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Original Contribution

Transmission Efficiency of Two Flea Species (*Oropsylla tuberculata cynomuris* and *Oropsylla hirsuta*) Involved in Plague Epizootics among Prairie Dogs

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Abstract: Plague, caused by *Yersinia pestis*, is an exotic disease in North America circulating predominantly in wild populations of rodents and their fleas. Black-tailed prairie dogs *(Cynomys ludovicianus)* are highly susceptible to infection, often experiencing mortality of nearly all individuals in a town as a result of plague. The fleas of black-tailed prairie dogs are *Oropsylla tuberculata cynomuris* and *Oropsylla hirsuta*. We tested the efficiency of *O. tuberculata cynomuris* to transmit *Y. pestis* daily from 24 to 96 h postinfection and compared it to previously collected data for *O. hirsuta*. We found that *O. tuberculata cynomuris* has over threefold greater transmission efficiency (0.18 infected fleas transmit *Y. pestis* at 24 h postinfection) than *O. hirsuta* (0.05 fleas transmit). Using a simple model of flea-borne transmission, we combine these laboratory measurements with field data on monthly flea loads to compare the seasonal vectorial capacity of these two flea species. Coinciding with seasonal patterns of flea abundance, we find a peak in potential for flea-borne transmission in March, during high *O. tuberculata cynomuris* abundance, and in September–October when *O. hirsuta* is common. Our findings may be useful in determining the timing of insecticidal dusting to slow plague transmission in black-tailed prairie dogs.

Keywords: prairie dog, flea, plague, seasonality, insecticidal dusting

Introduction

Yersinia pestis, the etiological agent of plague, was introduced to the west coast of North America around 1900 and spread eastward, establishing itself as a flea-borne disease of wild rodents (Ecke and Johnson, 1952; Gage and Kosoy, 2005). Black-tailed prairie dogs *(Cynomys ludovicianus)* are particularly susceptible to plague, with epizootics often resulting in decimation of towns and contributing to rapid spread of the disease among other small mammals and their fleas (Barnes, 1993; Cully and Williams, 2001). Declines in black-tailed prairie dog populations prompted a petition to list them as threatened (U.S. Fish and Wildlife Service, 2000), and plague has hindered the reintroduction of the black-footed ferret *(Mustela nigripes)*, a plague-susceptible, critically endangered species whose diet consists largely of prairie dogs (Fitzgerald, 1993; Biggins and Kosoy, 2001; Antolin

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et al., 2002). Plague epizootics also pose a human health risk. Many black-tailed prairie dog colonies are located near and within urban settings, and approximately 14% of North American human plague cases are associated directly with epizootics of prairie dogs (Seery et al., 2003).

The primary fleas of black-tailed prairie dogs are *Oropsylla tuberculata cynomuris* and *Oropsylla hirsuta* (Maupin, 1970; Ubico et al., 1988; Brinkerhoff et al., 2006), formerly *Opisocrostis tuberculatus* and *Opisocrostis hirsutus*. Although both species are competent vectors of *Y. pestis*, they have been shown to be inefficient in older literature (Eskey and Haas, 1940). Proventricular blockage, where *Y. pestis* forms a biofilm that occludes the proventriculus of the flea, is a process historically considered necessary for efficient flea-borne transmission of *Y. pestis* (Bacot and Martin, 1914). Blockages rarely form in most North American wild rodent fleas (Burroughs, 1947), including prairie dog fleas. Until recently, the single published study of *O. hirsuta* and *O. tuberculata* transmission efficiency examined the period following an infectious bloodmeal when blockage is likely to occur, between 5 and 130 days postinfection (p.i.) (Eskey and Haas, 1940). Transmission by both species was uncommon during this period, with 1 of 10 *O. tuberculata* and 3 of 70 *O. hirsuta* transmitting *Y. pestis* over the 125-day study period. Although feeding frequency has not been studied specifically in these species, *O. hirsuta* experiences high mortality in the lab when starved for longer than 1 day (Wilder, 2007). Similar feeding frequency was noted for *O. tuberculata cynomuris* in this study. Therefore, it is likely that fleas used in Eskey and Haas (1940) fed nearly daily.

Such low rates of flea-borne transmission cannot explain the rapid spread of plague among mammalian hosts during plague epizootics, and point to alternative routes of transmission, like direct transmission by respiratory droplets from infected animals or ingestion of infectious carcasses (Gage and Kosoy, 2005; Webb et al., 2006). Despite these indications that prairie dog fleas may not play a major role in epizootic spread, other evidence points to the importance of flea-borne transmission. Insecticidal dusting of prairie dog burrows appears to be effective in preventing plague epizootics (Seery et al., 2003; Hoogland et al., 2004), and climatic conditions that favor increased flea abundance are also associated with higher plague risk (Stapp et al., 2004). Further, prairie dog fleas rarely block, but instances of transmission in the absence of blockage have been reported in many species, even those that block frequently (Burroughs, 1947; Degtyareva et al., 1990; Gan et al., 1990; Engelthaler et al., 2000). Efficient early-phase transmission (between 1–4 days p.i.) of *Y. pestis* was first demonstrated explicitly in *Oropsylla montana*, and has also been demonstrated in *O. hirsuta* (Eisen et al., 2006; Wilder, 2007). Early-phase transmission appears to be more important than late-phase transmission (≥5 days p.i.) in these wild rodent fleas (Eskey and Haas, 1940; Engelthaler et al., 2000).

We hypothesized that early-phase transmission may also be important in *O. tuberculata cynomuris* because of the rarity of blockage and inefficient late-phase transmission in this species. By infecting wild-caught fleas with bacteremic blood and subsequently placing them on naïve mice daily from 24 to 96 h p.i., we examined the transmission efficiency of *O. tuberculata cynomuris*. Using field-collected data on prairie dog flea loads, laboratory transmission data, and a simple mathematical model, we compare the vectorial capacity of *O. tuberculata cynomuris* to that of *O. hirsuta*, and determine the potential for flea-borne transmission by each prairie dog flea in the context of seasonality of outbreaks and the timing of preventative measures.

Methods

Flea and Mouse Species and Bacterial Strain

Yersinia pestis used to infect fleas was the same fully virulent strain of *Y.* pestis CO963188 previously used in an *O. hirsuta* transmission study (Wilder et al., in press), and originally described by Eisen et al. (2006). Mice used in the study were 6-week-old Swiss-Webster females (Centers for Disease Control and Prevention, Division of Vector-Borne Infectious Diseases laboratory colony). All animal procedures were approved by the Centers for Disease Control and Prevention, Division of Vector-Borne Infectious Diseases, Institutional Animal Care and Use Committee. *O. tuberculata cynomuris* were collected from two black-tailed prairie dog colonies on the Pawnee National Grassland in northeastern Colorado in April 2007. Fleas were collected by burrow swab (Ubico et al., 1988), immediately returned to the lab, and identified to species using light microscopy. To test for *Y. pestis* infection acquired in the wild prior to the study, a subsample ($n = 84$) of wild-caught *O. tuberculata cynomuris* was frozen at −80°C and examined by PCR using methods described previously (Salkeld et al., 2007), shown to reliably detect 10–100 cfu per flea (Stevenson et al., 2003). Live fleas were maintained at 21°C and 85% RH throughout the study.

Infection of Fleas

Laboratory methods used for transmission studies by *O. tuberculata cynomuris* were identical to those used for *O. hirsuta* (Wilder et al., in press), and are described in detail by Eisen et al. (2006). Briefly, defibrinated Sprague-Dawley rat blood (Bioreclamation, Jericho, NY) was infected with *Y. pestis* to a final concentration of 1.3×10^9 cfu/mL. The infected blood was placed in glass feeders covered by a mouse skin, and heated to 37°C. Fleas were placed within a capsule on the mouse skin membrane and allowed to feed for 1 h. Following feeding, fleas were removed from the feeders and immobilized by chilling on ice. Using light microscopy, fleas were identified as having consumed infectious blood by the presence of fresh blood in the midgut. Fleas in which fresh blood did not appear in the midgut were removed from the study. Bacterial concentration in rat blood contained in each feeder was confirmed by plating (in duplicate) serial dilutions of the blood on 6% sheep blood agar immediately following infection of fleas.

Flea-borne Transmission to Naïve Mice

Fleas were allowed to feed in groups (7–20 fleas) on individual naïve mice at 24, 48, 72, and 96 h postinfection (p.i.) (Table 1). One group of fleas for each replicate was harvested each day and frozen at −80°C following the transmission feed to determine infection prevalence and bacterial load within the fleas. Mice were anesthetized by intraperitoneal injection of ketamine (Vedco, St. Joseph, MO)-Rompun (Xylazine; Anased, Bedford, OH). A capsule was affixed to the dorsal side of the mouse and fleas were placed for 1 h in the capsule to feed. Following the feeding period, fleas and flea fecal material were aspirated from the capsule. Fleas were immobilized by chilling on ice and the presence of fresh blood in the fleas' midguts was identified as described above. Fleas that failed to take a bloodmeal from the naïve mouse were removed to ensure all fleas feeding at each time point had taken the same number of bloodmeals.

Harvested fleas were frozen and maintained at −80°C until being ground individually in 100 µL HIB with 10% glycerol. Bacterial loads in fleas were quantified by plating (in duplicate) serial dilutions of the flea triturate on 6% sheep blood agar plates following Eisen et al. (2006).

After exposure to fleas, mice were held individually for 21 days in filter-top cages. Mice were monitored daily and euthanized at the onset of symptoms of *Y. pestis* infection. Infections were confirmed by direct fluorescence assay and *Y. pestis*-specific bacteriophage lysis of culture isolates (Chu, 2000). Mice that survived the 21-day postexposure period were euthanized and tested for antibody titers to *Y. pestis* F_1 antigen by passive hemagglutination and inhibition tests (PHA/HI) in serum (Chu, 2000).

Table 1. Transmission Study Summaries of *Oropsylla tuberculata cynomuris* (This Study) and *Oropsylla hirsuta* (Data from Wilder et al., in press)[a]

Hours postinfection	No. mice infected (no. mice exposed)	Flea infection prevalence	Average no. infected fleas fed on mouse	Median bacterial load (cfu) in fleas (range)	Transmission efficiency (95% CI)
O. tuberculata cynomuris					
24	18 (19)	0.88	13.5	1.52×10^4 (5×10^0–5.7×10^5)	17.88 (10.88, 37.22)
48	2 (15)	0.88	10.8	2.60×10^4 (5×10^0–9.1×10^5)	1.25 (0.23, 4.04)
72	0 (10)	0.87	11.1	6.65×10^4 (6.05×10^2–1.27×10^6)	0 (0.00, 2.87)
96	0 (5)	0.96	12.6	4.70×10^4 (8.30×10^2–3.60×10^5)	0 (0.00, 4.27)
O. hirsuta (data from Wilder et al., in press)					
24	10 (24)	1.00	12.9	7.33×10^4 (2.00×10^1–1.88×10^6)	4.54 (2.45, 7.93)
48	2 (18)	0.93	12.2	3.08×10^4 (1.00×10^1–1.71×10^6)	0.93 (0.17, 3.06)
72	0 (12)	0.91	10.8	1.87×10^4 (2.00×10^1–2.20×10^6)	0 (0.00, 2.50)
96	0 (6)	0.92	12	3.68×10^4 (2.50×10^1–1.71×10^6)	0 (0.00, 3.98)

[a]Transmission efficiency is the probability that one infected flea will transmit to a naïve host in one feed at a particular time point, and was estimated by maximum likelihood from the number of mice exposed to fleas, the number of mice that developed infection, and the number of infected fleas that fed on each mouse.

Statistical Analysis and Estimation of Transmission Efficiency

Pairwise comparisons of daily bacterial loads of fleas were performed using Dunn's multiple comparison procedure for nonparametric data (Dunn, 1964; Juneau, 2003). Data were analyzed using SAS statistical software (SAS Institute, Cary, NC). Daily transmission efficiency of *O. tuberculata cynomuris* was estimated based on the number of mice exposed to potentially infectious flea pools, the number of mice infected, and the number of infected fleas that fed on each mouse, by maximum likelihood using Microsoft® Excel® Add-In PooledInfRate, Version 3.0 (Biggerstaff, 2006). Because all flea groups fed daily, but groups assigned to later transmission trial days were not harvested in the days prior, infection prevalence data for unharvested groups on those days must be estimated. The number of infected fleas that fed on mice in unharvested groups was estimated by multiplying the infection prevalence (proportion of infected fleas) in harvested groups at a particular time point by the number of fed fleas in each unharvested group. For example, infection prevalence was 0.88 in harvested fleas at 24 h p.i. In an unharvested group at 24 h p.i. in which 14 fleas fed, the number of fed, infected fleas was estimated by multiplying 14 fed fleas by 0.88, or about 12 fleas. This method was repeated for each unharvested group at each time point.

Field Collection and Analysis of Black-tailed Prairie Dog Flea Loads

Black-tailed prairie dogs were captured between June 2004 and May 2007 from 12 towns showing no signs of plague activity on the Pawnee National Grassland (PNG) in Weld Co., CO, and from 10 towns during February and March 2004 in Boulder Co., CO. Trapping was not conducted in December or January because of low prairie dog activity during the winter months and low trapping success. Prairie dogs were trapped using Tomahawk live-traps (Tomahawk, WI) baited with sweet horse feed. Traps were set in the morning and checked several hours later before temperatures increased above approximately 25°C. Captured animals were anesthetized with IsoSol (Isoflurane, USP; Vedco Inc., St. Joseph, MO) and handled according to Colorado State University Animal Care and Use Committee protocols. Once anesthetized, prairie dogs were placed into a deep plastic tub and combed with a fine-tooth comb to remove fleas. All fleas were placed into Eppendorf tubes containing 1.5% saline with TWEEN. Prairie dogs were given time to recover and released at site of capture. Collected fleas were taken back to the laboratory and frozen at −70°C until being identified to species using light microscopy (Hubbard, 1968).

Estimation of Monthly Flea Load and Vectorial Capacity

Frequency distributions of flea loads, divided into monthly intervals, were fitted to a negative binomial distribution in the R statistical package (Version 2.5.0; R Development Core Team, 2007). The negative binomial parameter k describes how flea loads are aggregated onto relatively few individuals, with values of $k < 1$ indicative of aggregation, and was estimated by a maximum likelihood procedure (Crawley, 2007). Goodness of fit of the data to the negative binomial distribution was tested by χ^2.

A simplified model of flea-borne plague transmission was used to estimate vectorial capacity of fleas to transmit *Y. pestis*, using the notation of MacDonald (1957) and Garrett-Jones and Grab (1964):

$$V = mabp.$$

V is the number of infectious bites delivered to a susceptible host within a population by fleas that have fed on a single infectious host in a single day (Garrett-Jones and Grab, 1964). When $V \geq 1$, at least one infectious bite is delivered and the disease is spread to another host. In this model, m is the abundance of fleas on prairie dog hosts estimated by field-collected flea-load data collated from Weld and Boulder Co. The other three parameters were estimated from the present laboratory study *(O.t. cynomuris)* or from Wilder (in press; *O. hirsuta*): a is the probability that a flea will feed after encountering a susceptible host (daily feeding rate), b is the probability of a flea transmitting infection to a susceptible host (24-h transmission efficiency), p is the probability of surviving the period of time between feeding on an infectious and susceptible host (daily survival rate) (MacDonald, 1957).

Using the statistical program R, vectorial capacity was estimated by Monte Carlo simulation by generating 10,000 values of V for each flea species in each month. Flea load, m, was negative binomial with parameter k for each month, while a, b, and p were binomial probabilities with m fleas. Values for each of m, a, b and p were drawn sequentially from their underlying distributions, then multiplied to calculate V for that sample. Monthly confidence intervals for V and the proportion of $V \geq 1$ were estimated by

bootstrapping 2000 distributions of V, each with the number of prairie dog hosts sampled in each month.

This model assumes that if a prairie dog host becomes infectious to fleas (i.e., develops a high bacteremia), host density is sufficiently high that m infected fleas are able to find a susceptible host within 24 h. This is a valid assumption for prairie dog fleas considering the sociality and density of prairie dogs, and abundance of questing fleas in burrows (Hoogland, 1995; Seery et al., 2003), conditions likely to exist at the beginning of plague outbreaks. As epizootics proceed, more complex flea searching functions that include lowered host densities, fewer contacts between prairie dogs, and changing flea loads would have to be implemented to model flea-borne transmission (cf. Webb et al., 2006). The vectorial capacity model is used here is used as a gauge of the seasonal potential for flea-borne transmission of *Y. pestis*.

Results

The greatest transmission efficiency by *O. tuberculata cynomuris* was observed at 24 h p.i., when 18 of 19 mice exposed to groups of fleas were infected (Table 1). Transmission efficiency greatly decreased by 48 h p.i., when only 2 of 15 mice became infected. Transmission to any of the 10 mice exposed at 72 h p.i., or any of the 5 mice exposed at 96 h p.i., did not occur. Transmission efficiency, or the probability of an individual flea transmitting *Y. pestis* after feeding once, was 0.18 (95% CI 0.11, 0.37) for *O. tuberculata cynomuris* at 24 h p.i., significantly higher than the transmission efficiency of *O. hirsuta*, 0.05 (0.03, 0.08), at the same time point (Wilder et al., in press). The number of *O. tuberculata cynomuris* individuals in transmitting pools ranged from 5 to 17 fleas, and sizes of transmitting and nontransmitting flea pools were similar ($F = 1.1$, $df = 1$, $P = 0.31$), as were pool sizes over time ($F = 0.04$, $df = 1$, $P = 0.84$). Four of the 18 mice infected at 24 h p.i. survived to 21 days postexposure and developed antibody titers to *Y. pestis* F_1 antigen (two titers were 1:1024, two titers were 1:2048). All other mice that survived the 21-day postexposure study period did not develop detectable antibodies and were considered uninfected.

O. tuberculata cynomuris and *O. hirsuta* had similar daily bite rates over the experiment (0.82, SD 0.01 for *O. tuberculata cynomuris*; and 0.84, SD 0.01 for *O. hirsuta*), but differed in daily survival (0.88, SD 0.01 for *O. tuberculata cynomuris*; and 0.95, SD 0.01 for *O. hirsuta*).

All wild-caught, sub-sampled *O. tuberculata cynomuris* tested negative for *Y. pestis* infection by highly sensitive PCR (Stevenson et al., 2003). Based on maximum likelihood estimates, infection prevalence within the source population could have been as high as 4.37% (95% CI 0.00, 4.37). Even if *O. tuberculata cynomuris* used in the experiment were infected in the field, the late-phase transmission efficiency is so low (Eskey and Haas, 1940) it is unlikely that field-infected fleas would have altered these transmission results.

Infection prevalence of *O. tuberculata cynomuris* exposed to infectious blood in the laboratory is shown in Table 1 and did not differ significantly among time points (Fisher's exact test, $P = 0.28$). Bacterial loads are shown in Fig. 1. Median bacterial loads were 1.2, 2.6, 6.7, and 4.7×10^4 cfu, for 24, 48, 72, and 96 h p.i., respectively, but did not significantly differ at $\alpha = 0.05$ using Dunn's nonparametric multiple comparison procedure (Dunn, 1964). Maximum bacterial loads were similar among time points (ANOVA of $\log_{10}$-transformed data, $F = 2.2$, $df = 3$, $P = 0.14$). Median bacterial loads were similar between transmitting and nontransmitting groups (Wilcoxon, $\chi^2 = 1.4$, $df = 1$, $P = 0.24$), as were maximum bacterial loads (ANOVA of $\log_{10}$-transformed data, $F = 0.58$, $df = 1$, $P = 0.46$). The range in bacterial loads decreased over time, probably because less robust fleas that imbibed less of the infectious blood died or were removed from the study before later time points (Fig. 1).

Monthly average *O. tuberculata cynomuris* and *O. hirsuta* flea loads for captured black-tailed prairie dogs are shown in Fig. 2a. During trapping months, *O. tuberculata cynomuris* was found on animals between February and July, with greatest abundance in March (mean 9.1, 95% CI 5.8–12.5). Only five prairie dogs harbored any *O. tubercu-*

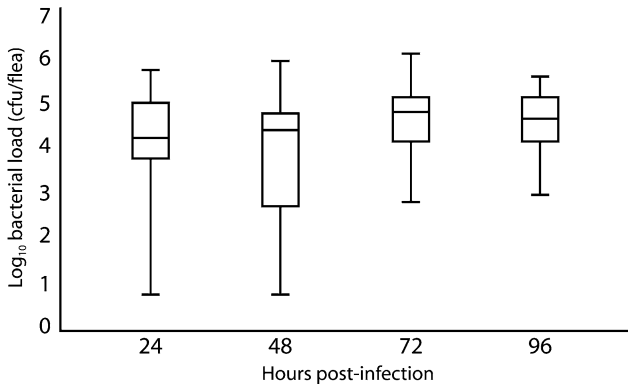

Figure 1. Box and whisker plots of $\log_{10}$-transformed bacterial loads (cfu) at each time point for *Oropsylla tuberculata cynomuris*. Lines represent medians, boxes represent first and third quartiles, and whiskers represent the range of data observed. Bacterial loads of fleas were similar at each time point ($P = 0.14$).

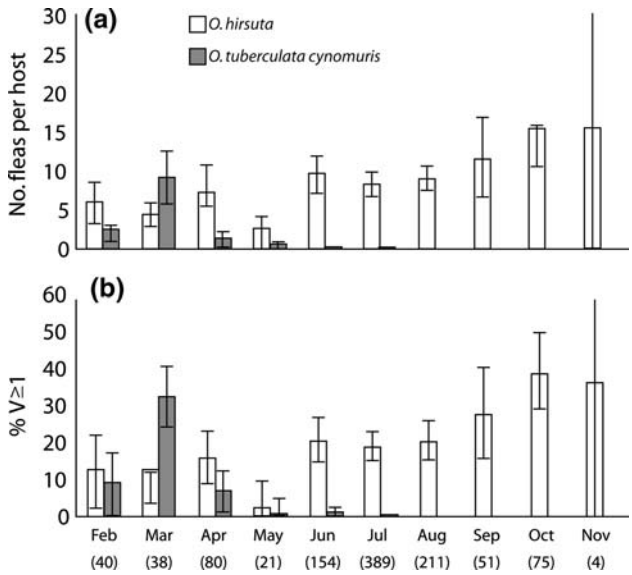

Figure 2. (**a**) Monthly flea loads of *Oropsylla tuberculata cynomuris* (gray) and *Oropsylla hirsuta* (white) on black-tailed prairie dogs captured from February 2004 to May 2007. Flea load was calculated by total number of fleas combed/total number of captured prairie dogs. The number of prairie dogs captured each month is shown in parentheses. Error bars are 95% confidence intervals. (**b**) Percent of hosts in simulated host populations with vectorial capacity which is sufficient to transmit infection to another host ($V \geq 1$); 95% confidence intervals (error bars) were estimated by bootstrap.

lata cynomuris in July, with one or two fleas per infested prairie dog. The other flea, *O. hirsuta*, was found on animals in all months trapped, with greatest abundance in October (15.4, 10.5–20.4). The monthly distributions of both flea species on hosts fit the negative binomial (many hosts harboring few or no fleas, and few hosts harboring many). The negative binomial parameter k ranged from 0.10 to 0.41 for *O. tuberculata cynomuris* and from 0.41 to 1.0 for *O. hirsuta*. Figure 2b shows the percent of host individuals expected to have $V \geq 1$ within a large, free-mixing population—that is the percentage harboring enough fleas to transmit *Y. pestis*. For *O. tuberculata cynomuris*, the greatest potential for transmission is expected in March (percent of $V \geq 1 = 33.6$, 95% CI 24.6–41.3), whereas for *O. hirsuta*, the potential for transmission steadily increases through late summer and peaks in October (39.8, 29.3–50.7).

Discussion

Both *O. tuberculata cynomuris* and *O. hirsuta* are competent vectors and transmission efficiency is higher at 24 h p.i. than at much later time points (Eskey and Haas, 1940; present study), suggesting that flea-borne transmission between hosts is more likely to occur shortly after the flea's ingestion of an infectious bloodmeal than after a long extrinsic incubation period. Interestingly, the pattern of efficient transmission 24 h p.i. followed by rapid decrease in efficiency in *O. hirsuta* and *O. tuberculata cynomuris* has not been seen in similar early-phase transmission studies of two other species (*Oropsylla montana* and *Xenopsylla cheopis*) (Eisen et al., 2006, 2007b). However, in those studies, fleas were not provided with uninfected bloodmeals on days between acquiring and transmitting infection, as were necessary in the present study. With *O. hirsuta* and *O. tuberculata cynomuris*, daily feedings were necessary because mortality in the lab was excessive when fleas were starved for more than 1 day. Thus, we do not know, based on these data, if the difference lies in the species of fleas or in the study designs. Further studies are needed to determine if prairie dog fleas may transmit with the efficiency seen at 24 h p.i. when starved until a later time. This information would help determine the infectious periods of the fleas, a factor shown to be important in epizootic spread among prairie dogs (Webb et al., 2006).

As with other early-phase transmission studies, daily bacterial loads were similar throughout the study, and median and maximum bacterial loads were not associated with transmission efficiency (Eisen et al., 2007b; Wilder et al., in press). Although a threshold bacterial load for transmission by fleas almost certainly exists (Engelthaler et al., 2000), bacterial loads were sufficiently high for 4 days after highly bacteremic bloodmeals to enable transmission by both *O. tuberculata cynomuris* and *O. hirsuta*. The mechanism responsible for the decrease in transmission efficiency despite high bacterial loads is unknown, but may be related to the distribution of bacteria within the flea or early biofilm formation (Eisen et al., 2007a; Wilder et al., in press). We do not feel that mechanical transmission via externally infected mouthparts is likely even at 24 h p.i., as *Y. pestis* was reported to be inviable after about 3 h on flea mouthparts (Bibikova, 1977). It is also unlikely that transmission is the result of a cumulative infectious dose delivered by several fleas to a single host because there was no relationship between experimental flea pool size and the likelihood of transmission for *O. hirsuta* (Wilder et al., in press) or *O. tuberculata cynomuris* (this study). Nevertheless, transmission studies using individual fleas would confirm this idea and also better pinpoint individual flea transmission rates for both species.

Slowing or stopping plague transmission in black-tailed prairie dogs would aid in conservation efforts and lower human health risks (Biggins and Kosoy, 2001; Antolin et al., 2002; Seery et al., 2003). Insecticidal dusting of burrows has been shown to reduce the risk of, and even prevent, epizootics (Seery et al., 2003; Hoogland et al., 2004). However, the timing of insecticidal application is important in its effectiveness (Fitzgerald, 1970). Our model incorporating flea load data from the field and flea transmission data from the lab predicts an increased potential for flea-borne transmission of *Y. pestis* among prairie dogs in March, when *O. tuberculata cynomuris* is present, in addition to the late-summer and fall months when *O. hirsuta* abundance increases and peaks in October. This pattern of flea-borne transmission potential is consistent with our observations on the seasonal timing of 22 outbreaks observed on the PNG in northern Colorado during 2003 to 2007. During these years, plague activity occurring in the early (February through July; 9 towns) and later months of the year (August through December; 13 towns) were roughly equal. Although we cannot be certain when outbreaks first begin (only when they reach detectable levels), the presence of the highly efficient vector *O. tuberculata cynomuris* in late winter and early spring provides an explanation for epizootics that occur when *O. hirsuta* is scarce.

Insecticidal application is likely to be most effective when timed to reduce both peaks in potential for flea-borne transmission: late February and August–September. Deltadust (Aventis Environmental Health, Montvale, NJ) containing deltamethrin, a synthetic pyrethroid, was shown to reduce flea populations for at least 84 days following a single application (Seery et al., 2003), spanning the window of the adult *O. tuberculata cynomuris* season and much of the fall peak of *O. hirsuta*. As with all chemical insecticides, development of resistance is a concern. Studies show resistance to deltamethrin in a number of vector species, including fleas (Bossard et al., 1998; Jirakanjanakit et al., 2007; Matambo et al., 2007; Romero et al., 2007; Tan et al., 2007), therefore colonies should be chosen for dusting prudently based on potential risk of plague exposure to humans and conservation importance.

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