X. cheopis* is the primary vector of *Y. pestis*, it is generally accepted that risk of epizootic spread and exposure to humans increases when the specific flea index is greater than one (Hirst 1926, Grubbs 1927, Pollitzer 1954, Gage 1999). Consistent with other studies (Pollitzer 1954, Seal 1960, Olson 1969, Njunwa et al. 1989, Gage et al. 2008, Pham et al. 2009), we showed that on average, the *Xenopsylla* flea index increased prior to the onset of the annual plague season and tended to be higher in years when plague activity was reported compared with years when it was not. These trends are consistent with plague transmission theories which predict that increased contact rates between susceptible hosts and *Y. pestis* vectors increases epizootic potential (Pollitzer 1954, Parmenter et al. 1999, Enscore et al. 2002, Lorange et al. 2005, Eisen et al. 2007, Gage et al. 2008). There is considerably less evidence to support the use of the flea index as a surveillance metric and limited guidance on how to implement such a program (Gage 1999). Thus, we sought to determine whether long-term monitoring of the *Xenopsylla* flea index in sentinel villages in the West Nile region of Uganda, accurately predicted plague occurrence in the surrounding parish. Based on observations spanning ~6 yr, this labor-intensive effort had limited spatial coverage and was a poor predictor of plague activity within sentinel parishes.

Accurately assessing the flea index requires capture and examination of a large number of rodents and identification of fleas to species (Schwan 1984, Gage 1999) making it a very labor-intensive means of surveillance. Moreover, the spatial scale over which flea indices can be monitored is often limited by resources available to devote to environmental surveillance. With a team of roughly a half dozen field staff, we were able to consistently monitor changes in rodent abundance and the *Xenopsylla* flea index in 10 villages spanning eight parishes over ~6 years. These villages were selected strategically to maximize the likelihood of observing plague activity (Eisen et al. 2014). Nonetheless, among the eight sentinel parishes included in our study, plague was reported through the RFS or human plague surveillance programs from only five. Meanwhile, during the same time period, plague was reported from six parishes from which we did not have sentinel villages situated. In addition to limited spatial coverage of observation, we found that within sentinel parishes the flea index often exceeded one without any evidence of plague activity in humans or rodents in the following season (PPV = 22.6%). The index performed even more poorly when plague activity and the flea index were measured in the same season. Indeed, several historical studies reviewed by Pollitzer (1954) showed that the timing of plague outbreaks did not necessarily coincide with when the flea index was elevated.

Among 31 observations when the flea index exceeded one in sentinel villages, in 24 cases, we did not observe plague activity in the sentinel parish the following season, yielding a false-positive rate of 77.4%. Conditions that are independent of plague epizootics that either increase flea abundance while keeping rodent abundance stable (e.g., favorable weather conditions; Gage et al. 2008) or that reduce rodent abundance while keeping flea abundance stable or increasing flea abundance (e.g., rodent control practices such as trapping or poisoning (Gratz 1999, Biggins and Eads 2019) could result in an elevated flea index in the absence of observed plague in humans or rats. The arbitrary threshold of a flea index greater than one may be of limited biological relevance and could limit the predictive capacity of this measure. Recent quantitative analyses of vectorial capacity suggest that the number of *X. cheopis* per *R. rattus* required to sustain *Y. pestis* transmission is more than 3.9 fleas per host (Lorange et al. 2005, Eisen et al. 2007). Notably, such high flea loads were never observed in our study, yet plague activity was recorded in nearby parishes. This could be explained by a detection bias, such that not all fleas are reliably collected from hosts in the field, or could suggest inaccuracies in modeling transmission dynamics based on laboratory transmission studies. Nonetheless, adjusting the threshold used in the flea index in our study did not improve the PPV.

Among 199 observations when the flea index was less than one in sentinel villages, plague activity was reported eight times in the surrounding parish, yielding a negative predictive value of 96%. A key limitation of the use of this metric as a predictor of plague activity is that the flea index represents a snapshot in space and time, and likely changes relative to when it is measured during an epizootic and where it is measured relative to the leading edge of the rodent die-off. The accuracy of the flea index as a predictor of plague activity is likely greater at smaller spatial and temporal scales; however, this is offset by the greater cost and effort to maintain such a surveillance effort.

In this study, we used plague positive rats submitted through the RFS program as a measure of plague activity. Because the detection of plague-infected rodent carcasses triggered activities that aimed to reduce risk of human exposure to *Y. pestis*, we were not able to measure the accuracy of plague positive rats as a predictor of human plague occurrence. However, we note that compared with surveillance efforts focused on the flea index, the RFS program had greater spatial coverage, was less labor intensive, yielded more timely results, and directly assessed the presence of *Y. pestis* rather than measuring a proxy (Boegler et al. 2018). In addition, the RFS design identified a more discrete area over which interventions could be applied in response to plague activity. Suggesting that identification of *Y. pestis* infected rats is a reasonable indicator of plague activity, Forrester et al. (2017) reported that 87% of laboratory confirmed or probable plague cases reported from the West Nile region from 2008 to 2016 reported rat die-offs in their village near the time of illness. They reported a PPV of rat die-offs for laboratory confirmed cases as 60% and a negative predictive value of 83%. As assessed in the present study, the flea index was not considered a reliable predictor of plague activity, and therefore, we did not implement prevention activities in response to it. We caution that application of insecticides in response to an elevated flea index as measured in this setting would likely have contributed to overuse of insecticides which could over time lead to insecticide resistance and undermine emergency flea control efforts (Miarinjara and Boyer 2016). Overall, we conclude that at coarse spatial

and temporal scales, the flea index can provide biologically plausible explanations for seasonal and interannual variability in plague occurrence. However, the poor PPV, limited spatial coverage and resources required to maintain such a program suggest it is a costly and ineffective strategy for early detection of plague.

Acknowledgments

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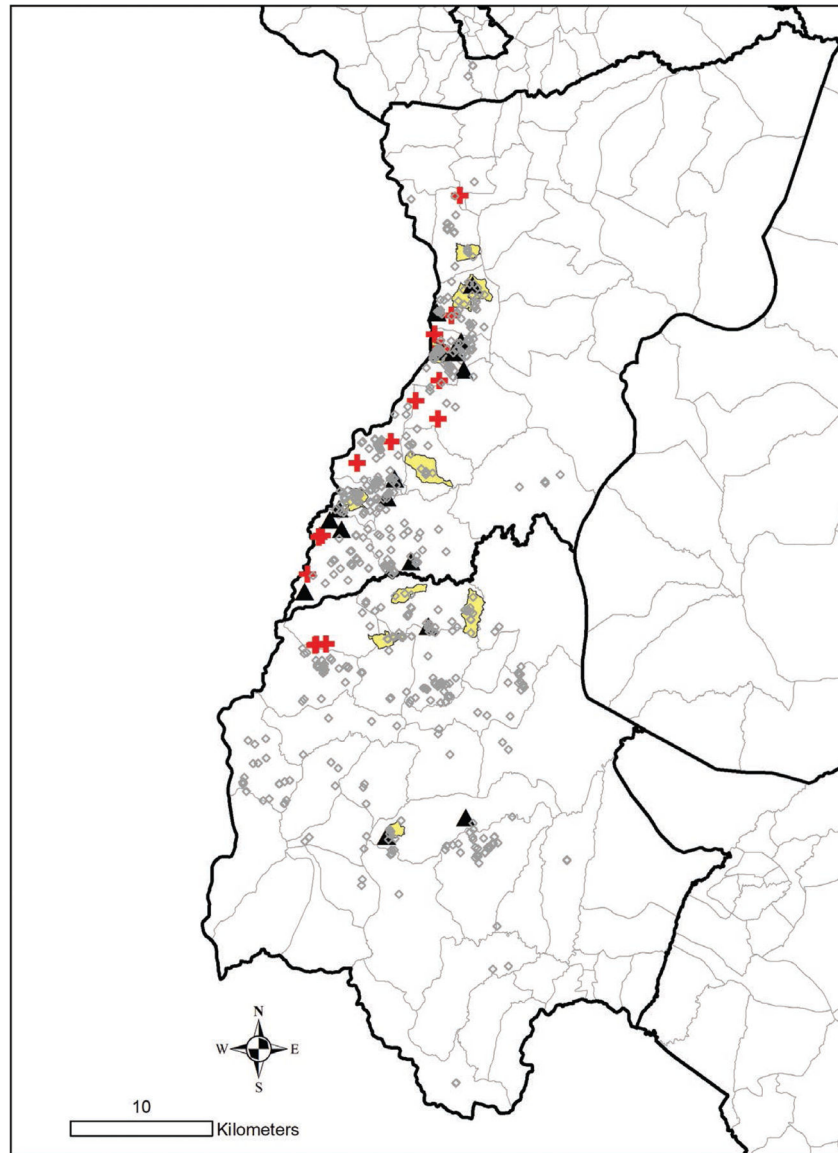
Fig. 1.
Locations of sentinel villages in which the flea index was monitored quarterly from June 2012 through January 2018 (yellow polygons). Plague positive small mammal carcasses submitted through the rat fall surveillance program from July 2013 through January 2018 are shown as gray diamonds (negative) or black triangles (positive). Probable and confirmed human plague cases with onset dates between June 2012 and January 2018 are shown as red crosses. Parish boundaries are indicated with light gray borders and Arua (north) and Zombo (south) district boundaries and depicted in black.

Table 1.

Seasonal variation in the *Xenopsylla* flea index and *Rattus rattus* abundance per 10 sentinel villages

Plague season	*Xenopsylla* flea index per sentinel village	*Ratus rattus* abundance per 100 trap nights per sentinel village
	Median (range)	Median (range)
Preplague season	0.35 (0.00–2.14)	12.75 (1.25–31.25)
Plague season	0.35 (0.00–2.86)	13.75 (3.75–26.25)
Late-plague season	0.10 (0.00–1.63)	13.75 (3.75–31.25)
Postplague season	0.19 (0.00–1.38)	13.75 (2.50–27.50)

Each village was sampled quarterly from June 2012 through Jan. 2018. Plague seasons are defined as follows: preplague season: June–Aug.; plague season: Sept.–Nov.; late-plague season: Dec.–Feb.; postplague season: Mar.–May.

Table 2.

Inter-annual variation in the *Xenopsylla* flea index and *Rattus rattus* abundance per sentinel village

Plague Year	*Xenopsylla* flea index per sentinel village Median (range)	*Rattus rattus* abundance per 100 trap nights per sentinel village Median (range)
2012–2013	0.45 (0.00–2.14)	17.5 (2.50–26.25)
2013–2014	0.44 (0.00–2.23)	14.38 (2.50–27.50)
2014–2015	0.21 (0.00–2.86)	13.75 (3.75–26.25)
2015–2016	0.09 (0.00–1.38)	15.00 (2.50–31.25)
2016–2017	0.14 (0.00–2.55)	11.25 (2.50–31.25)
2017–2018	0.04 (0.00–2.00)	8.75 (1.25–25.00)

The plague year is defined as June of year 1 through May of year 2. The 2017–2018 spanned from June 2017 through Jan. 2018 and therefore had only three trapping sessions, rather than four.

Table 3.

Comparison of submissions from the RFS program and reported probable and confirmed human plague cases by plague year for all areas and for sentinel parishes

Plague year	All submissions		Submission from sentinel parishes	
	Number of positive RFS submission/total RFS submissions tested (%)	Number of probable or confirmed human cases	Number of positive RFS submission/total RFS submissions tested (%)	Number of probable or confirmed human cases
2012–2013		11		4
2013–2014	18/261 (6.9)	4	13/138 (9.4)	0
2014–2015	5/173 (2.9)	0	1/99 (1.0)	0
2015–2016	1/145 (0.7)	1	1/87 (1.1)	1
2016–2017	0/85 (0.0)	0	0/49 (0.0)	0
2017–2018	0/64 (0.0)	0	0/34 (0.0)	0

Human cases are counted from June 2012 through Jan. 2018; RFS submissions span from July 2012 through Jan. 2018.

Table 4.

Comparison of submissions from the RFS program and reported probable and confirmed human plague cases by season for all submissions and for sentinel parishes

Plague Season	All submissions		Submission from sentinel parishes	
	Number of positive RFS submission/total RFS submissions tested (%)	Number of probable or confirmed human cases	Number of positive RFS submission/total RFS submissions tested (%)	Number of probable or confirmed human cases
Preplague season	0/154 (0.0)	1	0/83 (0.0)	0
Plague season	13/321 (4.0)	11	10/175 (5.7)	7
Late-plague season	8/127 (6.3)	9	4/75 (5.3)	2
Postplague season	3/126 (2.4)	3	1/74 (1.5)	0

Human cases are counted from June 2012 through Jan. 2018; RFS submissions span from July 2012 through Jan. 2018.

Table 5.

Evaluation of the flea index in 10 sentinel villages as a predictor of plague activity in the parish where the sentinel village was situated

Flea index value 1	Plague activity observed	
	No	**Yes**
No	191	8
Yes	24	7

Plague activity was classified as positive if either probable or confirmed human plague cases or plague positive small mammals were reported from the parish where the sentinel village was located. Plague activity is noted with a one season lag from observations of the flea index. Sensitivity = 7/15 (46.7%); PPV = 7/31 (22.6%).