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On-host flea phenology and flea-borne pathogen surveillance among mammalian wildlife of the pineywoods of East Texas

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ABSTRACT: Flea-borne diseases are endemic in Texas, U.S.A., with an increasing incidence of flea-borne typhus and cat scratch disease. Knowledge of flea natural history could provide information to protect public health, yet many knowledge gaps remain outside of plague-endemic regions. Our objective was to characterize seasonal activity patterns of fleas on common mammalian wildlife species and test fleas and wildlife for *Rickettsia* and *Bartonella* pathogens. We performed one year of monthly trapping for rodents and medium-sized mammals in a national forest with high recreational use and urban encroachment in East Texas. From 90 mammal captures representing seven species, 101 fleas were collected representing *Polygenis* spp., *Ctenocephalides felis*, and *Orchopeas* species. Virginia opossums (*Didelphis virginianus*) hosted 99% of the collected fleas (100 fleas) and a single flea was on an eastern woodrat (*Neotoma floridana*). Flea infestation prevalence of opossums was 79% (23/29). Mean flea abundance was 4.39 fleas, with intensity peaking in spring. One cat flea removed from an opossum was positive for *Bartonella henselae*. Furthermore, we identified tissue or blood of four raccoons (*Procyon lotor*) and one golden mouse (*Ochrotomys nuttalli*) positive for *Rickettsia amblyommatis*. These findings provide an ecological basis for the maintenance of vectors and pathogens from sylvatic settings. ***Journal of Vector Ecology* 49 (2): R39-49. 2024.**

Keyword Index: Flea, *Ctenocephalides felis, Rickettsia amblyommatis, Bartonella henselae,* opossum, *Didelphis virginianus,* phenology.

INTRODUCTION

Fleas (Siphonaptera) are arthropod vectors that transmit pathogens associated with significant morbidity and mortality in human and other animal populations (Salje et al. 2021). For example in the U.S. there has been a reemergence of flea-borne rickettsioses, including flea-borne spotted fever, caused by *Rickettsia felis* (Rickettsiales: Rickettsiaceae), and flea-borne typhus (also known as murine typhus or endemic typhus), caused by *Rickettsia typhi* in California since 2014 (Maina et al. 2016), in Hawaii since 2002 (Eremeeva et al. 2008), and in Texas since 2003 (Murray et al. 2017, Anstead 2020, Blanton 2023). Fleas are ubiquitous in diverse landscapes ranging from natural habitats to peri-domestic and domestic habitats with companion animal hosts. However, the majority of flea and flea-borne disease research concentrates on urban outbreaks. For example, urban flea-borne typhus outbreaks have been studied in Texas and California (Yomogida et al. 2024) and studies in sylvatic environments tend to focus on plague (e.g., vulnerable rodent population dynamics and *Yersinia pestis* (Enterobacterales: Yersiniaceae) surveillance in wildlife) (Hammond et al. 2019, Poje et al. 2020). Accordingly, flea and pathogen natural history in non-urban settings remains understudied.

Approximately 2,000 species and subspecies of flea exist, but only a subset are considered important vectors of pathogens (Eisen et al. 2009). These flea taxa can transmit pathogens through blood-feeding or via contact between contaminated flea feces and mucous membranes of a host (*Bartonella henselae, R. typhi, R. felis*). The cat flea, *Ctenocephalides felis* Bouché (Siphonaptera: Pulicidae), is a cosmopolitan species known to transmit *R. felis* and *R. typhi* (Blanton and Walker 2017). Traditionally, *Ct. felis* is considered both the reservoir and vector for *R. felis* given that vertical transmission from adult flea to progeny and flea co-feeding transmission has been documented; no wildlife reservoir has been incriminated (Wedincamp and Foil 2002, Brown et al. 2015). However, recent research has shown the capability of domestic dogs to serve as efficient reservoirs for *R. felis* (Ng-Nguyen et al. 2020). Urban and suburban flea hosts such as dogs, cats, and opossums have been documented to be seropositive for *Rickettsia* species (Boostrom et al. 2002, Case et al. 2006, Adjemian et al. 2010) and likely serve as pathogen reservoirs. *Ctenocephalides felis* can also transmit the causative agent of cat scratch disease, *Bartonella henselae* (Hyphomicrobiales: Bartonellaceae), for which felids serve as reservoirs (Schwartzman 2015). Other flea species have been documented to be positive for *Bartonella* sp. (Reeves et al. 2005, Millán et al. 2023), but their role in transmission is unclear given the route through infected flea feces (Schwartzman 2015).

Fleas are obligatory hematophagous insects dependent on hosts' blood during the adult life stage and are found on a diversity of vertebrates. Cats and dogs are known to

be common flea hosts in domestic spaces; however, most co-evolutionary flea-host associations were not with these hosts. Approximately 74% of flea species co-evolved with rodents, 8% with Soricomorpha (e.g., shrews), 5% each from Metatheria (e.g., opossums) and Chiroptera (e.g., bats), and a minimal 3% with Carnivora (e.g., cats and dogs) and Lagomorpha (e.g., rabbits) (Whiting et al. 2008). Historically, rats and cats have been associated with fleas in urban cycles of maintaining flea-borne pathogens, and opossums are gaining recognition for their importance in maintaining fleas and pathogens (Boostrom et al. 2002, Blanton et al. 2016, Anstead 2020). Due to increased urbanization, the key host and flea species responsible for circulating flea-borne rickettsioses has expanded dramatically (Azad et al. 1997, Friggens and Beier 2010). Sylvatic habitats with intermediate anthropogenic disturbance have been shown to have higher abundance of fleas on hosts (Friggens and Beier 2010).

Patterns of seasonal activity (i.e., phenology) and host associations for vectors are vital metrics to have defined prior to the deployment of vector management strategies aimed at reducing the transmission of flea-borne pathogens or reducing human or animal risk of exposure. Abiotic conditions are important for flea life cycles (Krasnov et al. 2001, Poje et al. 2020). However, given fleas often spend their life either on-host or in host burrows, any studies of flea activity inherently involve targeting hosts or host environments to capture fleas.

Texas, U.S.A. leads the nation in annual reports of human flea-borne typhus and cat-scratch disease cases (Schwartzman 2015, Anstead 2020). Flea-borne typhus cases have historically occurred in southern Texas in the Lower Rio Grande Valley and the Coastal Bend area, but in the past decade have increased in the larger metropolitan areas around Houston, San Antonio, and Austin (Reeves et al. 2008, Ruiz et al. 2020). While the Texas Department of State Health Services distinguishes flea-borne typhus as a reportable disease, other flea-borne rickettsioses and cat scratch disease are not reportable in the state, although cases occur in Texas (Boostrom et al. 2002, McCormick et al. 2023). The objective of this study was to characterize seasonal activity patterns of fleas on common mammalian wildlife species and test fleas and wildlife for pathogens in the genera *Rickettsia* and *Bartonella* in a transitional area where urban sprawl from Houston, TX, U.S.A. intersects pine-dominated forests.

MATERIALS AND METHODS

Field site description

Fieldwork was performed at the Sam Houston National Forest (SHNF) in central East Texas (Figure 1). SHNF, located 76 km north of Houston, TX receives high recreational use

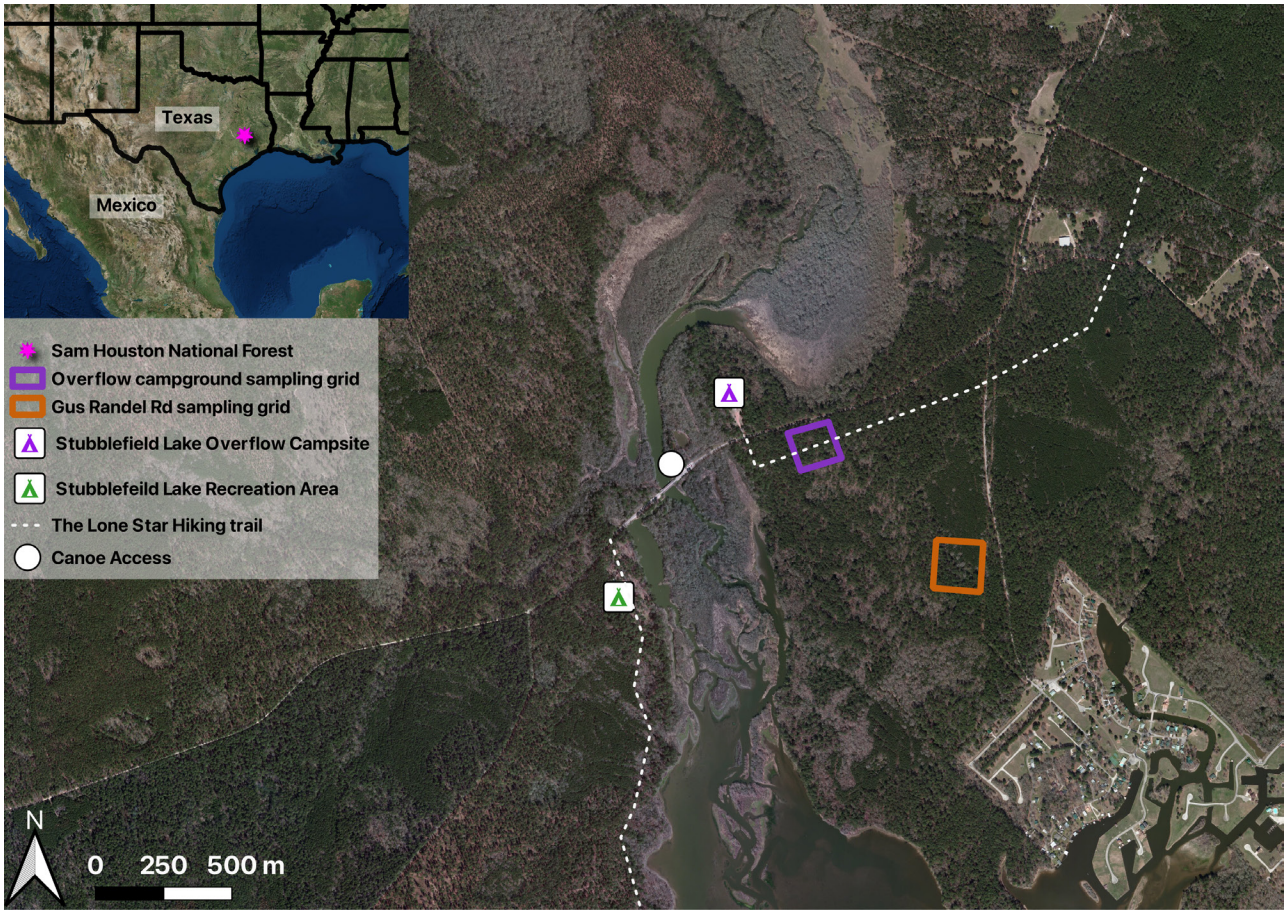

Figure 1. A map indicating the location of the sampling grids from May, 2020 to May, 2021 within a region of high human activity of Sam Houston National Forest, TX, U.S.A. The two sampling grids are indicated by the orange and purple squares. One inset map shows the context of the study sites in East Texas, U.S.A. Map was created in QGIS with Esri World Imagery (clarity) for the base map.

given the close proximity to Harris County, the third most populated county in the U.S.A., encompassing embedded residential private housing, multiple campgrounds, and Texas' largest trail system, the Lone Star Hiking Trail. SHNF is in the Piney Woods ecoregion which has uplands dominated by loblolly pines (*Pinus taeda*) and shortleaf pines (*Pinus echinata*) with lowlands mixed with deciduous hardwoods such as oak (*Quercus* sp.), elm (*Ulmus* sp.), and sweetgum (*Liquidambar* sp.). The understory vegetation primarily consists of dwarf palmetto (*Sabal minor*), yaupon holly (*Ilex vomitoria*), and American beautyberry (*Callicarpa americana*). The forest is managed by harvest for commercial logging and prescribed burning to reduce accumulation of fuel and enhance wildlife habitat.

Sample collection

Two sampling grids were established near the Stubblefield Campground in an unburned area of SHNF (Figure 1). The grids were 1 km apart and each consisted of six-by-six lines, each of 100 m, totaling 10,000 m^2 per grid. Each transect was spaced every 20 m apart and every intersection was demarcated with labeled plastic flags to standardize all trapping events each month of the year, starting in May, 2020 and ending May, 2021. Branches along the transect were removed to facilitate walking the transect for setting and collecting traps. One sampling grid was set up near the Overflow Campground off Stubblefield Lake Road and is intersected by the Lone Star Hiking Trail, while the second grid was off a power line right-of-way perpendicular to Gus Randel Road (Figure 1).

Live trapping and sampling of mammals was approved by the Texas A&M University Institutional Animal Care and Use Committee (IACUC 2021-0124 D C). A total of 14 large Tomahawk traps (122.7 × 32.3 × 32.3 cm; Tomahawk™ Trap, Hazelhurst, WI, USA), ten small Tomahawk traps (40.6 x 12.7 x 12.7 cm), 15 extra-large Sherman traps (10.2 x 11.4 x 38.1 cm; H.B. Sherman™ Traps, Tallahassee, FL, U.S.A.), and 49 Sherman traps (7.6 x 8.9 x 22.9 cm) were set at trapping stations on both of the grids for a single night of trapping on each grid. Each trapping station had a minimum of two small Sherman™ traps facing the opposite direction of each other, while all Tomahawk™ traps and large Sherman™ traps were placed around fallen logs or game trails near a trapping station. Sherman™ traps were baited with sunflower bird seed and Tomahawk™ traps were baited with wet cat food about 1 h before sunset. The following morning all traps were collected. Overall capture success was calculated as the number of animals captured divided by the capture effort (the number of traps set x by the number of nights trapped). Deviations from the standardized trapping regime are as follows: only Tomahawk™ traps were set, not Sherman traps, for May, 2020. In April and May of 2021, Sherman™ traps were set for a total of three days in a row, rather than just the one day, at one of the sampling sites to capture the potential spring boom of rodent populations.

All rodents were anesthetized via gaseous inhalation of Isofluorane (Sigma-Aldrich, St Louis, MO, USA) to effect. *Sciurus carolinensis* Gmelin were initially dosed with ketamine (0.06 mL) followed by inhalation of Isofluorane to effect. Meso-mammals were anesthetized with ketamine (10 to 20 mg/kg) and xylazine (2 to 4 mg/kg), and after processing were reversed with yohimbine (0.11 mg/kg) (Kreeger and Arnemo 2012). All animals were released upon recovery from anesthesia at the site of capture. Species weight, sex, age, and reproductive status were recorded for all animals. Ectoparasites were removed with flea combs or fine-tipped forceps and stored in 70% ethanol. To facilitate collection of fast-moving fleas on hosts, we sprayed 70% ethanol on the fur at the site where a flea was seen. Other ectoparasites were collected from hosts (ticks) and will be reported separately. A 2 mm ear punch biopsy was preserved in 70% ethanol, a blood sample was collected, and a metal numbered ear tag was applied. Blood samples were stored on ice no more than 48 h, until taken to the laboratory to be centrifuged. Individual serum and blood clots were stored separately at -20° C.

Flea morphological identification

Fleas were morphologically identified using two taxonomic keys (Fritz and Pratt 1947, Furman and Catts 1982), with final flea identification confirmed with molecular barcoding (see below).

DNA extractions of blood clot, ear punch biopsies, and fleas

For all collected ear punch biopsies, blood clots, and fleas, DNA was extracted (E.Z.N.A Tissue DNA Kit; Omega Bio-Tek, Norcross, GA, U.S.A.). Approximately 250 µL of blood clot for each meso-mammal was extracted, and for rodent blood clot samples, sterile PBS was added to bring the final volume up to 250 µL. Incubation time during the lysis step of DNA extraction was for ten min for blood clots and overnight for ear biopsies. Each individual flea was sliced repeatedly using a sterile number 11 scalpel blade prior to lysis incubation of at least 24 h but no more than 48 h. All tissue types had a two-step final elution bringing the final volume to 50 µL. A reagent-only extraction negative control was used in each extraction batch. After a single leg was removed for molecular identification, four individual flea specimens were submitted to the Texas A&M University Department of Entomology insect collection as voucher specimens (TAMUIC #765).

Flea and rodent molecular identification

All fleas were subjected to molecular identification based on amplification and sequencing of the cytochrome oxidase 1 (*CO1*) gene. First, we used a modified reaction based on previously described primers and methods (Ashwini et al. 2017, Folmer et al. 1994) (Table 1). The total volume for each reaction was 25 µL with 1 uL of DNA template, FailSafe™ 2X PCR Premix E, FailSafe™ enzyme (Lucigen, Middleton, WI, U.S.A.). Samples that did not amplify with this protocol were subsequently performed again with 3 µL or 5 µL of DNA template. Samples that did not amplify using the initial *CO1* protocol were subjected to a different PCR targeting the *CO1* gene with a different locus for the reverse primer (Cff-R loci) (Lawrence et al. 2014) (Table 1).

All mice and rats were molecularly identified to verify

Table 1. All primers used to confirm rodent species, identify flea species, and identify detect flea-borne pathogens (*Rickettsia* species and *Bartonella* species) in fleas and hosts from Sam Houston National Forest, May, 2020 - May, 2021.

Target gene	Primers	Nucleotide sequence (5'-3')	Amplicon size	Reference
Rodent species confirmation				
cytB	M1 M2	CCCCTCAGAATGATATTTGTCA CCATCCAACATCTCAGCATGATGAAA	383 bp	Meece et al. 2005
Flea species identification				
CO1	LCO1 HCO1	GGTCAACAAATCATAAAGATATTGG TAAACTTCAGGGTGACCAAAAAATCA	601 bp	Folmer et al. 1994
CO1	LCO1 Cff-R [S0368]	GGTCAACAAATCATAAAGATATTGG GAAGGGTCAAAGAATGATGT	727 bp	Lawrence et al. 2014
Flea-borne pathogen detection				
RrCS	RrCS.372 RrCS.989	TTTGTAGCTCTTCTCATCCTATGGC CCCAAGTTC CTTTAATACTTCTTTGC	617 bp	Kollars and Kengluecha 2001
OmpB	120-M59 120-807	CCGCAGGGTTGGTAACTGC CCTTTTAGATTACCGCCTAA	862 bp	Roux and Raoult 2000
OmpA	Rr190-70 Rr190-701 Rr190-602	ATG GCG AATATT TCTCCAAAA GTTCCGTTAATGGCAGCATCT AGTGCAGCATTCGCTCCCCCT	550 bp	Wikswo et al. 2008
Pap31	PAPn1 PAPn2	TTCTAGGAGTTGAAACCGAT GAAACACCACCAGCAACATA	269 bp	Zeaiter et al. 2002

field identifications through amplification of the cytochrome B (*cytB*) gene using M1 and M2 primers (Meece et al. 2005) (Table 1), as previously described (Busselman et al. 2021).

Detection of flea-borne pathogens

To test for the presence of *Rickettsia* species, we targeted three different genes: outer membrane protein A (*ompA*), outer membrane protein B (*ompB*), and *Rickettsia rickettsii* citrate synthase (*RrCS*) by PCR. The *ompA* PCR was performed using a semi-nested touchdown PCR as previously described (Salomon et al. 2022). The *ompB* PCR was performed using previously published primers but we adjusted thermocycling conditions and protocol (Roux et al. 1996, Roux and Raoult 2000, Raoult et al. 2001). Each reaction consisted of the total volume being 20 µL with 5.52 µL of water, 10 µL of FailSafe™ 2X PCR Premix E, 1.33 uL of 10uM *ompB*-120-M59 forward primer (Table 1), 1 µL of 10 µM *ompB* 120-807 reverse primer, 0.5 µL of FailSafe™ enzyme (Lucigen, Middleton, WI, U.S.A.), and 1.5 µL of DNA template. Cycling conditions were an initial denaturation step for 5 min at 95° C. Then 40 cycles of: a denaturation step running for 30 s at 95° C, followed by the annealing step starting at 50° C for 30 s, then followed by the extension step at 72° C for 1 min and 30 s. Following the 40 cycles an additional extension cycle was run at 72° C for 7 min. The *RrCS* PCR was performed using previously published primers and cycling conditions (Kollars and Kengluecha 2001, Williamson et al. 2010). Each reaction consisted of the total volume being 15 µL with the use of FailSafe™ 2X PCR Premix E, FailSafe™ enzyme (Lucigen, Middleton, WI, U.S.A.), and 1.5 µL of DNA template. A modification was made to the cycling conditions of an initial denaturation step for 2 min at 95° C.

To test for *B. henselae* we targeted the *Pap*31 gene (Zeaiter et al. 2002) (Table 1). Changes to the previously published protocol included only using 2.5 µL of DNA template rather than 10 µL in addition to changing the annealing temperature from 57° - 58° C, FailSafe™ reagents were again used. Each PCR included either a field-collected positive control of *R. parkeri* or *B. henselae* extracted from a tick and a flea, respectively (Cohen et al. 2015, Castellanos et al. 2016) and a negative control of PCR grade water.

DNA sequencing

All PCR products were visualized via gel electrophoresis and resulting amplicons were purified with ExoSAP-IT (Affymetrix, Santa Clara, CA, U.S.A.). Sanger sequencing was performed (Eton Bioscience Inc, San Diego, CA, U.S.A.). In Geneious (v 9.1.8), the sequences were trimmed and checked for quality; sequences were then compared to published sequences in NCBI GenBank. Criteria for concluding flea

molecular identification of the *CO1* PCR assay were that the sequences had a minimum of 97% identity to a previously submitted sequence in NCBI GenBank to make a species level match and a minimum of 91% identity for a genus level match, in addition to a morphological identification. The criterion for determining a sequence match for host species and pathogen species was at least 99% identity to a previously published sequence in NCBI GenBank.

Statistical analyses

Mean flea abundance, or the number of fleas removed per animal (Bush et al. 1997), was calculated across host species and for each month along with the corresponding standard error of the means. To test for significant associations between flea abundances and the seasons of the year (i.e., three-month bins of winter, spring, summer, and fall), we performed a generalized linear mixed effect model with sampling grid as the random effect and a negative binomial error distribution using R Version 1.4.1717, utilizing the '*glmer.nb*' function in the package '*lme4*' (Bates et al. 2015). Following the same model criteria, we tested if host sex or host age is associated with flea abundance. A p-value less than 0.05 has been considered a significant finding.

RESULTS

Host inventory

We conducted a total of 7,946 trapping nights (number of traps set per night of sampling) resulting in 97 rodents and meso-mammal captures, representing 61 unique individuals, with an overall trapping success of 1.18%. We captured seven different mammalian species, including 32 *Ochrotomys nuttalli* Harlan (golden mouse), 29 *Didelphis virginiana* Kerr (Virginia opossum), 17 *Peromyscus leucopus* Rafinesque (white-footed mouse), ten *Procyon lotor* Linnaeus (raccoon), five *Neotoma floridana* Ord (eastern woodrat), two *Glaucomys volans* Linnaeus (southern flying squirrel), and one *Sciurus carolinensis* (eastern gray squirrel). There were recaptures of the same animals over the year; 24 of the 49 mouse captures (*O. nuttalli* and *P. leucopus*) were unique individuals; 20 of the 29 *D. virginiana* were unique individuals; and three of the five *N. floridana* were unique individuals. Within each species, the largest number of independent monthly captures per individual was two for *N. floridana,* four for *P. leucopus,* four for *O. nuttalli*, and five for *D. virginiana*. Molecular identification of the rodents based on *cytB* amplification and sequencing confirmed the morphological identifications; representative sequences were deposited to NCBI GenBank (Accession numbers PP119073-PP119084).

Table 2. Molecular flea identification summary of fleas removed from wildlife hosts in Sam Houston National Forest, May, 2020 to May, 2021.

Flea identification	Abundance
Polygenis spp.	76
Ctenocephalides felis	8
Orchopeas sp.	1
Orchopeas caedens	2
Flea species (87% similar match to three genera: *Coptopsylla/ Hystrichopsylla/Ischnopsyllus*)	1
unknown	12
Total	100

Flea inventory

A total of 101 fleas were removed from 22 of the 97 hosts captured. Molecular identification of 100 fleas (one lost during extraction) resulted in: 76 *Polygenis* spp. (Siphonaptera: Rhopalopsylidae) (91-94% identity match to *Polygenis* sp. OU706244.1), eight *Ct. felis* (>99% identity matches), one *Orchopeas* Jordan & Rothschild. (Siphonaptera: Ceratophyllidae) (96% identity match to *Orchopeas agilis* HM398853.1), two *Orchopeas caedens* (97-99% identity matches), and one specimen produced a high-quality sequence but had low similarity (97-100% query cover and 87-88% identity) to *Coptopsylla* Lewis sp. (Siphonaptera: Coptopsyllidae) (Accession number MF000658.1), *Hystrichopsylla* Poole and Gentili sp. (Siphonaptera: Hystrichopsyllidae) (Accession number MG138325.1), and *Ischnopsyllus* Rothschild sp. (Siphonaptera: Ischnopsyllidae) (Accession number OR597495.1) (Table 2). Twelve fleas were not identified given that the PCR either didn't work or the sequence was of poor quality (Table 2). Representatives of flea sequences were submitted to NCBI GenBank (Accession numbers PP147411-PP147489).

Flea abundance

Overall mean flea abundance (the average number of fleas of all the checked animals in the study) was 1.04 fleas per host. Most fleas were removed from *D. virginiana* with an infestation prevalence of 76% (22/29) and a single *N. floridana* hosted a single flea for an infestation prevalence of 20% (1/5); no fleas were detected on the remaining host species. Among the mammals with fleas removed (22 opossums and one rat), they had a mean flea intensity (the average fleas removed from hosts that had at least one flea) of 4.39 fleas per host (Bush et al. 1997). Mean flea abundance among all the opossums was 3.45 (100/29) and among rats was 0.2 (1/5). The highest number of fleas removed from a host over the year was 16 fleas in May, 2022, which we captured from this same opossum the previous month (April) and it had the second highest overall flea abundance of 12 fleas (Figure 2). Mean flea abundance on opossums ranged between 0 and 9.75 among months and peaked in April (5.5 fleas) and May 2022 (9.75 fleas). We found a significant association between flea abundance on *D. virginiana* and spring (Estimate = 1.3, p-value = 0.009, Figure 2). However, we found no significant association between opossum sex or age and flea abundance (Sex: estimate = -0.63, p-value = 0.16, Age: estimate = 0.2, p-value = 0.87).

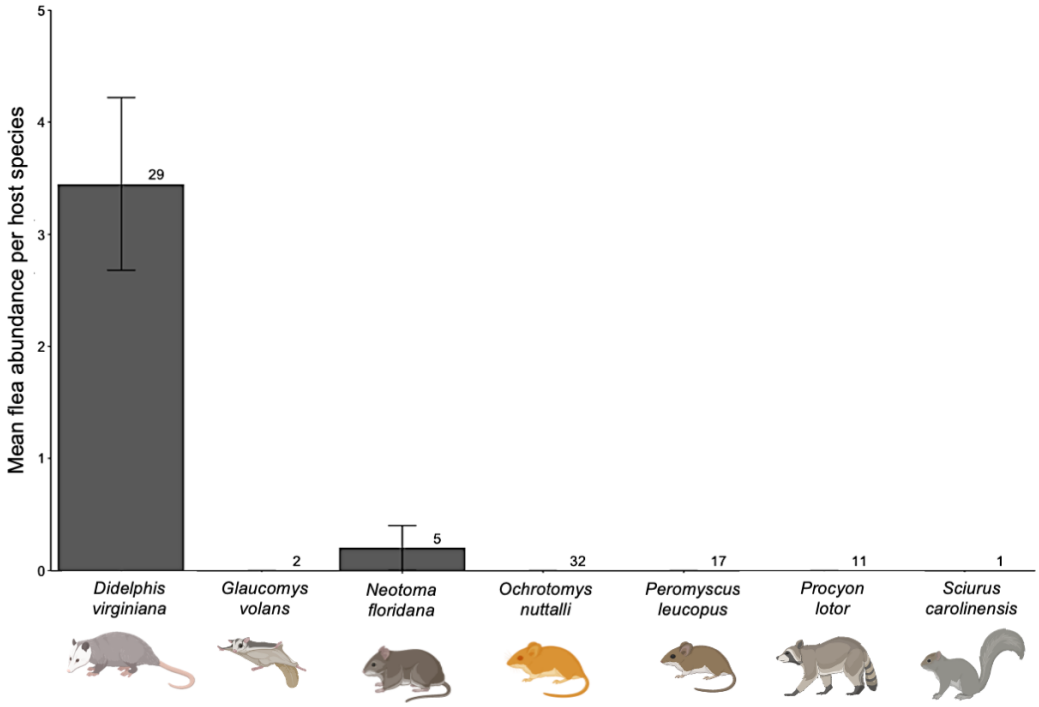

Figure 2. The mean number of fleas removed from all sampled hosts species across the year (May, 2020-May, 2021) in Sam Houston National Forest. Each host capture is treated individually, therefore any recaptures are considered a new independent flea abundance. The error bars represent the error of the mean. Black numbers above the bars indicate the number of each host species sampled across the year.

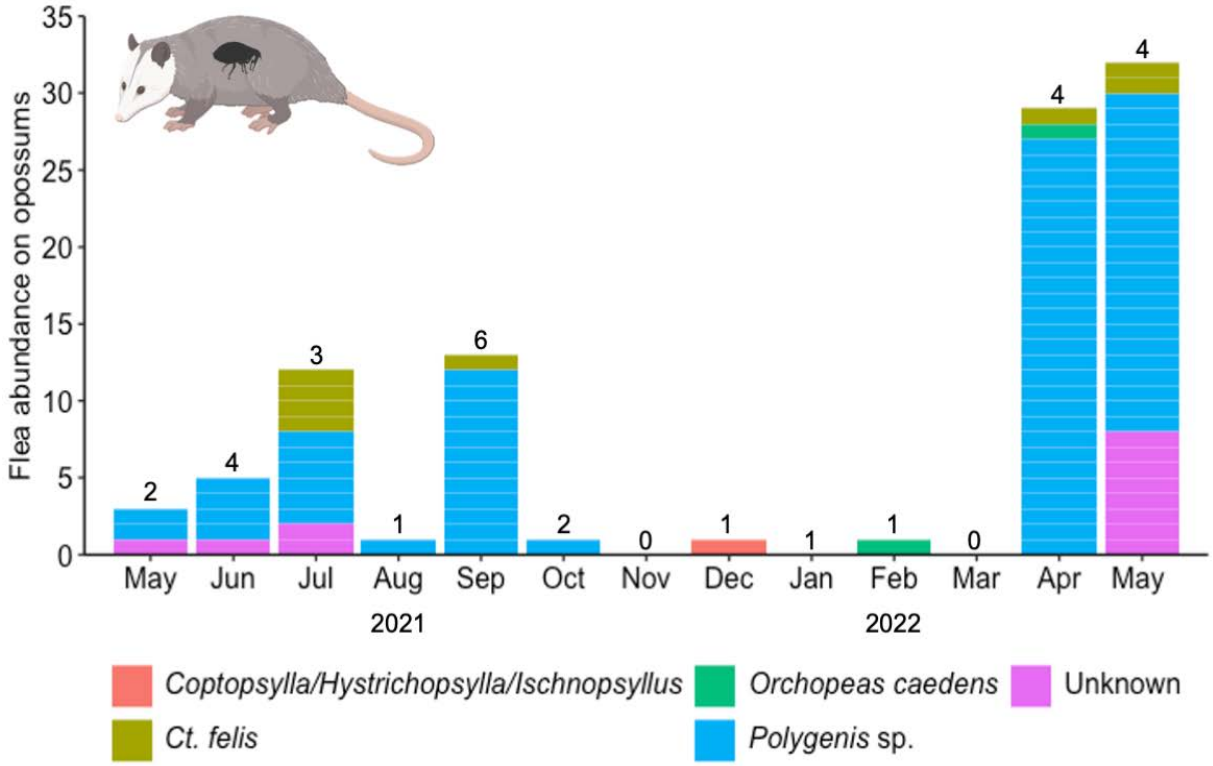

Figure 3. Abundance of fleas removed from opossums captured in San Houston National Forest each month across the year (May, 2020 – May, 2022). Each host capture is treated individually here, therefore any recaptures are considered a new independent flea abundance. Black numbers above the bars indicate the number of opossums sampled each month. The various colors represent the molecular results of the fleas.

Flea-borne infections

Ninety-six fleas were tested for flea-borne pathogens with four different gene targets focusing on *Rickettsia* and *Bartonella* species. We identified a single *Ct. felis* removed from an opossum as positive for *B. henselae* via the *Pap* 31 gene (1.04% infection prevalence of fleas overall (1/96) and 11.1% infection prevalence *of Ct. felis* (1/9); GenBank accession no. PP119086). The sequence of 189 bp was 100% identical to a published complete genome of *Bartonella henselae* isolated from a cat in Freiburg, Germany (Accession ID: CP072898.1). This opossum was captured first in July with three *Ct. felis*, and again in September with no fleas. The *B. henselae* positive *Ct. felis* was from July and all blood and ear tissue samples from this opossum were negative.

Of 87 blood clot and 90 ear biopsies sampled, none were positive for flea-borne pathogens. However, we did identify four *P. lotor* and one *O. nuttalli* to be positive for *R. amblyommatis* (a tick-associated *Rickettsia* species) via the *ompA* and *RrCS* PCR assays. Three of the five positives were amplified via the *ompA* PCR and two were amplified from the *RrCS* PCR. One of the *P. lotor* was positive via blood clot, while the other three *P. lotor* and the *O. nuttalli* were positive via ear biopsy. All sequences had an identity match over 99% and a query cover of 100% to previously submitted *R. amblyommatis* sequences in GenBank (Accession numbers PP119085, 87-89). All negative controls (extraction and PCR) were negative across all PCR assays.

DISCUSSION

During a year-long survey of mammalian wildlife in an East Texas national forest in close proximity to a large metropolitan area, we found opossums to be commonly infested with fleas (Figure 2), including a flea infected with the agent of cat scratch fever disease, *Bartonella henselae*. Opossums are recognized to serve as hosts for fleas implicated in flea-borne disease in urban environments (Boostrom et al. 2002, Reeves et al. 2005, Anstead 2020); here we show a similar role for opossums in a sylvatic forested habitat. Other than opossums, only one species, an eastern woodrat, was found to harbor a flea. We failed to detect fleas on all other surveyed mammal species across the year, including white-footed mice, golden mice, squirrels, and raccoons. Previous work documented nine taxa of fleas on rodents across five southwestern states including Texas (Haas et al. 2004), but comparative metrics of rodent infestation prevalence with fleas in Texas are unknown.

Although fleas were found nearly every month, peak abundance on opossums was in the spring (Figure 3,). Spring may provide an optimal temperature to humidity ratio that regulates flea development (Kreppel et al. 2016). Furthermore, opossums breed in the spring when males travel around to female dens, potentially distributing fleas from den to den (Ryser 1995, Woods II and Hellgren 2003) or during copulation. Future studies could monitor both on-opossum fleas and fleas recovered from den environments in relation to climatic factors.

Bartonella henselae is the causative bacterium of cat scratch disease, the most common infection among other *Bartonella* species in the U.S. (McCormick et al. 2023). Transmission to humans occurs primarily through contact of mucosa membranes and *B. henselae* contaminated surfaces initially from *Ct. felis* feces (i.e., a flea feces contaminated cat scratch or bite). In addition to *Ct. felis,* other vectors have been identified, including *Rhipicephalus sanguinis* and *Ixodes ricinis* (Cotté et al. 2008, Wechtaisong et al. 2020). Throughout the USA, Texas has the highest human cases of *B. henselae* (McCormick et al. 2023), with seropositive animals also documented including cats (Jameson et al. 1995), dogs (Lashnits et al. 2022), and mountain lions (Rotstein et al. 2000). We identified a single *Ct. felis* positive for *B. henselae* removed from an opossum in July. This opossum had two other concurrently infesting *Ct. felis* removed that were negative. Additionally, this opossum was recaptured in September, but had no fleas and did not test positive for *B. henselae* via blood clot or 2 mm ear biopsy either month of capture. Although house cats are the primary reservoir for *B. henselae*, this study and others have identified *Ct. felis* removed from opossums in the U.S. to be positive for *B. henselae* (Reeves et al. 2005), suggesting more research is necessary to monitor opossums as a potential reservoir for *B. henselae* in sylvatic and peri-domestic spaces.

The only *Rickettsia* species detected in our study was *R. amblyommatis*, found in the blood of one raccoon and the ear tissue of three other raccoons and a golden mouse. These host infections occurred in August, September, and May, and none of the hosts had fleas, but all of the hosts had ticks attached at the time of infection. The golden mouse was a previous capture from April but was negative at that time and again had no fleas (or ticks). *Rickettsia amblyommatis* is not officially recognized as a human pathogen, however evidence suggests it may be a pathogen (Richardson et al. 2023). It has been documented in numerous tick species around the world with *Amblyomma americanum* Linnaeus (Ixodida: Ixodidae) thought to be the vector (Sayler et al. 2014). Before this study, we are unaware of documentation of naturally occurring infections in wild animals (Richardson et al. 2023). However, there is some *R. amblyommatis* seroprevalence data from wildlife in Brazil (Serpa et al. 2021). Our findings of *R. amblyommatis* in both hosts' blood clot and ear punch biopsies, of two different mammalian species can be used to guide further investigations of wildlife reservoirs for this agent.

In contrast to a recent study of opossums and their fleas in the urban city of Galveston, TX (Blanton et al. 2016), we did not detect *R. typhi* or *R. felis* in opossum-associated fleas. Among a sample size of 12 opossums, Blanton et al. (2016) found all opossums had at least one *Ct. felis,* amounting to a total of 250 *Ctenocephalides felis*. Conversely, we found eight *Ct. felis* from 29 opossums captured in a non-urban habitat. Similar to our findings, they did not detect *Rickettsia* species within the opossums' blood clots via PCR, although they were able to identify 66.6% (8/12) of opossums had serological evidence of *R. typhi*, *R. felis,* and *Rickettsia rickettsii*. Moreover, they found molecular evidence of *R. typhi, R. felis,* and *Candidatus* Rickettsia senegalensis, a closely related

species to *R. felis*, within the fleas removed from all but one (91.6%,11/12) of the opossums. Likewise, Eremeeva et al. (2020) in Georgia found *Rickettsia asembonensis*, another close relative to *R. felis,* in 11 out of 19 *Ct. felis* from cats, dogs, opossums, or humans (Eremeeva et al. 2020). This study also indicates opossums are an important host since they inhabit both urban and rural areas while hosting both *R. felis* positive *Ct. felis* and *Polygenis gwyni* in large abundances (Eremeeva et al. 2020). Accordingly, natural maintenance of flea-borne *Rickettsia* species varies regionally, underscoring the importance of these disease ecology studies to be conducted across diverse landscapes.

In agreement with Friggens and Beiers (2010), we collected a diversity of flea species within an intermediately disturbed forest, likely comprising four different genera (Table 2). The majority of the fleas we collected had been molecularly identified as *Polygenis* spp. since they were all aligning to similar accessions of various *Polygenis* spp. but only at an identity of 91-94%. These specimens may be *Polygenis gwyni*, as *P. gwyni* does not have sequences submitted to GenBank and has been documented on *D. virginianus* (Pung et al. 1994), but the morphologic identification was not confirmed.

Ctenocephalides felis was the second most common flea encountered in our study. *Ctenocephalides felis* is a cosmopolitan species and a recognized vector of the flea-borne rickettsioses (*R. typhi* and *R. felis*), tape worm (i.e., *Dipylidium caninum* and *Hymenolepis diminuta*), and cat scratch disease (*B. henselae*). Studies show that in urban areas opossums can host high burdens of *Ct. felis*, with other species of fleas predominant in less urban areas, such as *Polygenis gwyni* (Blanton et al. 2016, Eremeeva et al. 2020). This pattern may be reflected in our data in which the majority of fleas on opossums were *Polygenis* sp. and *Ct. felis* was present at a lower abundance.

We detected a single *Orchopeas* sp.; this specimen was removed from an eastern woodrat in March, and its *CO1* sequence was most similar to *O. agilis*, although with low similarity. This genus of flea is known to infest many species of woodrats across the U.S. (Kosoy et al. 2017, McAllister et al. 2020, Hastriter 2023). For example, *O. agilis* has been removed from *N. albigula* in Arizona and both from *N. cinerea* and *N. lepida* in Colorado (Haas et al. 2004). Specifically in Texas, *O. neotomae* and *O. sexdentatus* have been removed from *Neotoma micropus* (Charles et al. 2012).

We obtained a single flea specimen removed from an opossum in December that had a *CO1* sequence with a near equally poor match to three different species of three different genera (*Coptopsylla lamelifer ardu, Hystrichopsylla multidentata,* and *Ischnopsyllus variabilis*) with a query cover of 97-100% and a low identity of 87-88%; none of these cryptic species have been documented in the U.S. All three species have been documented in Asia. *Coptopsylla lamelifer ardu* has been removed from *Rhombomys opimus* (great gerbil) and found to be PCR positive for *Bartonella elizabethae*, one of the many agents of human bartonellosis. It is, however, not a confirmed vector of any flea-borne pathogens (Yin et al. 2019). Furthermore, *C. lamelifer ardu* are found in winter seasons on wildlife in China (Shu et al. 2020), therefore additional wildlife sampling during the winter months in East Texas may be useful in recovering additional specimens of this cryptic species for morphologic and molecular description. The *H. multidentata* sequence that had a similar identity was documented to have been removed from a rodent in China but is not associated with a publication (accession number MG138325.1). As for *I. variabilis*, it is associated with bats, can be found in Russia, and is not known to be a vector for any pathogens (Orlova et al. 2022). Whether this Texas flea sequence represents an unknown species or one which is known to exist but not yet represented in a sequence database cannot be differentiated in this study, but expansion of sequences databases in the future to encompass the known diversity of fleas will be useful.

Given the emergence of flea-borne diseases, ecological studies to elucidate flea-wildlife associations, flea seasonality, and infection prevalence are required to understand the natural pathogen maintenance cycles that create risk of spillover to humans as well as other animals. Especially given changing land use and urbanization, the key role of the Virginia opossum we identified here may be expected to expand, as the opossum is a synanthrope that thrives in human-dominated landscapes (Bezerra-Santos et al. 2021, Mays Maestas et al. 2023). Moreover, research of flea control strategies involving either host-targeted interventions (Brianti et al. 2016, Eads et al. 2019) or host habitats (Miller et al. 1970) may wish to consider the variation in flea activity that we show here, so as to target hosts at key times of flea abundance. This study contributes to the knowledge of flea populations in the sylvatic habitat of East Texas to improve public health messaging and other flea-borne disease intervention activities.

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Author contributions

Conceptualization JS, SH, GH. Project funding SH, GH. Sample collection JS, CD, EL. Sample lab processing JS, HM, CD, EL, SB, LA. Data analysis JS. Manuscript First draft JS. Manuscript editing JS, EL, HM, CD, SB, GH, SH.

Data availability

These data have been published for public download with PHAROS (Pathogen Harmonized Observatory) of the Verena Institute. https://pharos.viralemergence.org/projects/?prj=prjVWw6jLhFXw3

REFERENCES CITED

Adjemian, J., S. Parks, K. McElroy, J. Campbell, M.E. Eremeeva, W.L. Nicholson, J. McQuiston, and J. Taylor. 2010. Murine typhus in Austin, Texas, USA, 2008. Emerg. Infect. Dis. 16: 412–417.

Anstead, G.M. 2020. History, rats, fleas, and opossums. II. The decline and resurgence of flea-borne typhus in the United States, 1945–2019. Trop. Med. Infect. Dis. 6: 2.

Ashwini, M., G. Puttalakshmamma, G. Mamatha, O. Rakshith, B. Chandranaik, P. Thimmareddy, E. Placid, and T. Venkatshan. 2017. Studies on morphology and molecular characterization of oriental cat flea infesting small ruminants by barcoding. J. Entomol. Zool. Stud. 5: 301–305.

Azad, A.F., S. Radulovic, J.A. Higgins, B.H. Noden, and J.M. Troyer. 1997. Flea-borne rickettsioses: Ecologic considerations. Emerg. Infect. Dis. 3: 319–327.

Bates, D., M. Mächler, B. Bolker, and S. Walker. 2015. Fitting linear mixed-effects models using lme4. J. Stat. Softw. 67: 1-48.

Bezerra-Santos, M.A., R.A.N. Ramos, A.K. Campos, F. Dantas-Torres, and D. Otranto. 2021. *Didelphis* spp. opossums and their parasites in the Americas: A One Health perspective. Parasitol. Res. 120: 4091–4111.

Blanton, L.S., B.M. Idowu, T.N. Tatsch, J.M. Henderson, D.H. Bouyer, and D.H. Walker. 2016. Opossums and cat fleas: New insights in the ecology of murine typhus in Galveston, Texas. Am. J. Trop. Med. Hyg. 95: 457–461.

Blanton, L.S. and D.H. Walker. 2017. Flea-borne rickettsioses and rickettsiae. Am. J. Trop. Med. Hyg. 96: 53–56.

Blanton, L.S. 2023. Murine typhus: A review of a reemerging flea-borne rickettsiosis with potential for neurologic manifestations and sequalae. Infect. Dis. Rep. 15: 700–716.

Boostrom, A., M.S. Beier, J.A. Macaluso, K.R. Macaluso, D. Sprenger, J. Hayes, S. Radulovic, and A.F. Azad. 2002. Geographic association of *Rickettsia felis* -infected opossums with human murine typhus, Texas. Emerg. Infect. Dis. 8: 549–554.

Brianti, E., E. Napoli, G. Gaglio, L. Falsone, S. Giannetto, F. Solari Basano, R. Nazzari, M.S. Latrofa, G. Annoscia, V.D. Tarallo, et al. 2016. Field evaluation of two different treatment approaches and their ability to control fleas and prevent canine leishmaniosis in a highly endemic area. PLoS Negl. Trop. Dis. 10: e0004987.

Brown, L.D., R.C. Christofferson, K.H. Banajee, F. Del Piero, L.D. Foil, and K.R. Macaluso. 2015. Cofeeding intra- and interspecific transmission of an emerging insect-borne rickettsial pathogen. Mol. Ecol. 24: 5475–5489.

Bush, A.O., K.D. Lafferty, J.M. Lotz, and A.W. Shostakll. 1997. Parasitology meets ecology on its own terms: Margolis et al. revisited. J. Parasitol. 83: 575–583.

Busselman, R.E., M.F. Olson, V. Martinez, E. Davila, C. Briggs, D.S. Eldridge, B. Higgins, B. Bass, T.L. Cropper, T.M. Casey, et al. 2021. Host bloodmeal identification in cave-dwelling *Ornithodoros turicata* dugès (Ixodida: Argasidae), Texas, USA. Front. Vet. Sci. 8: 1–8.

Case, J.B., B. Chomel, W. Nicholson, and J.E. Foley. 2006. Serological survey of vector-borne zoonotic pathogens in pet cats and cats from animal shelters and feral colonies. J. Feline Med. Surg. 8: 111–117.

Castellanos, A.A., M.C.I. Medeiros, G.L. Hamer, M.E. Morrow, M.D. Eubanks, P.D. Teel, S.A. Hamer, and J.E. Light. 2016. Decreased small mammal and on-host tick abundance in association with invasive red imported fire ants (*Solenopsis invicta*). Biol. Lett. 12 (9): 20160463.

Charles, R.A., S. Kjos, A.E. Ellis, J.P. Dubey, B.C. Shock, and M.J. Yabsley. 2012. Parasites and vector-borne pathogens of southern plains woodrats (*Neotoma micropus*) from southern Texas. Parasitol. Res. 110: 1855–1862.

Cohen, E.B., L.D. Auckland, P.P. Marra, and S.A. Hamer. 2015. Avian migrants facilitate invasions of neotropical ticks and tick-borne pathogens into the United States. Appl. Environ. Microbiol. 81: 8366–8378.

Cotté, V., S. Bonnet, D. Le Rhun, E. Le Naour, A. Chauvin, H.J. Boulouis, B. Lecuelle, T. Lilin, and M. Vayssier-Taussat. 2008. Transmission of *Bartonella henselae* by *Ixodes ricinus*. Emerg. Infect. Dis. 14: 1074–1080.

Eads, D.A., D.E. Biggins, J. Bowser, K. Broerman, T.M. Livieri, E. Childers, P. Dobesh, and R. L. Griebel. 2019. Evaluation of five pulicides to suppress fleas on black-tailed prairie dogs: Encouraging long-term results with systemic 0.005% Fipronil. Vector-Borne Zoonotic Dis. 19: 400–406.

Eisen, R.J., L. Eisen, and K.L. Gage. 2009. Studies of vector competency and efficiency of North American fleas for *Yersinia pestis*: State of the field and future research needs. J. Med. Entomol. 46: 737–744.

Eremeeva, M.E., W.R. Warashina, M.M. Sturgeon, A.E. Buchholz, G.K. Olmsted, S.Y. Park, P.V. Effler, and S.E. Karpathy. 2008. *Rickettsia typhi* and *R. felis* in rat fleas (*Xenopsylla cheopis*), Oahu, Hawaii. Emerg. Infect. Dis. 14: 1613–1615.

Eremeeva, M.E., D. Capps, C.L. McBride, A.J. Williams-Newkirk, G.A. Dasch, J.S. Salzer, L. Beati, and L.A. Durden. 2020. Detection of *Rickettsia asembonensis* in fleas (Siphonaptera: Pulicidae, Ceratophyllidae) collected in five counties in Georgia, United States. J. Med. Entomol. 57: 1246–1253.

Folmer, O., M. Black, W. Hoeh, R. Lutz, and R. Vrijenhoek. 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Mol. Mar. Biol. Biotechnol. 3: 294–299.

Friggens, M.M. and P. Beier. 2010. Anthropogenic disturbance and the risk of flea-borne disease transmission. Oecologia 164: 809–820.

Fritz, R. and H. Pratt. 1947. *Fleas: Pictorial Key to Species*

Found on Domestic Rats in Southern United States. U.S. Department of Health, Education and Welfare, Public Health Service, Communicable Disease Center, Atlanta, GA. 196 pp.

Furman, D. and E. Catts. 1982. *Manual of Medical Entomology*. Cambridge University Press, Cambridge. 224 pp.

Haas, G.E., N. Wilson, and C.T. McAllister. 2004. Fleas (Siphonaptera: Ceratophyllidae, Ctenophthalmidae) from rodents in five southwestern states. West. North Am. Nat. 64: 514–517.

Hammond, T.T., C.I. Hendrickson, T.L. Maxwell, A.L. Petrosky, R. Palme, J.C. Pigage, and H.K. Pigage. 2019. Host biology and environmental variables differentially predict flea abundances for two rodent hosts in a plague-relevant system. Int. J. Parasitol. Parasites Wildl. 9: 174–183.

Hastriter, M.W. 2023. An assemblage of fleas (Siphonaptera) from Canada, México, and the United States of America, in the Hastriter Collecton (Brigham Young University). Ann. Carnegie Museum. 88: 13–90.

Jameson, P., C. Greene, R. Regnery, M. Dryden, A. Marks, J. Brown, J. Cooper, B. Glaus, and R. Greene. 1995. Prevalence of *Bartonella henselae* antibodies in pet cats throughout regions of North America. J. Infect. Dis. 172: 1145–1149.

Kollars, T.M. and A. Kengluecha. 2001. Spotted fever group Rickettsia in *Dermacentor variabilis* (Acari: Ixodidae) infesting raccoons (Carnivora: Procyonidae) and opossums (Marsupialia: Didelphimorphidae) in Tennessee. J. Med. Entomol. 38: 601–602.

Kosoy, M., P. Reynolds, Y. Bai, K. Sheff, R.E. Enscore, J. Montenieri, P. Ettestad, and K. Gage. 2017. Small-scale die-offs in woodrats support long-term maintenance of plague in the U.S. Southwest. Vector-Borne Zoonotic Dis. 17: 635–644.

Krasnov, B.R., I.S. Khokhlova, L.J. Fielden, and N.V. Burdelova. 2001. Development rates of two *Xenopsylla* flea species in relation to air temperature and humidity. Med. Vet. Entomol. 15: 249–258.

Kreeger, T.J. and J.M. Arnemo. 2012. *Handbook of Wildlife Chemical Immobilization*. 4th ed. Terry J. Kreeger, Sybille, Wyoming. 418 pp.

Kreppel, K.S., S. Telfer, M. Rajerison, A. Morse, and M. Baylis. 2016. Effect of temperature and relative humidity on the development times and survival of *Synopsyllus fonquerniei* and *Xenopsylla cheopis*, the flea vectors of plague in Madagascar. Parasit. Vectors 9: 82.

Lashnits, E., B. Thatcher, A. Carruth, A. Mestek, J. Buch, M. Beall, P. Neupane, R. Chandrashekar, and E.B. Breitschwerdt. 2022. *Bartonella* spp. seroepidemiology and associations with clinicopathologic findings in dogs in the United States. J. Vet. Intern. Med. 36: 116–125.

Lawrence, A.L., G.K. Brown, B. Peters, D.S. Spielman, V. Morin-Adeline, and J. Šlapeta. 2014. High phylogenetic diversity of the cat flea (*Ctenocephalides felis*) at two mitochondrial DNA markers. Med. Vet. Entomol. 28: 330–336.

Maina, A.N., C. Fogarty, L. Krueger, K.R. Macaluso, A. Odhiambo, K. Nguyen, C.M. Farris, A. Luce-Fedrow, S. Bennett, J. Jiang, et al. 2016. Rickettsial infections among *Ctenocephalides felis* and host animals during a flea-borne rickettsioses outbreak in Orange County, California. PLoS One. 11: e0160604.

Mays Maestas, S.E., L.P. Campbell, S.M. Wisely, P.A. Dingman, L.E. Reeves, and P.E. Kaufman. 2023. Comparison of ectoparasite communities of sylvatic and urban wild mesomammals and unowned community cats in north-central Florida. J. Med. Entomol. 60: 460–469.

McAllister, C.T., L. Durden, J. Hnida, and H. Robison. 2020. Ecto- and endoparasites of the Texas deermouse, *Peromyscus attwateri* and eastern woodrat, *Neotoma floridana* (Rodentia: Cricetidae) from Polk County, Arkansas. J. Ark. Acad. Sci. 74.

McCormick, D.W., S.L. Rassoulian-Barrett, D.R. Hoogestraat, S.J. Salipante, D. SenGupta, E.A. Dietrich, B.T. Cookson, G.E. Marx, and J.A. Lieberman. 2023. *Bartonella* spp. infections identified by molecular methods, United States. Emerg. Infect. Dis. 29: 467–476.

Meece, J.K., C.E. Reynolds, P.J. Stockwell, T.A. Jenson, J.E. Christensen, and K.D. Reed. 2005. Identification of mosquito bloodmeal source by terminal restriction fragment length polymorphism profile analysis of the cytochrome B gene. J. Med. Entomol. 42: 657–667.

Millán, J., P. Sepúlveda-García, S. Di Cataldo, N. Canales, N. Sallaberry-Pincheira, J. Painean, A. Cevidanes, and A. Müller. 2023. Molecular identification of *Bartonella* spp. and *Rickettsia felis* in fox fleas, Chile. Comp. Immunol. Microbiol. Infect. Dis. 96: 101983.

Miller, B.E., D.L. Forcum, K.W. Weeks, J.R. Wheeler, and C.D. Rail. 1970. An evaluation of insecticides for flea control on wild mammals. J. Med. Entomol. 7: 697–702.

Murray, K.O., N. Evert, B. Mayes, E. Fonken, T. Erickson, M.N. Garcia, and T. Sidwa. 2017. Typhus group rickettsiosis, Texas, USA, 2003–2013. Emerg. Infect. Dis. 23: 645–648.

Ng-Nguyen, D., S.F. Hii, M.T.T. Hoang, V.A.T. Nguyen, R. Rees, J. Stenos, and R.J. Traub. 2020. Domestic dogs are mammalian reservoirs for the emerging zoonosis flea-borne spotted fever, caused by *Rickettsia felis*. Sci. Rep. 10: 4151.

Orlova, M.V., V.D. Thong, D.G. Smirnov, A.V. Zabashta, and O.L. Orlov. 2022. New geographical and host records of bat fleas (Siphonaptera: Ischnopsyllidae) in Russia. Ann. Parasitol. 68: 121–128.

Poje, J.E., T.E. Rocke, and M.D. Samuel. 2020. Impacts of environmental conditions on fleas in black-tailed prairie dog burrows. J. Vector Ecol. 45: 356–365.

Pung, O.J., L.A. Durden, C.W. Banks, and D.N. Jones. 1994. Ectoparasites of opossums and raccoons in southeastern Georgia. J. Med. Entomol. 31: 915–919.

Raoult, D., B. La Scola, M. Enea, P.E. Fournier, V. Roux, F. Fenollar, M.A.M. Galvao, and X. De Lamballerie. 2001. A flea-associated *Rickettsia* pathogenic for humans. Emerg. Infect. Dis. 7: 73–81.

Reeves, W.K., M.P. Nelder, and J.A. Korecki. 2005. *Bartonella* and *Rickettsia* in fleas and lice from mammals in South Carolina, U.S.A. J. Vector Ecol. 30: 310–315.

Reeves, W.K., K.O. Murray, T.E. Meyer, L.M. Bull, R.F. Pascua, K.C. Holmes, and A.D. Loftis. 2008. Serological evidence of typhus group rickettsia in a homeless population in Houston, Texas. J. Vector Ecol. 33: 205–207.

Richardson, E.A., R.M. Roe, C.S. Apperson, and L. Ponnusamy. 2023. *Rickettsia amblyommatis* in ticks: A review of distribution, pathogenicity, and diversity. Microorganisms 11: 11–13.

Rotstein, D.S., S.K. Taylor, J. Bradley, and E.B. Breitschwerdt. 2000. Prevalence of *Bartonella henselae* antibody in Florida panthers. J. Wildl. Dis. 36: 157–160.

Roux, V., P.E. Fournier, and D. Raoult. 1996. Differentiation of spotted fever group rickettsiae by sequencing and analysis of restriction fragment length polymorphism of PCR-amplified DNA of the gene encoding the protein rOmpA. J. Clin. Microbiol. 34: 2058–2065.

Roux, V. and D. Raoult. 2000. Phylogenetic analysis of members of the genus *Rickettsia* using the gene encoding the outer-membrane protein rOmpB (*ompB*). Int. J. Syst. Evol. Microbiol. 50: 1449–1455.

Ruiz, K., R. Valcin, P. Keiser, and L.S. Blanton. 2020. Rise in murine typhus in Galveston County, Texas, USA, 2018. Emerg. Infect. Dis. 26: 1044–1046.

Ryser, J.T. 1995. Activity, movement and home range of Virginia opossum (*Didelphis virginiana*) in Florida. Bull. Florida Mus. Nat. Hist. 38, PT II: 177–194.

Salje, J., T. Weitzel, P.N. Newton, G.M. Varghese, and N. Day. 2021. Rickettsial infections: A blind spot in our view of neglected tropical diseases. PLoS Negl. Trop. Dis. 15: 1–7.

Salomon, J., N.A. Fernandez Santos, I.B. Zecca, J.G. Estrada-Franco, E. Davila, G.L. Hamer, M.A. Rodriguez Perez, and S.A. Hamer. 2022. Brown dog tick (*Rhipicephalus sanguineus* sensu lato) infection with endosymbiont and human pathogenic *Rickettsia* spp., in Northeastern México. Int. J. Environ. Res. Publ. Hlth. 19: 6249.

Sayler, K.A., H.L. Wamsley, M. Pate, A.F. Barbet, and A.R. Alleman. 2014. Cultivation of *Rickettsia amblyommii* in tick cells, prevalence in Florida lone star ticks (*Amblyomma americanum*). Parasites Vectors 7: 270.

Schwartzman, W.A. 2015. Cat scratch disease and other *Bartonella* infections. In: *Clinical Infectious Disease*. Cambridge University Press. pp. 853–858.

Serpa, M.C. de A., H.R. Luz, F.B. Costa, B.C. Weck, H.R. Benatti, T.F. Martins, L.S. Correa, D.G. Ramirez, V. Rocha, T.C. Dias, et al. 2021. Small mammals, ticks and rickettsiae in natural and human-modified landscapes: Diversity and occurrence of Brazilian spotted fever in Brazil. Ticks Tick Borne Dis. 12: 101805.

Shu, C., M. Jiang, M. Yang, J. Xu, S. Zhao, X. Yin, B. Wang, J. Sheng, and Y. Wang. 2020. Flea surveillance on wild mammals in northern region of Xinjiang, northwestern China. Int. J. Parasitol. Parasites Wildl. 11: 12–16.

Wechtaisong, W., S.I. Bonnet, Y.Y. Lien, S.T. Chuang, and Y.L. Tsai. 2020. Transmission of *Bartonella henselae* within *Rhipicephalus sanguineus*: Data on the potential vector role of the tick. PLoS Negl. Trop. Dis. 14: e0008664.

Wedincamp, J. and L.D. Foil. 2002. Vertical transmission of *Rickettsia felis* in the cat flea (*Ctenocephalides felis* Bouché). J. Vector Ecol. 27: 96–101.

Whiting, M.F., A.S. Whiting, M.W. Hastriter, and K. Dittmar. 2008. A molecular phylogeny of fleas (Insecta: Siphonaptera): origins and host associations. Cladistics 24: 677–707.

Wikswo, M.E., R. Hu, G.A. Dasch, L. Krueger, A. Arugay, K. Jones, B. Hess, S. Bennett, V. Kramer, and M.E. Eremeeva. 2008. Detection and identification of spotted fever group Rickettsiae in *Dermacentor* species from southern California. J. Med. Entomol. 45: 509–516.

Williamson, P.C., P.M. Billingsley, G.J. Teltow, J.P. Seals, M.A. Turnbough, and S.F. Atkinson. 2010. *Borrelia*, *Ehrlichia*, and *Rickettsia* spp. in ticks removed from persons, Texas, USA. Emerg. Infect. Dis. 16: 441–446.

Woods II, H.A. and E.C. Hellgren. 2003. Seasonal changes in the physiology of male Virginia opossums (*Didelphis virginiana*): Signs of the Dasyurid semelparity syndrome? Physiol. Biochem. Zool. 76: 406–417.

Yin, X., S. Zhao, B. Yan, Y. Tian, T. Ba, J. Zhang, and Y. Wang. 2019. *Bartonella rochalimae*, *B. grahamii*, *B. elizabethae*, and *Wolbachia* spp. in fleas from wild rodents near the China-Kazakhstan border. Korean J. Parasitol. 57: 553–559.

Yomogida, K., A. Kjemtrup, B. Martínez-López, M. Ibrahim, Z. Contreras, V. Ngo, H. Umme-Aiman, S. Balter, M. Feaster, M. Zahn, et al. 2024. Surveillance of flea-borne typhus in California, 2011–2019. Am. J. Trop. Med. Hyg. 110: 142–149.

Zeaiter, Z., P.E. Fournier, and D. Raoult. 2002. Genomic variation of *Bartonella henselae* strains detected in lymph nodes of patients with cat scratch disease. J. Clin. Microbiol. 40: 1023–1030.