

the disease) compared with the Sahel savanna vegetation in the central and northern parts of Africa (6,7). Furthermore, the intensive agricultural activities in the southern districts and the common postharvest practice of drying crops in open spaces in these hilly areas probably favor food contamination by the disease vector.

Our finding that a delay in seeking care of ≥ 24 hours after onset of bleeding was a strong predictor of death among cases is a concern. LF has some common early symptoms similar to those of other febrile diseases, especially malaria, that are frequently encountered in most LF-endemic settings (8). An LF diagnosis is often delayed because health workers suspect these other febrile diseases (8). Furthermore, Bauchi State has the highest CFR in Nigeria, nearly double the national average (28.9%) (2,9). A similarly high CFR was reported in a previous study in Sierra Leone in a region affected by conflicts where the health infrastructure was poor (5). Finally, the designated LF treatment center in Bauchi State lacks adequately trained personnel and other essential resources to effectively manage complications once they occur. If one considers that a delay in seeking care has been demonstrated to be a predictor of death in this study, the high CFR in Bauchi State could have been lower if the treatment center was better equipped or if cases could be diagnosed earlier.

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Plague Epizootic Dynamics in Chipmunk Fleas, Sierra Nevada Mountains, California, USA, 2013–2015

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We describe *Yersinia pestis* minimum infection prevalence in fleas collected from *Tamias* spp. chipmunks in the Sierra Nevadas (California, USA) during 2013–2015. *Y. pestis*-positive fleas were detected only in 2015 (year of plague epizootic), mostly in *T. speciosus* chipmunks at high-elevation sites. Plague surveillance should include testing vectors for *Y. pestis*.

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To better forecast vectorborne infection dynamics, characterizing disease cycles in both hosts and vectors is critical. The rate of infection of vector species can serve as a good indicator for risk during epizootic events, especially in areas with high human-wildlife overlap, but vectors are often poorly sampled. *Yersinia pestis*, the bacterium that causes plague, is carried by multiple flea species in western North America, where sciurids are often the primary reservoirs (1). Although human plague cases in this area are rare, in 2015, two cases were linked to exposures in Yosemite National Park, California, USA (2). In the investigation conducted to determine the source of these exposures, multiple *Y. pestis*-positive flea and rodent species were documented, and the lodgepole chipmunk (*Tamias speciosus*) was the host that was most frequently seropositive (2).

Plague surveillance in the western United States typically involves serologic testing of rodents and carnivores. Positive serologic results indicate prior plague activity. A *Y. pestis*-positive flea, however, indicates current plague transmission and is more likely to trigger control activities (3). Here, we sought to characterize *Y. pestis* infection in fleas of alpine (*T. alpinus*) and lodgepole (*T. speciosus*) chipmunks in Yosemite National Park and surrounding areas during 2013–2015. We focused on *T. speciosus* chipmunks because of their documented role in the 2015 epizootic (2) and on *T. alpinus* chipmunks because they co-occur with *T. speciosus* chipmunks (4) and little is known about their role in plague ecology. Our goals were to describe the proportion of *T. speciosus* and *T. alpinus* chipmunks harboring *Y. pestis*-positive fleas and the minimum infection prevalence of *Y. pestis* in fleas collected from these species across multiple sites and in years with and without known epizootic activity.

During June–October 2013–2015, we collected fleas from tagged chipmunks. Using a metal-pronged comb, we combed each animal 5 times down the dorsum, the tail, and each hind leg and placed collected fleas into vials containing 100% ethanol. These procedures were approved by the University of California, Berkeley, Animal Care and Use Committee (Berkeley, California, USA).

We identified key flea specimens (N = 122) (5–9) and then cleared, dehydrated, and mounted them on microscope slides (Denver Museum of Nature and Science accession nos. ZP.2000–176). For the remaining fleas, we microscopically observed and identified the species (5) using keys (6–9) and mounted some fleas as references. For each host, we pooled all

conspecific fleas, which resulted in 162 pools (with 291 fleas total) from 121 *T. alpinus* chipmunks and 538 pools (with 1,096 fleas total) from 389 *T. speciosus* chipmunks (Appendix Table 1, <https://wwwnc.cdc.gov/EID/article/26/4/19-0733-App1.pdf>). We used molecular methods to detect *Y. pestis* DNA in flea pools (Appendix).

We found *Y. pestis*-positive fleas exclusively in 2015 at 5 of the 6 sites surveyed (Figure; Appendix Table 2). In 2015, 7.29% (14/192) of *T. speciosus* hosts carried ≥ 1 *Y. pestis*-positive flea. The minimum infection prevalence of *Y. pestis* in *T. speciosus* chipmunk-hosted fleas was 3.28% (assuming 1 positive flea per positive pool, 18 positive pools/548 total fleas in 280 pools tested). All 3 of the flea species (*Ceratophyllus ciliatus mononis*, *Eumolpianus eumolpi*, and *E. eutamias*) most commonly found on *T. speciosus* and *T. alpinus* chipmunks were found to be positive for *Y. pestis* (Appendix Table 1) (10). In 2015, a total of 5.13% (2/39) of *T. alpinus* hosts carried ≥ 1 *Y. pestis*-positive flea (Appendix Table 2). The infection prevalence (not minimum infection prevalence because each positive pool contained a single flea) of *Y. pestis* in *T. alpinus* chipmunk-hosted fleas was 2.47% (2 positive pools/81 total fleas in 50 pools tested). Unfortunately, these fleas were too damaged to identify morphologically, and molecular species identification was not possible.

Y. pestis-positive flea pools were detected at 5 of 6 high-elevation (2,650–3,200-m) study sites in 2015. Many of these sites are areas of high human activity, with popular hiking trails or established campgrounds. In 2015, plague risk assessments, including testing flea pools and rodent carcasses for *Y. pestis* DNA and rodent serology, also took place at lower elevation sites (1,778 \pm 553 m) in and around the park; these surveys detected *Y. pestis* at 4 of 17 locations (2).

Altogether, our data indicate a dramatic shift in *Y. pestis* prevalence in fleas during a plague epizootic year in California. Our results support integrating flea testing, especially those at high-elevation sites, into regular surveillance.

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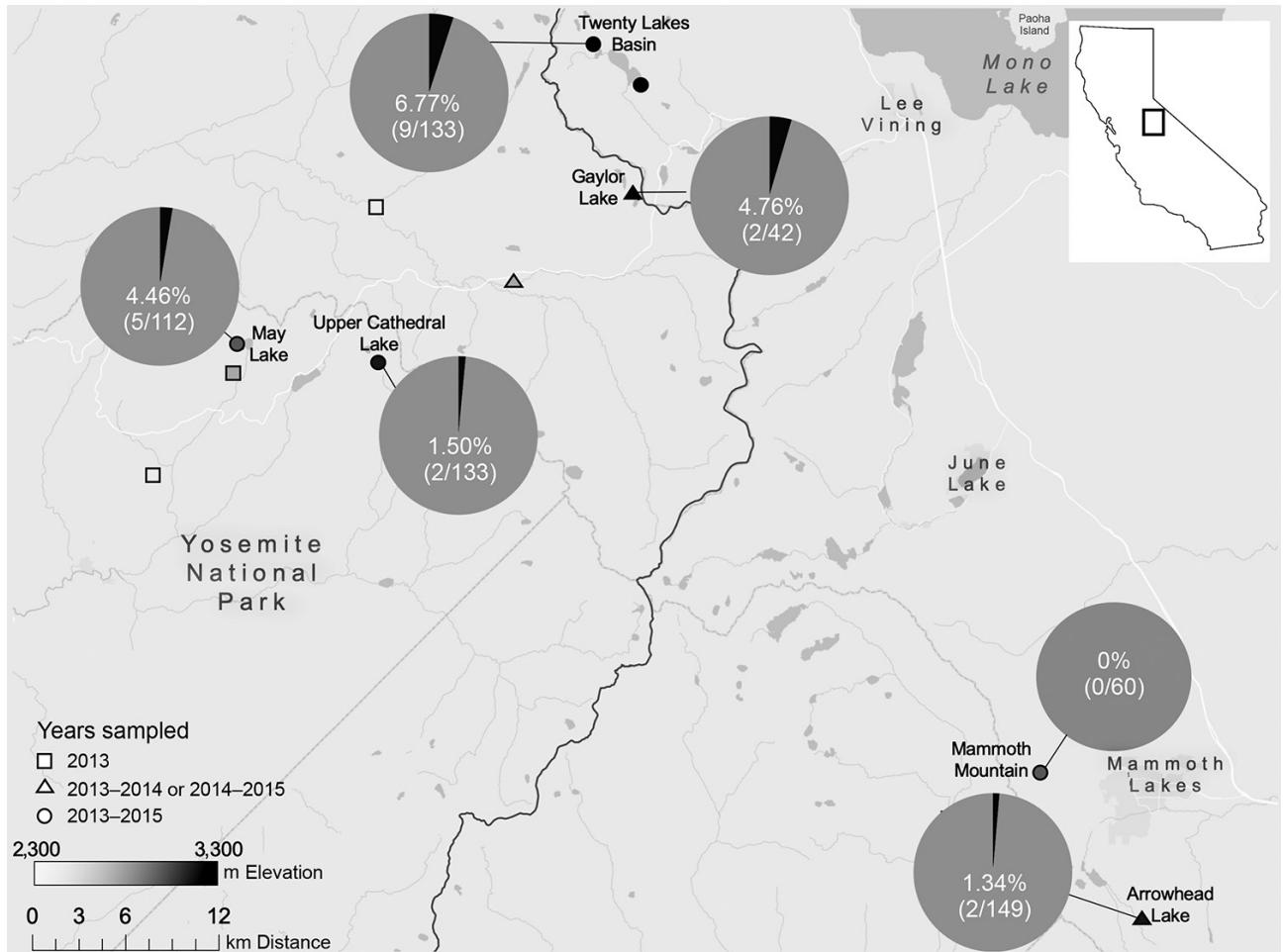


Figure. Minimum *Yersinia pestis* infection prevalence in fleas harbored by *Tamias speciosus* and *T. alpinus* chipmunks, by study site, Sierra Nevada, California, 2013–2015. We visited sites for different numbers of years: 1 study year (2013 only), 2 study years, or all 3 study years. Plague prevalence was zero in 2013 and 2014, and map shows plague prevalence only in 2015. Pie charts show percentage of minimum infection prevalence (no. *Y. pestis* DNA-positive pools/no. fleas in pools tested). Sites without pie charts were either not visited in 2015 or had no flea pools collected there in 2015 because of low chipmunk prevalence. The irregular black line shows the eastern border of Yosemite National Park. Inset shows location of study sites in California. See Appendix Tables 1, 2 (<https://wwwnc.cdc.gov/EID/article/26/4/19-0733-App1.pdf>) for more details on fleas tested.

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***Brucella melitensis* in Asian Badgers, Northwestern China**

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We isolated *Brucella melitensis* biovar 3 from the spleen of an Asian badger (*Meles leucurus*) in Nilka County, northwestern China. Our investigation showed that this isolate had a common multilocus variable-number tandem-repeat analysis 16 genotype, similar to bacterial isolates from local aborted sheep fetuses.

¹These authors contributed equally to this article.

Brucellosis can be transmitted between domestic animals and wildlife (1). *Brucella melitensis* has been isolated from wildlife, such as chamois (*Rupicapra rupicapra*) (2), Alpine ibex (*Capra ibex*) (3), and Iberian wild goat (*Capra pyrenaica*) (4). Badgers are major predators in forests and consume a broad spectrum of food items, including small terrestrial vertebrates and their cadavers (5), which might result in contact with pathogens from tissues of these vertebrates. We report an Asian badger (*Meles leucurus*) in China naturally infected with *B. melitensis* biotype 3.

This study was approved by the Animal Ethics Committee of Shihezi University (approval no. AEC-SU2017–04). In 2017, a total of 7 illegally hunted and dying badgers in Nilka County, northwestern China, were confiscated by the local government.

We identified the animals as Asian badgers by using a PCR targeting the 16S rDNA gene (GenBank accession no. MH155253). We collected different organs or tissues, including heart, liver, spleen, lung, kidney, small intestine, large intestine, and blood, from all badgers. We separated serum from blood samples by centrifugation at 1,000 × *g* for 15 min and tested serum by using the rose bengal test (RBT) and serum agglutination test (SAT) (6). To detect *Brucella* antigens, we used immunohistochemical staining of liver and spleen tissue sections by pipetting mouse anti-*Brucella melitensis* IgG diluted 1:100 in 30% bovine serum albumin/phosphate-buffered saline onto each section. For comparison, we collected samples from 14 aborted sheep fetuses from Nilka County.

We extracted genomic DNA from all samples by using a commercial kit (Blood and Cell and Tissue Kit; BioTeke, <http://www.bioteke.com>). We used the partial *omp22* gene (238 bp) encoding 22-kD outer membrane protein to identify the *Brucella* genus and the *IS711* gene to identify *Brucella* species. We used PCRs that have been described (7). We used *Brucella* reference strains (*B. melitensis* 16M and *B. abortus* 2308) as positive controls and double-distilled water as a negative control.

We homogenized spleen samples of badgers and the 14 aborted sheep fetuses and inoculated these homogenates onto individual *Brucella* agar plates, which we then incubated at 37°C in an atmosphere of 5% CO₂ for 5 days. We tested putative *Brucella* colonies using by H₂S production, dye inhibition, agglutination by monospecific serum, and sensitivity to bacteriophages (Appendix Table, <https://wwwnc.cdc.gov/EID/article/26/4/19-0833-App1.pdf>). We analyzed colonies by using a multilocus variable-number tandem-repeat analysis (MLVA) typing assay (8).

Only serum from badger no. 2 was positive for smooth *Brucella* antigen by RBT and SAT; the specific

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Appendix

Molecular Methods

Fleas were suspended in 180 μ L Buffer AL + 20 μ L proteinase K (Qiagen, Hercules, CA) in cryovials containing three 2.8 mm ceramic beads. Pools were then homogenized using a Bead Ruptor (Omni Inc, Kennesaw, GA). DNA was extracted using Qiagen DNeasy Blood and Tissue Kit per the manufacturer's protocol (Qiagen, Hercules, CA). DNA samples were eluted to a final volume of 200 μ L. A real-time PCR protocol using TaqMan primers and probe targeting the *cafI* gene was used to detect *Y. pestis* DNA (1). The volume of nucleic acid for each PCR reaction was 1 μ L. Each 20 μ L reaction included the sample and the master mix, which was a combination of 10 μ L Bio-rad SsoAdvanced Universal Probes Supermix, 8.4 μ L Sterile Water, 0.2 μ L primer F, 0.2 μ L Primer R, and 0.2 μ L probe. Cycling conditions were as described in (1). Samples were run in singlicate. Any samples that were positive for CAF1 in initial testing were included in a second confirmatory PCR with CAF-1, YPO and LCRV primers/probes. Samples were considered positive at CT values of <38.

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Appendix Table 1. Number of flea pools tested in each year, arranged by host and flea species*

Year	Host species (no.)	<i>C. ciliates mononis</i>	<i>E. eumolpi</i>	<i>E. eutamiadis</i>	<i>Oropsylla montana</i>	<i>Aetheca wagneri</i>	Species unknown
2013	<i>T. alpinus</i> (34)	9 (0)	39 (0)	1 (0)	-	-	1 (0)
	<i>T. speciosus</i> (76)	58 (0)	26 (0)	21 (0)	1(0)	-	5 (0)
2014	<i>T. alpinus</i> (48)	22 (0)	36 (0)	3 (0)	-	-	1 (0)
	<i>T. speciosus</i> (121)	104 (0)	30 (0)	12 (0)	-	-	1 (0)
2015	<i>T. alpinus</i> (39)	9 (0)	34 (0)	1 (0)	-	-	6 (2)
	<i>T. speciosus</i> (192)	156 (7)	74 (7)	36 (3)	-	1 (0)	13 (1)
Totals		358 (7)	239 (7)	74 (3)	1 (0)	1 (0)	27 (3)

*For each flea species, the number of *Y. pestis*-positive pools is shown in parenthesis. Mean (\pm SE) pool size was 1.80 ± 0.11 fleas for *T. alpinus* and 2.04 ± 0.11 for *T. speciosus*.

Appendix Table 2. 2015 Study site locations and numbers of fleas, flea pools, hosts, and information regarding *Y. pestis* status*

2015 Sites	Latitude	Longitude	Elevation, m	Host species	No. fleas (no. pools, no. hosts)	No. pools <i>Y. pestis</i> -positive (no. hosts)	Minimum <i>Y. pestis</i> infection prevalence in fleas	% Hosts with at least 1 <i>Y. pestis</i> -positive flea
AL	37.584044	-118.97638	2970	<i>T. speciosus</i>	149 (85, 58)	2 (2)	1.34%	3.45%
CL	37.838859	-119.41795	2960	<i>T. alpinus</i>	47 (27, 21)	0 (0)	0%	0%
				<i>T. speciosus</i>	86 (41, 29)	2 (2)	2.33%	6.90%
GL	37.91332	-119.26717	3190	<i>T. alpinus</i>	11 (9, 6)	2 (2)	18.18%	33.33%
				<i>T. speciosus</i>	31 (14, 9)	0 (0)	0%	0%
MA	37.651181	-119.03418	2650	<i>T. speciosus</i>	60 (41, 33)	0 (0)	0%	0%
ML	37.845177	-119.49606	2870	<i>T. alpinus</i>	19 (10, 8)	0 (0)	0%	0%
				<i>T. speciosus</i>	93 (44, 27)	5 (3)	5.38%	11.11%
TL	37.981377	-119.2918	3110	<i>T. alpinus</i>	4 (4, 4)	0 (0)	0%	0%
				<i>T. speciosus</i>	129 (55, 36)	9 (7)	6.98%	19.44%

*AL, Arrowhead Lake; CL, Cathedral Lake (upper); GL, Gaylor Lakes; MA, Mammoth; ML, May Lake; TL, Twenty Lakes Basin.