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Early-phase Transmission of *Yersinia pestis* by Cat Fleas (*Ctenocephalides felis*) and Their Potential Role as Vectors in a Plague-endemic Region of Uganda

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Abstract. In recent decades, the majority of human plague cases (caused by *Yersinia pestis*) have been reported from Africa. In northwest Uganda, which has had recent plague outbreaks, cat fleas (*Ctenocephalides felis*) have been reported as the most common fleas in the home environment, which is suspected to be a major exposure site for human plague in this country. In the past, *C. felis* has been viewed as only a nuisance-biting insect because limited laboratory studies suggested it is incapable of transmitting *Y. pestis* or is an inefficient vector. Our laboratory study shows that *C. felis* is a competent vector of plague bacteria, but that efficiency is low compared with another flea species collected in the same area: the oriental rat flea, *Xenopsylla cheopis*. On the other hand, despite its low vector efficiency, *C. felis* is the most common flea in human habitations in a plague-endemic region of Uganda (Arua and Nebbi Districts), and occasionally infests potential rodent reservoirs of *Y. pestis* such as the roof rat (*Rattus rattus*) or the Nile rat (*Arvicanthis niloticus*). Plague control programs in this region should remain focused on reducing rat flea populations, although our findings imply that cat fleas should not be ignored by these programs as they could play a significant role as secondary vectors.

INTRODUCTION

Plague is a highly virulent and primarily flea-borne zoonotic disease. Although laboratory studies have confirmed that *Y. pestis*, the etiological agent of plague, is transmitted by at least 80 different flea species[1] transmission efficiency is highly variable among competent vectors.[2,3] Likewise, the plague bacterium can infect a wide range of vertebrate hosts, but disease outbreaks are primarily associated with rodents.[1,2,4] Because of the diversity of competent vectors and broad array of susceptible hosts, understanding local plague cycles and transmission dynamics depends on our ability to first identify 1) which flea and rodent species are driving local enzootic transmission cycles of *Y. pestis*, and 2) which fleas serve as bridging vectors from zoonotic hosts to humans within specific plague endemic regions. Such information is critical for evaluating the most effective methods of controlling pathogen transmission and ultimately reducing the number of human cases.

Our lack of knowledge of local plague transmission cycles is particularly acute in much of Africa, where the majority of human cases have occurred in recent decades.[5,6] In many parts of East Africa, rat fleas (*Xenopsylla cheopis*, *X. brasiliensis*) are believed to play a crucial role in plague epizootics because they commonly infest susceptible rodent hosts,[1,7,8] readily feed on humans when encountered,[9–11] and laboratory studies have demonstrated that they are efficient vectors of *Y. pestis*.[1,2,4,12–15] However, in some regions, other fleas are also commonly encountered in huts. For example, recent studies have indicated that *Pulex irritans* is commonly found in human habitations in Tanzania.[16] In northwestern Uganda, the cat flea (*Ctenocephalies felis*) has been reported as the most common species in human habitations. This last flea species is typically perceived as a nuisance biter, rather than as an insect of public health importance, despite limited studies indicating that it can transmit plague bacteria under some circumstances.[1,7,17]

Although there have been surprisingly few empirical evaluations of its vector competency,[11,18,19] *C. felis* is typically referred to as incapable of transmitting *Y. pestis* or as an inefficient vector.[1,3,20,21] However, this flea species is a catholic feeder and will readily bite humans and rats.[10,11] It therefore could serve as a bridging vector between zoonotic and anthroponotic cycles. High flea densities and frequent feeding could compensate for a low transmission efficiency rate and result in high vectorial capacity for *C. felis*[22–26]; thus, cat fleas may pose a greater risk to public health than previously believed.

In the West Nile region of Uganda, Arua and Nebbi Districts have consistently reported human plague cases. From 1999 through 2007, for example, clinics from these districts reported to the Ugandan Ministry of Health a mean of approximately 223 (range: 76–467) suspect human cases per year (CDC, unpublished data). In the field component of this study, we demonstrate that *C. felis* is the most common flea in human habitations in this plague-endemic region, and that it occasionally infests potential rodent reservoirs of *Y. pestis*. Our laboratory study shows that *C. felis* is a competent vector of plague bacteria, but that efficiency is low compared with another flea species collected in the same area, *Xenopsylla cheopis*. We introduce a preliminary model of vectorial capacity for *C. felis,* which estimates that the number of cat fleas per host required to maintain *Y. pestis* in human habitations is within the range of field-derived values.

MATERIALS AND METHODS

Field evaluation of flea infestations of small mammals. Small mammals were captured from northwestern Uganda (Arua and Nebbi districts) in Sherman and Tomahawk live traps baited with equal portions of corn, ground peanuts, and dried fish. Each district was sampled every other month on an alternating schedule (i.e., Arua was sampled one month, then Nebbi the next) from January through August 2006 and then

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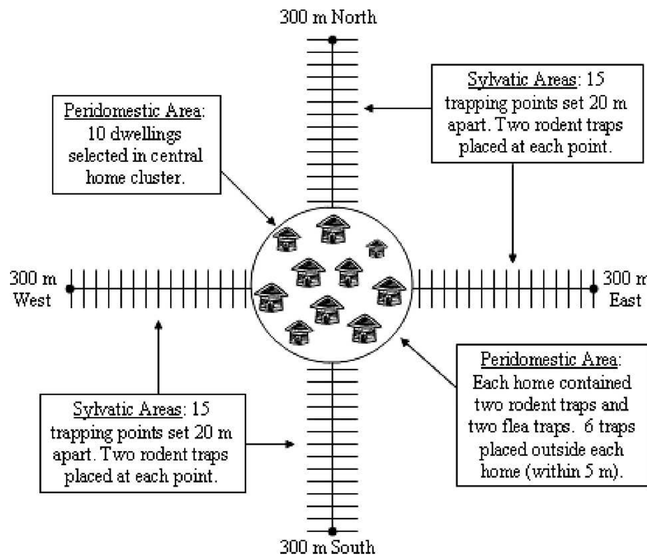

FIGURE 1. Small mammal and flea trapping design.

from December 2006 through June 2007. For each district, six villages were sampled using the following design. Within each village, 10 residences were sampled by placing two Sherman and two Tomahawk traps inside and three Sherman and three Tomahawk traps within 5 m of the outside of each residence. Henceforth, these trap locations are referred to as "peridomestic." Orienting from the center of the village, one Tomahawk and one Sherman trap was set every 20 m for 300 m away from the edge of villages in each of the four cardinal directions (i.e., a total of 15 Sherman and 15 Tomahawk traps were set along the northern trap line and this design was repeated for the eastern, southern, and western directions) (Figure 1). These trap locations are later referred to as "sylvatic." These areas represent a mixture of agricultural plots, fallow fields, and natural vegetation.

For each trapping session, each trap was operated for two nights with animals recovered each morning following a night of trapping. Upon capture, animals were anesthetized by inhalation of halothane, identified to species based on morphological measurements (e.g., length of body, tail, ear, hind foot),[27] and combed to recover fleas. All fleas collected from small mammals were stored in individual glass collection tubes containing 2% saline with Tween 80 and later identified to species following published taxonomic keys.[17,28,29] Blood was collected on a Nobuto strip and tested for serological evidence of exposure to *Y. pestis* using passive hemagglutination and inhibition tests.[30]

Field evaluation of flea infestations in human habitations. In the villages sampled in Arua and Nebbi districts, a typical residence consists of a mud and brick structure with a grass thatched roof and earthen floors. Within each of the 10 residences in villages selected for small mammal trapping, two flea traps placed on the floor were set for two nights per trapping session. The traps were a modification of the Kilonzo flea trap[31] for collection of photosensitive fleas. One trap consisted of a flashlight suspended over a metal pan (25.4 cm diameter) containing 2% saline with Tween 80. The other trap targeted non-photosensitive fleas and consisted of a metal pan containing 2% saline with Tween 80. On both types of traps, Vaseline was applied to the rim of the pan to prevent fleas from exiting. Village residents were instructed to turn the flashlights on at night and to leave on throughout the night. Fresh batteries were used for each night of collection. After two days, fleas were collected from the pans and stored in microcentrifuge tubes containing 2% saline with Tween 80 and later identified to species following published taxonomic keys.[17,28,29] In Nebbi district, five villages were sampled on two occasions (August 2006 and February–March 2007) and in Arua district four villages were sampled on two occasions (December 2006 and March 2007).

Laboratory evaluation of flea-borne transmission of *Y. pestis.* The ability of *C. felis* to transmit *Y. pestis* 1–4 d p.i. (early-phase transmission) was tested following methods published previously.[14,22,32] *Oropsylla montana*, a species evaluated previously,[22] was used as a positive control for flea-borne transmission efficiency. This species was selected because it has demonstrated the highest transmission efficiency 1–4 d p.i. of all species examined in our laboratory. Methods for infecting *C. felis* and *O. montana* with *Y. pestis* (CO96-3188), confirming transmission from fleas to naive Swiss Webster mice, quantification of bacterial loads in fleas and evaluation of vector competency 1–4 d post infection (p.i.) were described in detail previously.[22] The strain was selected so vector-efficiency could be directly compared among flea species that were evaluated previously[14,22,32–34] and infected with the same bacterial strain. Briefly, on day 0 four batches of colony-reared female *C. felis* obtained from a commercial vendor (Heska, Fort Collins, CO) and one batch of *O. montana* from a colony maintained by the Division of Vector-Borne Infectious Diseases, Centers for Disease Control and Prevention (N = 50–60 fleas per feeder) were allowed to feed for 1 h on artificial feeders containing defibrinated Sprague-Dawley strain rat blood (Bioreclamation, Jericho, NY) heated to 37°C and containing a fully virulent North American strain of *Y. pestis*, biovar orientalis, designated CO96-3188 (1.09×10^9 – 2.61×10^9 cfu/mL). Aliquots of bacteria used in the present study came from the same source population as those used previously.[14,22,32–34] Similarly, the *O. montana* controls were derived from the same colony as those used previously.[22,32] Fed fleas containing red blood in the proventriculus or midgut were differentiated from unfed fleas using light microscopy.[22] Any flea that did not take a potentially infectious bloodmeal was discarded; the remaining fleas were held at 23°C and 85% relative humidity for 1–4 d p.i. On each of the days 1–4 p.i., pools of approximately 10 potentially infectious *C. felis* were placed in feeding capsules for 1 h on anesthetized 6-wk-old Swiss-Webster strain mice. On day 1 p.i. only, the procedure was replicated with pools of 10 *O. montana*. After 1 h, fleas were removed from the feeding capsule using a mechanical aspirator. Flea feeding success was determined using light microscopy[22]; fed fleas were stored in individual microcentrifuge tubes at −80°C until infection status and bacterial loads were determined by serial dilutions of fleas triturated in heart infusion broth supplemented with 10% glycerol and plated in duplicate on blood agar plates containing 6% sheep blood.[22] Following exposure to fleas, recipient mice were housed individually in filter-top cages, monitored daily and euthanized when signs of *Y. pestis* exposure were evident (e.g., slow response to stimuli, shivering, ruffled fur). Transmission from flea to mouse was demonstrated if the exposed mouse showed clinical symptoms of plague followed by presumptive identification of *Y. pestis* in the liver or spleen by direct fluores-

cence antibody test targeting the F1 antigen and was confirmed by culture isolation and subsequent bacteriophage lysis. Alternatively, mice surviving to 21 d p.i. were euthanized and blood was collected for testing for serological evidence of exposure to *Y. pestis* using passive hemagluttination and inhibition tests.[30] Animal procedures were approved by the Division of Vector-Borne Infectious Diseases (Centers for Disease Control and Prevention) Institutional Animal Care and Use Committee.

Estimate of vectorial capacity. The density of fleas in relation to humans that are required for person-to-person transmission of *Y. pestis* was determined following recently published studies of vectorial capacity.[14,22,25] The model assumes that 1) host density is sufficiently high for every infected flea to find a susceptible host upon which to feed, and 2) the first bite following infection results in transmission. The resulting model predicts the number of fleas required per host to maintain an infection in a population, as:

$$m = R_0(r/abp^n)$$

where R_0 represents vectorial capacity or force of infection (e.g., the number of secondary infections arising from a focal infection). When person-to-person transmission of *Y. pestis* by cat fleas could be maintained, $R_0 = 1$. The flea density per person, m, represents the infestation in human habitations. The daily biting rate of infected fleas, a was set at 0.92 following previously published studies.[22] The probability of a flea acquiring an infection after feeding on a septicemic host and transmitting the infection during a subsequent feeding on a susceptible host, b, was derived from the range in vector efficiency yielded from our laboratory study. The probability of the flea surviving the extrinsic incubation period (defined as the duration of time from which a flea is infected until it can transmit) is symbolized as p^n. This was estimated to be 1.0 because the extrinsic incubation period is believed to be very short, allowing all or nearly all fleas to survive to become infectious. Finally, $1/r$ defines the life expectancy of the host after it reaches threshold septicemia and following previous studies was set as equaling 2.[22,25]

Statistical analysis. For *C. felis* tested during each time point (1–4 d p.i.), transmission efficiency per individual flea was estimated using maximum likelihood based on the number of infected fleas that fed on an individual mouse and whether transmission was observed for that recipient mouse using the Microsoft® Excel® Add-In PooledInfRate, Version 3.0.[14] Mean maximum bacterial loads for infected fleas fed per animal at each time point were compared using ANOVA. Kruskal-Wallis and Wilcoxon rank sums tests were used to compare median numbers of colony-forming units per flea among and between treatments, respectively. Wilcoxon signed rank tests were used to compare the percentage of fleas classified as *C. felis* between trapping sessions. All statistical comparisons were run using JMP statistical software (SAS Institute, Cary, NC) and results were considered significant if $P < 0.05$.

RESULTS

Field-derived evaluation of on-host flea infestations. In total, 236 and 409 small mammals representing 11 and 13 species were collected from peridomestic and sylvatic areas, respectively, in Arua district. Among all individuals tested, a single Nile rat (*Arvicanthis niloticus*) from each group tested seropositive for previous exposure to *Y. pestis* with titers of 1:128 and 1:1,024. *A. niloticus* and the roof rat (*Rattus rattus*) comprised 70% of the sample in peridomestic areas, but only 40% in sylvatic areas. In peridomestic areas, 40% of *A. niloticus* and 80% of *R. rattus* were infested with either *C. felis*, *X. cheopis*, *X. brasiliensi,* or a combination of these. The average number of *X. cheopis* per *R. rattus* (6.5 fleas per host) was approximately twice the average for *A. niloticus* (3.1 fleas per host). Among all rodents captured in peridomestic areas in Arua district, only *R. rattus* was infested with *C. felis* and the average number of fleas per rat (0.02) was 325-fold lower than for *X. cheopis* (Table 1).

In Nebbi district, 392 small mammals comprised of 9 species were collected from peridomestic areas whereas 316 samples representing 13 species were collected from sylvatic areas. Among individuals collected in peridomestic areas, one *A. niloticus* showed serological evidence of exposure to *Y. pestis* (1:32). Likewise, one *Crocidura turba* collected in a sylvatic location was also seropositive (1:64). Similar to sites in Arua district, *A. niloticus* and *R. rattus* represented a higher proportion of the total capture in peridomestic (88%) relative to sylvatic areas (48%). In peridomestic areas, 46% and 72% of *A. niloticus* and *R. rattus*, respectively, were infested with either *C. felis*, *X. cheopis*, *X. brasiliensis*, or a combination of these. In contrast to Arua district where *X. cheopis* was the most commonly collected flea from small mammals, *X. brasiliensis* predominated in Nebbi district. The average number of *X. brasiliensis* per host was 10.7 for *A. niloticus* and 5.9 for *R. rattus*. Similar to Arua district, *C. felis* was rare on small mammals in Nebbi district with an average of only 0.04 fleas per *R. rattus* (Table 2).

Field-derived evaluation of off-host flea infestations in human habitations. Pan trap data revealed that *C. felis* was the most common host-seeking flea in human habitations in Arua and Nebbi districts. In Arua district, 37% of traps captured at least 1 flea whereas in Nebbi district, 56% collected at least 1 flea (Fisher's exact test; $P = 0.05$). Among traps from which fleas were collected, 81% ($N = 227$ of 281) and 94% ($N = 666$ of 707) of fleas collected from Arua and Nebbi districts, respectively, were identified as *C. felis* (Table 3). Significantly more *C. felis* were collected from Nebbi than Arua district (Wilcoxon rank sum test with χ^2 approximation; $\chi^2 = 9.4$, d.f. = 1, $P = 0.0021$). In Arua, the remaining 54 fleas were classified as *Ctenophthalmus* spp. ($N = 2$), *Dinopsyllus* spp. ($N = 4$), *Pulex irritans* ($N = 2$), *Tunga penetrans* ($N = 5$), and *X. cheopis* ($N = 41$). In Nebbi, additional fleas collected in pan traps included *Ctenocephalides canis* ($N = 6$), *Echidnophaga gallinacea* ($N = 6$), *T. penetrans* ($N = 12$), and *X. brasiliensis* ($N = 17$). In Arua district, the proportion of fleas classified as *C. felis* was significantly lower in session 1 compared with session 2 (one-tailed Wilcoxon signed rank test; $P = 0.03$). No differences were detected between trapping sessions in Nebbi district.

Laboratory evaluation of flea-borne transmission of *Y. pestis.* Among 203 fleas allowed to feed on mice 1–4 d p.i., none showed evidence of proventricular block formation. Transmission of *Y. pestis* to susceptible mice by unblocked *C. felis* was observed for a single flea pool in only 1 of the 4 time points (2 d p.i.; Table 4). Transmission of *Y. pestis* to mice was confirmed by recovery of plague bacteria from the liver and

TABLE 1
Flea infestation data for small mammals collected in peridomestic or sylvatic areas in Arua District, Uganda. Average number of fleas recovered is the total number of fleas collected divided by the total number of rodents examined.

Peridomestic or sylvatic	Small mammal species	No. hosts examined	No. hosts infested	Total (average) no. fleas recovered		
				C. felis	*X. cheopis*	*X. brasiliensis*
Peridomestic						
	Aethomys kaiseri	1	0	0 (0.0)	0 (0.0)	0 (0.0)
	Arvicanthis niloticus	84	34	0 (0.0)	257 (3.1)	8 (0.1)
	Crocidura spp.	38	19	0 (0.0)	159 (4.2)	16 (0.42)
	Lophuromys flavopunctatus	1	0	0 (0.0)	0 (0.0)	0 (0.0)
	Mastomys natalensis	19	12	0 (0.0)	274 (14.4)	20 (1.1)
	Mastomys spp.	1	1	0 (0.0)	8 (8.0)	0 (0.0)
	Mus spp.	2	1	0 (0.0)	4 (2.0)	0 (0.0)
	Myomys fumatus	4	3	0 (0.0)	32 (8.0)	4 (1.0)
	Praomys spp.	2	2	0 (0.0)	4 (2.0)	28 (14.0)
	Rattus rattus	81	69	2 (0.02)	525 (6.5)	16 (0.2)
	Tatera spp.	3	2	0 (0.0)	5 (1.7)	0 (0.0)
Sylvatic						
	Aethomys kaiseri	20	6	0 (0.0)	29 (1.5)	0 (0.0)
	Arvicanthis niloticus	139	21	0 (0.0)	73 (0.5)	29 (0.2)
	Crocidura spp.	44	24	0 (0.0)	96 (2.2)	12 (0.3)
	Dasymys spp.	5	1	0 (0.0)	1 (0.2)	0 (0.0)
	Lemniscomys striatus	11	1	4 (0.4)	0 (0.0)	0 (0.0)
	Lophuromys flavopunctatus	12	0	0 (0.0)	0 (0.0)	0 (0.0)
	Mastomys natalensis	71	30	0 (0.0)	189 (2.7)	0 (0.0)
	Mastomys spp.	8	4	0 (0.0)	40 (5.0)	20 (2.5)
	Mus spp.	4	0	0 (0.0)	0 (0.0)	0 (0.0)
	Myomys fumatus	13	11	0 (0.0)	16 (1.2)	40(3.1)
	Praomys spp.	27	9	2 (0.1)	68 (2.5)	0 (0.0)
	Rattus rattus	22	20	0 (0.0)	183 (8.3)	0 (0.0)
	Tatera spp.	33	2	0 (0.0)	5 (0.2)	0 (0.0)

spleen of a mouse that showed clinical symptoms of infection. On that day (day 2 p.i.), transmission efficiency was estimated to be 2.15% (95% CI: 0.13–10.64). Because transmission efficiency was similar from 1–4 d p.i. (Table 4), we estimated the per flea transmission efficiency for the entire early-phase observation period using maximum likelihood. This yielded an estimate for transmission efficiency of 0.57% (95% CI: 0.03–2.78%). Demonstrating the ability of this strain of *Y. pestis* to be transmitted from flea to mouse, each of the five *O. montana* flea pools infected with plague bacteria from the same aliquot of CO96-3188 transmitted *Y. pestis* to susceptible mice (Table 4).

TABLE 2
Flea infestation data for small mammals collected in peridomestic or sylvatic areas in Nebbi District, Uganda

Peridomestic or sylvatic	Small mammal species	No. hosts examined	No. hosts infested	Total no. fleas recovered		
				C. felis	*X. cheopis*	*X. brasiliensis*
Peridomestic						
	Aethomys kaiseri	1	1	0 (0.0)	0 (0.0)	12 (12.0)
	Arvicanthis niloticus	109	50	0 (0.0)	4 (0.03)	1169 (10.7)
	Crocidura spp.	15	0	0 (0.0)	0 (0.0)	0 (0.0)
	Lemniscomys striatus	1	0	0 (0.0)	0 (0.0)	0 (0.0)
	Lophuromys flavopunctatus	5	2	0 (0.0)	0 (0.0)	6 (1.2)
	Mastomys natalensis	21	9	0 (0.0)	0 (0.0)	75 (3.6)
	Mus spp.	2	0	0 (0.0)	0 (0.0)	0 (0.0)
	Praomys spp.	2	1	0 (0.0)	0 (0.0)	2 (1.0)
	Rattus rattus	236	172	10 (0.04)	30 (0.1)	1400 (5.9)
Sylvatic						
	Aethomys kaiseri	5	0	0 (0.0)	0 (0.0)	0 (0.0)
	Arvicanthis niloticus	141	28	18 (0.1)	4 (0.03)	263 (1.9)
	Crocidura spp.	28	1	0 (0.0)	0 (0.0)	3 (0.1)
	Dasymys spp.	3	0	0 (0.0)	0 (0.0)	0 (0.0)
	Lemniscomys striatus	9	1	3 (0.3)	0 (0.0)	0 (0.0)
	Lophuromys flavopunctatus	34	1	3 (0.1)	0 (0.0)	0 (0.0)
	Mastomys natalensis	56	5	0 (0.0)	0 (0.0)	23 (0.4)
	Mastomys spp.	1	0	0 (0.0)	0 (0.0)	0 (0.0)
	Mus spp.	4	1	0 (0.0)	0 (0.0)	3 (0.8)
	Oenomys spp.	2	0	0 (0.0)	0 (0.0)	0 (0.0)
	Praomys spp.	10	1	0 (0.0)	0 (0.0)	3 (0.3)
	Rattus rattus	10	6	3 (0.3)	0 (0.0)	54 (5.4)
	Tatera spp.	13	0	0 (0.0)	0 (0.0)	0 (0.0)

TABLE 3
Summary of flea infestations in human habitations in Arua and Nebbi districts. In each village and for each session, ten residences were sampled

Village	Trap Session 1*			Trap Session 2†		
	% Infested	Total no. *C. felis* (% of all fleas)	Total no. other flea species	% Infested	Total no. *C. felis* (% of all fleas)	Total no. other flea species
Arua						
Kaza	70	92 (72)	36	40	51 (93)	4
Olli	30	8 (66)	4	20	4 (100)	0
Pembeleku	40	14 (78)	4	N/A	N/A	N/A
Pomosi	10	16 (80)	4	70	42 (95)	2
Nebbi						
Agore	60	93 (94)	6	40	24 (100)	0
Anyiku	50	60 (100)	0	70	48 (96)	2
Gbalia	70	105 (97)	3	30	10 (83)	2
Sokonzi	60	81 (78)	24	40	22 (85)	4
Uyaru Upper	40	27 (100)	0	70	196 (100)	0

N/A = site not sampled
* Session 1 refers to December 2006 for Arua district and August 2006 for Nebbi district.
† Session 2 refers to March 2007 for Arua district and February or March 2007 for Nebbi district.

Mean maximum bacterial loads increased significantly from 24 to 48 h p.i. and then reached a plateau ($F = 6.05$, d.f. = 3, 13, $P = 0.008$; Figure 2). Mean maximum bacterial loads for *O. montana* harvested 24 h p.i. were similar to those in *C. felis* for 48 to 96 h p.i., but were significantly higher than for *C. felis* at 24 h p.i. ($F = 9.08$, d.f. = 4, 14, $P = 0.0004$).

Median bacterial loads per flea differed among time points ($\chi^2 = 68.5$, d.f. = 4, $P < 0.0001$; Table 4). Bacterial loads for *C. felis* were lower at 24 h p.i. compared with all other time points ($\chi^2 \geq 18.12$, d.f. = 1, $P < 0.0001$), but similar among each of the other time points. Compared with *O. montana*, bacterial loads were lower in *C. felis* for each of the different time points ($\chi^2 > 7.88$ d.f. = 1, $P < 0.005$).

Estimate of vectorial capacity. We sought to determine if flea loads observed in human habitations would be sufficient to sustain person to person transmission if a single individual in the hut became infectious. Using the range in transmission rates estimated in our laboratory study (Table 4), we determined that to maintain person to person transmission of *Y. pestis*, an average of 25 cat fleas per person would be required (95% CI: 5–418). When vectorial capacity is based on the pooled early-phase transmission estimate (days 1–4 p.i. com-

TABLE 4
Bacterial loads and transmission efficiency of *C. felis* and *O. montana* for *Y. pestis**

Time point (days after infectious feed) and mouse number	No. of infected fleas fed on naive mouse (total no. fed of total no. exposed to mouse)	Median (range) bacterial load per flea fed on naive mouse (cfu per flea)	Transmission from flea to mouse	Estimated transmission efficiency (95% CI) per time point *C. felis*†	Estimated transmission efficiency (95% CI) per time point *X. cheopis*‡
O. montana 1 d				ND	
1	9 (9/10)	3.60×10^6 (2.6×10^5–3.9×10^6)	Yes		
2	10 (10/10)	3.06×10^6 (7.4×10^4–4.46×10^6)	Yes		
3	10 (10/10)	2.68×10^6 (5.1×10^5–5.9×10^6)	Yes		
4	9 (9/10)	7.60×10^5 (9.95×10^4–4.19×10^6)	Yes		
5	10 (10/10)	2.78×10^6 (3×10^5–4.6×10^6)	Yes		
C. felis 1 d				0.00 (0.00–6.70)	4.71 (0.34–21.06)
1	8 (8/8)	4.25×10^4 (1×10^2–3.05×10^5)	No		
2	9 (11/11)	3.60×10^5 (2.5×10^1–2.09×10^6)	No		
3	7 (10/10)	8.90×10^4 (2.0×10^2–4.4×10^6)	No		
4	8 (10/10)	1.42×10^5 (6.5×10^3–5.55×10^5)	No		
5	9 (9/9)	2.80×10^5 (1.55×10^4–6.35×10^5)			
C. felis 2 d				2.15 (0.13–10.64)	6.32 (0.38–28.54)
1	10 (10/10)	6.0×10^5 (1.65×10^4–3.05×10^6)	No		
2	9 (10/10)	1.30×10^6 (3×10^2–5.0×10^6)	No		
3	9 (9/9)	1.19×10^6 (7.5×10^3–2.8×10^6)	Yes		
4	9 (9/9)	1.02×10^6 (2.5×10^1–1.35×10^6)	No		
5	9 (11/11)	4.50×10^5 (1.56×10^4–2.15×10^6)	No		
C. felis 3 d				0.00 (0.00–5.84)	9.14 (2.65–25.94)
1	8 (8/10)	1.38×10^6 (4.95×10^5–3.95×10^6)	No		
2	7 (7/9)	2.8×10^6 (4.6×10^3–7.1×10^6)	No		
3	10 (10/10)	1.03×10^6 (1.65×10^4–3.02×10^6)	No		
4	12 (13/14)	7.93×10^5 (1.00×10^3–2.18×10^6)	No		
5	10 (10/11)	7.10×10^5 (5.00–8.05×10^5)	No		
C. felis 4 d				0.00 (0.00–6.50)	2.54 (0.16–12.47)
1	9 (9/10)	1.53×10^6 (5.75×10^3–6.35×10^6)	No		
2	10 (10/10)	1.94×10^6 (2.95×10^4–2.5×10^6)	No		
3	11 (11/11)	1.71×10^6 (1.50×10^2–2.28×10^6)	No		
4	10 (11/11)	1.56×10^6 (1.29×10^5–2.60×10^6)	No		

* Fleas were infected in artificial feeders containing defibrinated rat blood infected with *Y. pestis* at a concentration of 1.09–2.61×10^9 cfu/mL. To compare vector efficiency using the same strain of *Y. pestis* with *X. cheopis*, a flea commonly encountered in Uganda, transmission efficiency data from a previous study are included.
† ND, not determined because all flea pools transmitted, thus maximum likelihood could not be used.
‡ Data from Eisen and others.[14]

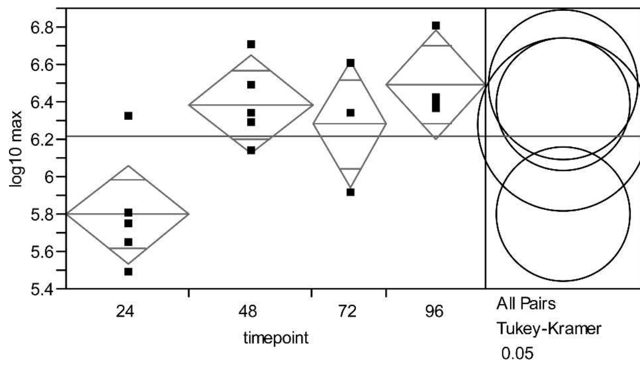

FIGURE 2. Mean maximum number of *Y. pestis* colony forming units in *C. felis* flea pools at 24, 48, 72, and 96 h p.i. Data points are means of each replicate.

bined where transmission efficiency was 0.57% (95% CI: 0.03–2.78%)), the average number of fleas per host required for a single focal infection to give rise to a secondary infection was estimated to be approximately 95 fleas per person (95% CI: 20–1,811).

DISCUSSION

In contrast to previous studies,[18,19] we demonstrated that *C. felis* is a competent vector of *Y. pestis*. Although the observed transmission efficiency was low, our field study revealed that *C. felis* is the most abundant flea species in human habitations in a plague endemic region of Uganda. Furthermore, we showed that this flea occasionally infests rodents (*A. niloticus* and *R. rattus*) that are 1) abundant in peridomestic environments, 2) susceptible to *Y. pestis* infection,[1,7,21,22] and 3) commonly harbor highly efficient vectors of *Y. pestis* (*X. cheopis* and *X. brasiliensis*).[1,2,4,12–15] Our findings imply that cat fleas should not be ignored in vector control programs aimed at reducing human risk of exposure to *Y. pestis*, but that focusing broadly on ridding rats of their fleas may be more effective at disrupting transmission.

Previous studies have speculated that *P. irritans*, a human-biting flea that is commonly found in human habitations in Tanzania, Kenya and the Democratic Republic of Congo, may serve as a vector in anthroponotic cycles of *Y. pestis* in Africa and elsewhere.[16,35–37] It is possible that *C. felis*, which replaces *P. irritans* as the most common flea in human habitations in Uganda,[17,35] plays a similar role in *Y. pestis* transmission. A high proportion of human habitations in the Arua and Nebbi districts were infested with host-seeking fleas and the overwhelming majority of those fleas were *C. felis*. Although pan trapping gave us an estimate of the relative abundance of fleas in human habitations among villages, we are uncertain what proportion of the population is captured by this method and therefore the abundance values reported in this study represent a minimum estimate of flea abundance. Interestingly, our estimates of the number of cat fleas per person required for maintenance of *Y. pestis* by person-to-person transmission (approximately 25 fleas per person) was within the range observed in our study. Among sampled huts that yielded fleas, the number of *C. felis* ranged from 1 to 62 cat fleas for a single sampling occasion. To more precisely estimate the density of fleas required for maintenance of the pathogen in human habitations, field-derived values of daily biting rates, host preference, and daily survivorship are needed. Our data revealed a great deal of variability among huts and among villages in the abundances of fleas captured. Perhaps understanding the underlying mechanisms explaining flea abundance could be used to make recommendations on how to reduce flea numbers and disrupt transmission in the home environment.

Cat fleas were rarely collected from rodents susceptible to plague infection, such as *R. rattus* and *A. niloticus*, and transmission efficiency for *Y. pestis* was very low for *C. felis*. Thus, it is unlikely that this flea alone could sustain enzootic transmission among rodents, and it is an improbable bridging vector to humans. However, it is quite likely that *X. cheopis* could serve as a bridging vector to humans because of its high efficiency of transmission and willingness to bite humans when its typical rodent hosts perish from plague infection.[1,2,4,9,12–15,29,38] Furthermore, because of the high abundance of rats and their heavy infestations with *X. cheopis*, rat fleas are probably contributing more strongly than cat fleas to transmission in peridomestic settings. Previous studies have estimated that to sustain enzootic transmission of *Y. pestis* by *X. cheopis*, an average of 4.7–7.8 fleas per host are needed.[14,25] In Arua district, *R. rattus* harbored an average of 8.3 *X. cheopis* in sylvatic areas, a quantity theoretically high enough to sustain transmission in an *X. cheopis-R. rattus* cycle. In peridomestic areas, *X. cheopis* infestation levels were slightly above one[25] and slightly below another[14] theoretical estimate of the threshold infestation needed for enzootic transmission. Because several small mammal species that are commonly infested with *X. cheopis* were collected in sylvatic and peridomestic areas (Tables 1 and 2), it is possible that *X. cheopis* could serve as a bridging species between sylvatic and peridomestic cycles in the Arua district. In the Nebbi district, *X. cheopis* was often replaced by another competent vector of *Y. pestis*, *X. brasiliensis*,[1,2] and probably plays a similar role in that district. A future study will evaluate ecological differences between these districts that could account for the observed distribution.

Future studies are needed to elucidate the roles of *X. cheopis*, *X. brasiliensis*, and *C. felis* in the transmission of plague bacteria in Arua and Nebbi districts. First, our field collection was conducted during an inter-epizootic period. It is possible that flea loads and the composition of flea species on hosts differ between epizootic and inter-epizootic periods. Thus, a similar study conducted during a plague epizootic is needed. Second, the abundance of host-seeking fleas in human habitations may increase during epizootics and *X. cheopis* and *X. brasiliensis* could be more common in these areas when their rodent hosts succumb to plague infection. Interestingly, the proportion of fleas captured in pan traps in Arua district that were classified as *C. felis* was significantly lower during the first trapping session, which represented the peak of the plague season than the second session when few human cases are typically reported. The majority of non-cat flea captures included *X. cheopis*. These data show that flea species composition is not temporally static and comparisons between epizootic and inter-epizootic periods are necessary. Although *C. felis* is the most common flea in human habitations, it is unclear how frequently they feed on humans in this setting. Molecular identification of blood meals of fleas, similar to those produced for ticks and mosquitoes,[39–44] would be useful

for assessing how commonly cat fleas bite humans compared with other potential hosts such as rodents in the homes or livestock in peridomestic settings. Third, we made the assumption that most human exposures occur in peridomestic areas, which is reportedly the situation in many of the world's plague foci, but this has not been confirmed by epidemiological studies for our study sites in Uganda. Finally, our vector efficiency study focused on *C. felis felis*, whereas *C. felis strongylus* is the most common subspecies in East Africa.[17] It is possible that vector competency could differ between these subspecies. On the other hand, a molecular study questioned the validity of a sub-species characterization,[45] raising the question of how dissimilar they are biologically. Regardless of taxonomic classification, use of fleas and bacteria from different geographical regions could result in differing vector efficiency outcomes. Therefore, future studies using fleas and *Y. pestis* strains originating from Uganda are necessary.

Infrequent transmission of *Y. pestis* by *C. felis* compared with *X. cheopis* 1–4 d p.i. (Table 4) sheds light on possible mechanisms of early-phase transmission. In 1914, Bacot and Martin[12] described a flea-borne transmission mechanism that has been the dominant paradigm for nearly a century. Under this scenario, the plague bacilli multiply and form a blockage in the proventriculus of the infected flea. This blockage prevents newly ingested blood from reaching the midgut, causing the flea to starve. As a result, the flea increases its feeding attempts and ingested blood combined with infectious material cleaved from the blockage are occasionally regurgitated into the bite site resulting in transmission. Although Bacot and Martin[12] proposed that a partially blocked flea may be even more efficient as a vector, the blocked-flea scenario has dominated the plague literature. For example, vector efficiency is often equated with a flea's ability to block.[1,3,4,16,21,25,46] However, recent studies using aliquots from the same stock of *Y. pestis* used in the present study have demonstrated high vector efficiency by unblocked fleas of all species tested to date using the experimental system described in this study (i.e., *X. cheopis*, *O. montana*, and *O. tuberculata cynomuris*, *O. hirsuta*.[14,32–34,47]). Among these studies, bacterial loads were similarly as high as those observed in *C. felis* in the present study. As suggested previously,[14,32,33] bacterial loads in fleas are not predictive of a flea's ability to transmit plague bacteria. Unlike other species evaluated 1–4 d p.i., *C. felis* appeared to have a higher digestive rate, as indicated by copious excretion of partially digested blood while feeding, and other researchers have noted that this species commonly defecates large quantities of partially digested blood on the host while feeding.[9] It is possible that more rapid digestion or increased peristalsis could force bacteria further posterior in the digestive tract, making transmission by regurgitation less probable than when it is in a more anterior position.[32] Interestingly, bacterial loads were significantly lower in cat fleas 24 h p.i. compared with all other time points. In addition, at 24 h p.i., bacterial loads were significantly lower in *C. felis* than *O. montana* fed the same source of infectious blood. These observations suggest that bacteria may have been eliminated in the feces within 24 h of feeding, followed by replication of the bacteria that remained in the digestive tract. Although useful in providing data contributing to a continuum of vector efficiency that could be used in comparative physiological studies, the mechanism of early-phase transmission remains the focus of future work.

Our study suggests that plague control programs should continue to focus on reducing the abundance of rat fleas (*X. cheopis* and *X. brasiliensis*). However, because of their abundance in human habitations, catholic feeding habits, and ability to transmit *Y. pestis*, cat fleas could serve as secondary vectors and should not be ignored. In the United States, plague prevention and control focuses primarily on reducing rodent abundance through elimination of harborage (e.g., piles of wood, brush, or debris), food sources (e.g., pet food, garbage), and access to human dwellings.[48–50] Due to differences in housing structure and cultural practices, this is less feasible in rural Uganda, where rodent-targeted vector control or indoor residual spraying may be more effective.[50] Initiatives aimed at reducing the incidence of malaria, such as use of insecticide-treated bednets and indoor residual spraying, may also be effective in reducing human contact with fleas in human habitations, providing flea vectors do not develop resistance to the given insecticides. In some areas where malaria control programs were implemented in the absence of modifying housing conditions the number of human plague cases have been reduced.[51] Integrated vector control programs aimed at reducing transmission of multiple vector-borne pathogens may be cost effective, but the efficacy of such a strategy for reducing the incidence of plague requires further investigation and will require the kind of knowledge provided by our study.

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REFERENCES

1. Pollitzer R, 1954. Plague. World Health Organization Monograph Series No. 22. Geneva, Switzerland: World Health Organization.
2. Gage KL, Kosoy MY, 2005. Natural history of plague: perspectives from more than a century of research. *Annu Rev Entomol 50:* 505–528.
3. Hinnebusch BJ, 2005. The evolution of flea-borne transmission in *Yersinia pestis. Curr Issues Mol Biol 7:* 197–212.
4. Eskey CR, Haas VH, 1940. Plague in the western part of the United States. *Publ Hlth Bull 254:* 1–83.
5. WHO, 2004. Human plague in 2002 and 2003. *Wkly Epidemiol Rec 79:* 301–308.
6. WHO, 2005. Outbreak news index 2005. *Wkly Epidemiol Rec 80:* 433–440.
7. Hopkins GHE, 1949. Report on rats, fleas and plague in Uganda: East African Standard, Ltd., 52.
8. Kilonzo BS, Makundi RH, Mbise TJ, 1992. A decade of plague epidemiology and control in the western Usambara mountains, north-east Tanzania. *Acta Trop 50:* 323–329.
9. Dryden MW, Rust MK, 1994. The cat flea: biology, ecology and control. *Vet Parasitol 52:* 1–19.
10. Kwochka KW, 1987. Fleas and related disease. Veterinary Clinics of North America. *Small Animal Practice 17:* 1235–1262.

11. Verjbitski DT, 1908. The part played by insects in the epidemiology of plague. *J Hyg (Lond) 8:* 162–208.
12. Bacot AW, Martin CJ, 1914. Observations on the mechanism of the transmission of plague by fleas. *J Hyg (Lond) 13 (Plague Suppl. III):* 423–439.
13. Burroughs AL, 1947. Sylvatic plague studies: the vector efficiency of nine species of fleas compared with *Xenopsylla cheopis. J Hyg (Lond) 43:* 371–396.
14. Eisen RJ, Wilder AP, Bearden SW, Montenieri JA, Gage KL, 2007. Early-phase transmission of *Yersinia pestis* by unblocked *Xenopsylla cheopis* (*Siphonaptera: pulicidae*) is as efficient as transmission by blocked fleas. *J Med Entomol 44:* 678–682.
15. Engelthaler DM, Hinnebusch BJ, Rittner CM, Gage KL, 2000. Quantitative competitive PCR as a technique for exploring flea-*Yersina pestis* dynamics. *Am J Trop Med Hyg 62:* 552–560.
16. Laudisoit A, Leirs H, Makundi RH, Van Dongen S, Davis S, Neerinckx S, Deckers J, Libois R, 2007. Plague and the human flea, Tanzania. *Emerg Infect Dis 13:* 687–693.
17. Hopkins GHE, 1947. Annotated and illustrated keys to the known fleas of East Africa. *Ugandan Journal 11:* 133–191.
18. Wheeler CM, Douglas JR, 1945. Sylvatic plague studies. V. The determination of vector efficiency. *J Inf Dis 77:* 1–12.
19. Devignat R, 1946. Aspects de l'epidemiologie de al peste au Lac Albert. *Ann Soc Belg Med Trop 26:* 13–54.
20. Herms WB, 1953. Medical Entomology. New York: MacMillan.
21. Hirst LF, 1953. The Conquest of Plague. Oxford: Claredon Press.
22. Eisen RJ, Bearden SW, Wilder AP, Montenieri JA, Antolin MF, Gage KL, 2006. Early-phase transmission of *Yersinia pestis* by unblocked fleas as a mechanism explaining rapidly spreading plague epizootics. *Proc Natl Acad Sci USA 103:* 15380–15385.
23. Fine PEM, 1981. Epidemiological principles of vector-mediated transmission. McKelvey JJ, Eldridge BF, Maramorosch K, eds. Vectors of Disease Agents: Interactions with Plants, Animals, and Man. New York: Praeger Publishers.
24. Garrett-Jones C, Schidrawi GR, 1969. Malaria vectorial capacity of a population of *Anopheles gambiae*: an exercise in epidemiological entomology. *Bull World Health Organ 40:* 531–545.
25. Lorange EA, Race BL, Sebbane F, Hinnebusch BJ, 2005. Poor vector competence of fleas and the evolution of hypervirulence in *Yersinia pestis. J Infect Dis 191:* 1907–1912.
26. Macdonald G, 1961. Epidemiologic models in studies of vector-borne diseases. *Public Health Rep 76:* 753–764.
27. Delany MJ, 1975. The Rodents of Uganda. Kettering Northamptonshire: The George Press.
28. Haselbarth E, 1966. Siphonaptera. Zumpt F, ed. The Arthropod Parasites of Vertebrates in Africa South of the Sahara (Ethiopia Region). Johannesburg: South African Institute of Medical Research, 117–212.
29. Smit FGAM, 1973. Siphonaptera (Fleas). Smith KGV, ed. Insects and Other Arthropods of Medical Importance. London: British Museum of Natural History, 325–371.
30. Chu MC, 2000. Laboratory Manual of Plague Diagnostics. Geneva: Centers for Disease Control and Prevention and World Health Organization, 129.
31. Kilonzo BS, 1977. A simple light trap for field collections of adult fleas: studies on its efficiency and suitability in north-east Tanzania. WHO/VBC/77.673, 11.
32. Eisen RJ, Lowell JL, Montenieri JA, Bearden SW, Gage KL, 2007. Temporal dynamics of early-phase transmission of *Yersinia pestis* by unblocked fleas: secondary infectious feeds prolong efficient transmission by *Oropsylla montana* (Siphonaptera: Ceratophyllidae). *J Med Entomol 44:* 672–677.
33. Wilder AP, Eisen RJ, Bearden SW, Montenieri JA, Gage KL, Antolin MF, 2008. *Oropsylla hirsuta* (Siphonaptera: Ceratophyllidae) can support plague epizootics in black-tailed prairie dogs (*Cynomys ludovicianus*) by early-phase transmission of *Yersinia pestis. Vector Borne Zoonotic Dis*.
34. Wilder AP, Eisen RJ, Bearden SW, Montenieri JA, Tripp DT, Brinkerhoff RJ, Gage KL, Antolin MF, 2008. Transmission efficiency of two flea species (*Oropsylla tuberculata cynomuris* and *Oropsylla hirsuta*) involved in plague epizootics among prairie dogs. *EcoHealth*.
35. Davis DH, 1953. Plague in Africa from 1935 to 1949; a survey of wild rodents in African territories. *Bull WHO 9:* 665–700.
36. Drancourt M, Houhamdi L, Raoult D, 2006. *Yersinia pestis* as a telluric, human ectoparasite-borne organism. *Lancet Infect Dis 6:* 234–241.
37. Hopla CE, 1980. A study of the host associations and zoogeography of *Pulex*. Traub R, Starcke H, eds. Fleas. Rotterdam: A.A. Balkema, 185–207.
38. Hunter KW Jr, Campbell AR, Sayles PC, 1979. Human infestation by cat fleas, *Ctenocephalides felis* (Siphonaptera: Pulicidae), from suburban raccoons. *J Med Entomol 16:* 547.
39. Beati L, Humair PF, Aeschlimann A, Raoult D, 1994. Identification of spotted fever group rickettsiae isolated from *Dermacentor marginatus* and *Ixodes ricinus* ticks collected in Switzerland. *Am J Trop Med Hyg 51:* 138–148.
40. Kent RJ, Thuma PE, Mharakurwa S, Norris DE, 2007. Seasonality, blood feeding behavior, and transmission of *Plasmodium falciparum* by *Anopheles arabiensis* after an extended drought in southern Zambia. *Am J Trop Med Hyg 76:* 267–274.
41. Kirstein F, Gray JS, 1996. A molecular marker for the identification of the zoonotic reservoirs of Lyme borreliosis by analysis of the blood meal in its European vector *Ixodes ricinus. Appl Environ Microbiol 62:* 4060–4065.
42. Moran Cadenas F, Rais O, Humair PF, Douet V, Moret J, Gern L, 2007. Identification of host bloodmeal source and *Borrelia burgdorferi* sensu lato in field-collected *Ixodes ricinus* ticks in Chaumont (Switzerland). *J Med Entomol 44:* 1109–1117.
43. Pichon B, Egan D, Rogers M, Gray J, 2003. Detection and identification of pathogens and host DNA in unfed host-seeking *Ixodes ricinus* L. (Acari: Ixodidae). *J Med Entomol 40:* 723–731.
44. Pichon B, Rogers M, Egan D, Gray J, 2005. Blood-meal analysis for the identification of reservoir hosts of tick-borne pathogens in Ireland. *Vector Borne Zoonotic Dis 5:* 172–180.
45. Vobis M, D'Haese J, Mehlhorn H, Mencke N, Blagburn BL, Bond R, Denholm I, Dryden MW, Payne P, Rust MK, Schroeder I, Vaughn MB, Bledsoe D, 2004. Molecular phylogeny of isolates of *Ctenocephalides felis* and related species based on analysis of ITS1, ITS2 and mitochondrial 16S rDNA sequences and random binding primers. *Parasitol Res 94:* 219–226.
46. Krasnov BR, Shenbrot GI, Mouillot D, Khokhlova IS, Poulin R, 2006. Ecological characteristics of flea species relate to their suitability as plague vectors. *Oecologia 149:* 474–481.
47. Eisen RJ, Bearden SW, Wilder AP, Montenieri JA, Antolin MF, Gage KL, 2006. Early-phase transmission of *Yersinia pestis* by unblocked fleas as a mechanism explaining rapidly spreading plague epizootics. *Proc Natl Acad Sci USA 103:* 15380–15385.
48. MMWR, 2006. Human plague-four states, 2006. *MMWR 55:* 1–3.
49. Poland JD, Barnes AM, 1979. Plague. Steele JH, ed. CRC Handbook Series in Zoonoses. Section A: Bacterial, rickettsial and mycotic diseases. Volume I. Boca Raton, FL: CRC Press Inc., 515–559.
50. Gratz NG, 1999. Control of Plague Transmission. Plague Manual: Epidemiology, Distribution, Surveillance and Control. Geneva: World Health Organization, 97–134.
51. Akiev AK, 1982. Epidemiology and incidence of plague in the world, 1958–79. *Bull WHO 60:* 165–169.