

Burrowing owls, *Pulex irritans* and plague

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Running Head: *Owls, fleas and Yersinia pestis*

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Abstract

Western burrowing owls (*Athene cunicularia hypugaea*) are small, ground-dwelling owls of western North America that frequent prairie dog (*Cynomys* spp.) towns and other grasslands. As they rely on rodent prey and occupy burrows once or concurrently inhabited by fossorial mammals, the owls often harbor fleas. We examined the potential role of fleas found on burrowing owls in plague dynamics by evaluating prevalence of *Yersinia pestis* in fleas and in owl blood. During 2012-2013 fleas and blood were collected from burrowing owls in portions of five states with endemic plague: Idaho, Oregon, Washington, Colorado, and South Dakota. Fleas were enumerated, taxonomically identified, pooled by nest and assayed for *Y. pestis* using culturing and molecular (PCR) approaches. Owl blood underwent serological analysis for plague antibodies and nested PCR for detection of *Y. pestis*. Of >4750 fleas collected from owls, *Pulex irritans*, a known plague vector in portions of its range, comprised more than 99.4%. However, diagnostic tests for *Y. pestis* of flea pools (culturing and PCR) and owl blood (PCR and serology) were negative. Thus, despite that fleas were prevalent on burrowing owls, and the potential for a relationship with burrowing owls as a phoretic host of infected fleas exists, we found no evidence of *Y. pestis* in sampled fleas or in owls that harbored them. We suggest that studies similar to those reported here during plague epizootics will be especially useful for confirming these results.

Key Words: plague – western burrowing owls – *Athene cunicularia hypugaea* – fleas – *Pulex irritans* – *Yersinia pestis* – United States.

Introduction

Plague is caused by the Gram-negative bacterium *Yersinia pestis* and infects primarily rodents, but a wide array of other mammalian hosts, including humans, can be infected.

Hematophagous fleas are the main vectors of *Y. pestis*, as transmission events occur predominantly through bites. Because of concern for human and wildlife health, relationships among plague, fleas, and small mammals in western North America have been investigated (Gage and Kosoy 2005). Epizootic outbreaks have been common in prairie dog (*Cynomys* sp.) colonies and other settings with colonial mammals (Stapp et al. 2009, Tripp et al. 2009, Salkeld et al. 2010, Jones et al. 2011, Brinkerhoff et al. 2011). Although they are not susceptible to disease, the relationship of *Y. pestis* with birds is less well understood. Birds are believed to be among the possible agents for spreading plague between rodent populations (Stenseth et al. 2008, Gage 2012). Gage (2012) explains that in the course of capturing, killing and feeding on rodents, birds of prey especially may acquire fleas that are carried to new sites where the fleas leave their accidental host and find new rodent hosts. The successful transport of *Y. pestis*-infected fleas from one rodent population to another by predators is thought to occur only rarely, however (Gage 2012). In an alternate scenario, birds may be carriers of *Y. pestis*, as at least one species of bird, the northern wheatear (*Oenanthe oenante*), was found to be infected with plague in Mongolia (Galdan et al. 2010). The potential for birds to serve as a source of infection for fleas that feed on the birds is not well known but may be low because fleas typically need to feed on more highly bacteremic hosts to become infected (Gage 2012).

Avian species that inhabit prairie dog (*Cynomys* sp.) colonies and other mammal burrows, and that have potential to harbor fleas, are important candidates for investigation. One such species is the western burrowing owl (*Athene cunicularia hypugaea*; hereafter burrowing owls), which are small, ground-dwelling owls of western North America. Burrowing owls have the potential to act as vectors or reservoirs of *Y. pestis* for a number of reasons, including: their geographic range (see Poulin et al. 2011) overlaps areas with endemic plague, they nest widely in prairie dog colonies and burrows (Desmond et al. 2000, Restani et al. 2001, Lantz et al. 2007, Alverson and Dinsmore 2014), outside the range of prairie dogs the owls use fossorial mammal burrows including mammals with frequent exposure to *Y. pestis* (e.g., American badgers, *Taxidea taxus*, Messick et al. 1983), owls hunt rodent prey and harbor fleas (Smith and Belthoff 2001) and lastly, the owls make widespread migratory (e.g., Holroyd et al. 2010) and other movements that have the potential to move fleas among plague regions.

Previously, Jellison (1939) recovered 109 live rodent fleas of six species from a burrowing owl nest in a plague area near Dillon, Montana. He quoted Rucker (1909) who stated “There is reason to believe that the booby owl, which is a constant companion of the ground squirrel, occupying the same burrows with him, may play an important role in the dissemination of the epizootic. It is thought that this bird, flying from burrow to burrow, may carry infected fleas for long distances.” Wheeler et al. (1941) subsequently collected a burrowing owl following a plague epizootic in California from which 70 sticktight fleas (*Echidnophaga gallinacea*) were retrieved. Upon mass inoculation into a test guinea pig, the fleas were said to be infected with plague organisms. Wheeler et al. (1941) concluded that this was the first record of a bird host as a carrier of plague-infected ecto-parasites, and the first demonstration of natural plague

infection in this species of flea. Finally, Brown (1944) reported that burrowing owls were unusually abundant in a plague epizootic area (Hanna-Youngstown) in Alberta, Canada. In 1940 and 1941, a small number of fleas (n = 47 and 37, respectively; species not identified) were collected from burrowing owl burrows, but they tested negative for *Pasteurella (Yersinia) pestis*. In 1942 Brown recovered a single flea from each of two live burrowing owls and identified the fleas as *Oropsylla idahoensis* and (tentatively) *Rectofrontia fraterna*. Brown (1944) noted that *Oropsylla idahoensis* was a known plague vector.

These studies point to a potential role of fleas found on burrowing owls in plague dynamics, although numerous aspects remain poorly understood. We were interested in determining (1) if, and how commonly, fleas on burrowing owls harbor *Y. pestis*, (2) the geographic variation in flea infestations and *Y. pestis* detection in owl fleas, and (3) if burrowing owls might be exposed to *Y. pestis* via flea bites, even though owls presumably are not susceptible to plague.

Materials and Methods

During the 2012 and 2013 breeding seasons, burrowing owls (Fig. 1) were captured to collect fleas from their plumage and blood samples for diagnostic testing. Owls were captured in 10 study sites (Fig. 2) in five states: (1) Morley Nelson Snake River Birds of Prey National Conservation Area, Ada and Elmore Co., Idaho (**NCA**); (2) Umatilla Chemical Depot, Morrow and Umatilla Co., Oregon (**DEPOT**); East of Baker City, Baker Co., Oregon (**BAKER**); (4) Yakima Training Center, Yakima Co., Washington (**YTC**); (5) Near Pasco, Franklin Co., Washington (**PASCO**); (6) Umatilla National Wildlife Refuge, Benton Co., Washington (**UNWR**); (7) Private lands NE of Naches, Yakima Co., Washington (**NACHES**); (8) Fitzer/Eberhardt Arid Lands Ecology Reserve, Benton Co., Washington (**ALE**); (9) Rocky Mountain Arsenal National Wildlife Refuge,

Adams Co., Colorado (**RMA**); (10) Conata Basin, Buffalo Gap National Grassland, Pennington Co., South Dakota (**BGNG**). Each study site had the potential for enzootic plague, but to our knowledge there were no observable or widespread epizootic outbreaks, although there were two confirmed human cases of plague in 2012 in nearby portions of Oregon during the time of our study (Oregon Public Health Division CD Summary, Vol 62, No. 11, May 2013, see also Kugeler et al. 2015). Prior to our study, the BGNG study site experienced an explosive plague epizootic prompting remaining prairie dog habitat to be treated annually with insecticide to reduce fleas and plague transmission in prairie dogs. Such treatments could have reduced the occurrence of fleas on burrowing owls and potential exposure of owls to *Y. pestis* in this area, but not our other study areas.

Capture of owls

Burrowing owls were located by scanning appropriate habitat with binoculars from roads and while traversing fields on foot. Whereas the owls in Idaho, Oregon, and Washington either nested in artificial burrows (Belthoff and Smith 2003) or in natural burrows often dug by American badgers, owls in Colorado and South Dakota inhabited black-tailed prairie dog (*C. ludovicianus*) towns and nested in natural burrows. Adult owls tending nests were captured using traps placed at or near the mouth of the nest burrow or with bow nets baited with a pet-shop mouse. Owls nesting in artificial burrows were captured by hand after opening the artificial burrows or in traps as they exited nests. All captured owls were fitted with U.S. Geological Survey aluminum leg bands for identification.

Flea collection and identification

Fleas were collected directly from nestlings and adult owls with tweezers or an aspirator and stored by nest site in Ziploc bags or scintillation vials on ice in a portable cooler. Samples were frozen at -20°C upon return from the field. Fleas were subsequently thawed, enumerated, sexed, and taxonomically identified under a dissecting microscope. Taxonomic identification included a standard clearing procedure to lighten the flea for more efficient morphological evaluation (Hastriter and Whiting 2003, Whiting et al. 2008). For clearing, fleas were soaked for several hours in a dilute concentration of potassium hydroxide and then underwent a series of ethanol washes. Once fleas were sufficiently lightened to visualize internal organ structures, they were mounted in Canada balsam on a microscope slide for final identification. This preparation allowed us to taxonomically identify reference individuals but prevented including the reference individuals in PCR tests for *Y. pestis*.

Nearly all fleas collected (see results) from burrowing owls were *Pulex* sp. (Siphonaptera: Pulicidae). Morphological features allow researchers to distinguish male *Pulex* fleas as *P. irritans* or *P. simulans*. There are currently no known morphological features in female *Pulex* fleas to distinguish species. Thus we used DNA extracted from fleas, as described later, for secondary molecular confirmation to distinguish *Pulex* fleas. Previously published primers by Gamerschlag et al. (2008) were used to initially amplify and sequence the internal transcribed spacer (ITS) region of morphologically identified male *P. irritans* and comparative *P. simulans* specimens. Single nucleotide polymorphism (SNP) loci were identified in the ITS region of the male *P. irritans* and *P. simulans*. These SNPs were used to develop an allele-specific PCR. Allele-specific primers (5'-GTCGAATCGCATTTTCCA-3', 5'-ACGCTTTCGGTATTTATC-3') were identified to discriminate between SNP alleles and *P. irritans* and *P. simulans* based on size or

melting temperature (Okimoto and Dodgson 1996). Melting curve PCR (Urdaneta-Marquez et al. 2008) was performed to confirm species in male and female *Pulex* fleas.

Flea cultures and DNA extraction

Fleas were pooled in 1.5 ml screw-cap vials by species according to nest site. For homogenization of fleas, two glass beads were added to each vial. For vials containing fewer than 10 fleas, we added 100 μ l of sterile heart infusion broth (HIB) with 20% glycerol. Flea pools containing 10-200 fleas received 10 μ l of HIB per flea (up to 500 μ l). Pools containing more than 200 fleas were divided into smaller aliquots and processed accordingly. Using a mini bead-beater, we then homogenized pooled fleas for 1-3 min. A 20 μ l aliquot from each freshly-homogenized flea pool was then inoculated onto sheep blood agar (SBA). Cultures were incubated at 28°C for 48-72 hours and visually examined for presence of gray/white and non-hemolytic colonies morphologically suggestive of *Y. pestis*.

DNA was extracted from an aliquot of the remaining volume of flea homogenates using the MagNA Pure Compact Nucleic Acid Isolation Kit (Roche Life Sciences, Branford CT). Sample volumes of 20-200 μ l were extracted using the manufacturer-supplied DNA Blood External Lysis protocol. Sample volumes of less than 200 μ l were adjusted to that volume with PBS prior to extraction. DNA was eluted in volumes of 100 or 200 μ l.

Burrowing owl blood

To assess potential exposure of burrowing owls to *Y. pestis*, ~200-300 μ l of whole blood was obtained from either or both the adult male and the adult female tending the nests. A 26-gauge needle was used to puncture a wing vein to collect blood using microhematocrit tubes. Blood was immediately transferred to 1.5 ml polypropylene centrifuge tubes which were stored

on ice until returned from the field and placed in long term storage at -20°C. When they were ~2-4 weeks of age, 70 µl of whole blood was collected from nestling burrowing owls. Blood from nestlings at a nest was pooled prior to DNA extraction/analysis if there was more than one nestling captured. In 2013, a portion (100 µl) of adult blood samples was added to separate Nobuto filter paper strips for serology, which were allowed to air dry before storing separately in coin envelopes. Occasionally there was sufficient blood from adult owls for serology but not PCR testing, so sample sizes for analyses differed.

For DNA extraction, frozen blood samples were thawed to room temperature and then 10-50 µl of whole blood was added to 300 µl Queen's Lysis Buffer (0.01M Tris-Cl, 0.01M NaCl, 0.01M EDTA, 1% n-lauroylsarcosine, pH 8.0) and 10 µl Proteinase K, and each sample was allowed to incubate overnight in a water bath at 65°C. After incubation, the mixture was added to 200 µl lysis binding buffer (EZ BioResearch EZ Blood/Cell DNA Isolation Kit, EZ BioResearch LLC, St. Louis, MO), and genomic DNA was purified according to manufacturer's instructions. Successful DNA extraction and concentration was determined using a Nanodrop ND-2000 UV-VIS spectrophotometer (Wilmington, DE).

Amplification of Y. pestis DNA in flea and owl blood samples

Detection of *Y. pestis* plasminogen activator (pla) (Table 1) was performed using nested PCR in 25 µl reaction volumes following an approach similar to Hanson et al. (2007). Negative (no template) and positive (*Y. pestis* genomic DNA, Idaho Bureau of Laboratories, Boise, ID) controls were amplified and run with experimental samples. Owl1 primers (Table 1) were used to confirm bird DNA was extracted from the blood. Primers to amplify the *Y. pestis* low calcium response gene (*lcrV*) were also constructed (Table 1) but yielded inconsistent results and, thus,

were not used on all samples. Any equivocal results obtained from the *pla* nested PCR analyses were confirmed with two rounds of PCR using primers specific to the *Y. pestis caf* and *yop* genes found on a separate larger plasmid (Table 1). Amplification of all genomic targets was performed under the same conditions, and PCR products were analyzed on 9% or 1.5% agarose gels.

Plague serology on burrowing owl serum

Owl blood samples stored on paper strips (Nobuto) were tested for the presence of antibodies against the *Y. pestis*-specific F1 capsule antigen using the passive hemagglutination/inhibition assay (PHA/HI) (Chu 2000). A Nobuto titer $\geq 1:32$ is considered positive for evidence of past or current infection. Serological analysis was focused on adult owls to increase the chance of detecting any historical antibody evidence of *Y. pestis* exposures either on their breeding grounds or from possible recent exposure on wintering grounds/migratory routes; the owls in our study populations were primarily migratory.

RESULTS

Fleas from burrowing owls

From 29 May - 30 June 2012 and 12 April - 22 June 2013, more than 4750 fleas were collected from 86 burrowing owl nest sites located in Idaho, Oregon, Washington, and Colorado (Table 2). The median number of fleas collected per nest site was 36.5 in 2012 (range: 1 - 244, $n = 40$) and 40.5 in 2013 (range: 1 - 282, $n = 46$). While fleas were prevalent on burrowing owls in study sites in Idaho, Oregon, and Washington, just one of 60 adult burrowing owls captured at RMA (Colorado), and none of 55 adult burrowing owls captured at BGNG (South Dakota) harbored fleas (Table 2).

More than 99.4% of the fleas we collected from burrowing owls were *Pulex* sp. (Table 2). Morphological (males) and molecular (males and females) analyses indicated that they were *P. irritans*, the human flea. The remaining ~0.6% of fleas (n = 28, Table 2) included individuals that keyed to *Meringis*, *Dactylopsylla*, and potentially *Oropsylla thrassis*.

All homogenized flea samples from both 2012 and 2013 yielded bacterial growth on non-selective blood agar media, but none of the cultures contained colonies morphologically indicative of *Y. pestis*. Therefore, we considered cultures of fleas collected from burrowing owls in both years of study negative for *Y. pestis*.

In 2012, there were 46 separate pools of fleas analyzed by PCR for *Y. pestis*. These included 40 pools of *P. irritans* from 40 burrowing owls nests located in NCA, DEPOT, YTC, NACHES, and ALE, and 6 pools of 'non-*Pulex* species' from 6 nests in NCA, DEPOT, and ALE (Table 2). For 2013, 48 pools of *P. irritans* from 48 different nests in NCA, DEPOT, BAKER, PASCO, ALE, and RMA were analyzed for *Y. pestis*, and one pool of 'non-*Pulex* species' from one nest in the NCA was analyzed. Five nest sites in 2013 each contained a single flea that was not *P. irritans*, and these individuals underwent the clearing procedure for identification that precluded testing for *Y. pestis*.

Thus, 95 flea pools collected at 86 burrowing owls nests in Idaho, Oregon, Washington, and Colorado during 2012-2013 were tested for *Y. pestis* by PCR. Just as in culturing results, there was no *Y. pestis* detected in the fleas found on burrowing owls.

Burrowing owl blood

During 2012-2013, blood was obtained from adult burrowing owls (Table 3) in Idaho (NCA), Oregon (DEPOT, BAKER), Washington (PASCO, UNWR, NACHES, ALE), Colorado (RMA) and

South Dakota (BGNG). Blood from nestlings (Table 3) was obtained in Idaho (NCA), Oregon (DEPOT), and Washington (NACHES, ALE). While numerous adult burrowing owls were captured in Colorado (RMA) and South Dakota (BGNG) during 2013, there were no nestlings observed at these study sites during trapping. Thus, blood from 170 adult (n = 99 females, 71 males) and >400 nestling burrowing owls of unknown sex was obtained. Blood samples/pools (n = 239) collected at 143 nests were subjected to PCR testing for *Y. pestis* (2012: n = 5 adult male, 29 adult female, and 48 nestling pools; 2013: n = 61 adult male, 69 adult female, and 26 nestling pools). None of the owl blood samples tested positive for *Y. pestis*.

Serum samples from 128 adult burrowing owls (Table 3) were also collected and analyzed for *Y. pestis* antibodies. These samples came from owls in Idaho (NCA, n = 25 females, 19 males), Oregon (DEPOT, n = 11 females, 13 males; BAKER, n = 4 females, 6 males), Washington (ALE, n = 2 females, 1 male; PASCO, n = 5 females, 2 males), Colorado (RMA, n = 9 females, 11 males), and South Dakota (BGNG, n = 10 females, 8 males). All burrowing owl serum samples tested negative with titers of <1:32.

Discussion

We found that fleas were prevalent on burrowing owls in Oregon, Idaho, and Washington, where we collected more than 4750 fleas from owls at 86 nests during two owl breeding seasons. Examination by traditional culturing techniques and molecular methods (PCR testing) provided no evidence of *Y. pestis* in these fleas. We also obtained blood from more than 500 burrowing owls at >140 nest sites in five states. Molecular testing of owl blood for *Y. pestis* and serologic examination for *Y. pestis* antibodies were also consistent with the absence of *Y. pestis*. Thus, while fleas were common and owls inhabited areas with endemic plague, unlike Wheeler

et al. (1941), we detected no direct relationship between owl fleas and plague in the areas we studied. It is possible that the low numbers of bacteria present during enzootic cycles are undetectable using these methods, and that any such relationships in these areas are more detectable during plague epizootics (Biggins et al. 2010, Matchett et al. 2010), but if and how infection rates would vary during a plague epizootic remains unknown.

The most prevalent species of flea on burrowing owls in Oregon, Washington, and Idaho was *P. irritans*. Moreover, the only flea collected from a burrowing owl in Colorado was this same species. Smith and Belthoff (2001) found that *P. irritans* was the most common ectoparasite among lice and fleas previously enumerated from a small number of burrowing owls in Idaho. Although geographic variation in flea abundance and the flea species that burrowing owls harbor throughout their range remain poorly understood, our study extends results about the prevalence of *P. irritans* on burrowing owls to other states in the region beyond Idaho. In North America, *P. irritans* is mainly a parasite of large mammals showing a particular preference for carnivores (Lewis et al. 1988). For instance, Harrison et al. (2003) reported that *P. irritans* was among the most common fleas collected from foxes in New Mexico, where 58.3% of kit foxes (*Vulpes macrotis*), 73.3% of swift foxes (*V. velox*), and 11.1% of gray foxes (*Orocyon cinereoargenteus*) harbored *P. irritans*. McGee et al. (2006) found that *P. irritans* was the most prevalent and abundant flea on swift foxes in Rita Blanca National Grassland, Texas during a plague epizootic. Harris et al. (2014) found *P. irritans* on black-footed ferrets (*Mustela nigripes*) in Conata Basin, South Dakota but in far fewer numbers than the most abundant species *Oropsylla hirsuta*. Finally, Lewis et al. (1988) indicated that the majority of their records of *P. irritans* from the Pacific Northwest were from mule deer (*Odocoileus*

hemionus columbianus), but indicated occurrence was likely accidental rather than reflecting a true host preference.

In some cases, fleas on burrowing owls may be accidental associates from either the burrow environment or from their prey. For instance, Smith and Belthoff (2001) speculated that the small number of *Aetheca wagneri* and *Meringis hubbardi* on burrowing owls in Idaho were accidental associates because these species most commonly parasitize small rodents. Indeed, we also found a small number (<0.6%) of non-*Pulex* fleas characteristic of small mammals on owls in Idaho, Oregon, and Washington.

In that *P. irritans* occurs on burrowing owls so commonly, in widespread regions, and at times in large numbers, suggests that this flea species may have developed a closer relationship with burrowing owls that could include feeding. In fact, recent evidence from molecular blood meal assays has revealed burrowing owl DNA in *P. irritans* collected from burrowing owls (Graham et al., in prep.). Thus, rather than interacting with owls exclusively as a phoretic host that may move *P. irritans* fleas potentially infected with *Y. pestis* from place to place, *P. irritans* may have potential to bite and infect burrowing owls with *Y. pestis*. We know that *P. irritans* can frequently be infected with *Y. pestis* in portions of its range (Dennis et al. 1999, Ratovonjato et al. 2014), but it may not always be infective because it is not an easily blocked species (Burroughs 1947). However, Eisen et al. (2009) indicate that the importance of common human biting fleas (including *P. irritans*) as vectors of *Y. pestis* in places such as Africa may have been underestimated because early-phase transmission by unblocked fleas (cf. Eisen et al. 2006) was not considered. Indeed, Eisen et al. (2009) stress that *P. irritans* tops the list of additional flea species needing to be evaluated for early-phase transmission of *Y. pestis*.

In contrast to its habit of infesting carnivores in North America, the cosmopolitan *P. irritans* commonly infests human dwellings elsewhere. It is considered a potential plague vector in regions of Asia and Africa and was likely a primary vector in Europe during the Black Death (Drancourt et al. 2006). Laudisoit et al. (2007) found that *P. irritans* is the predominant species among domestic fleas in studies of plague in Tanzania, and it was the only species that inhabited every village and the only species to be significantly higher in high plague frequency villages. In Madagascar, *P. irritans* was also recently implicated in human-to-human transmission of plague, where it was the most commonly collected flea species, and both engorged and unfed male and female *P. irritans* carried *Y. pestis* (Ratovonjato et al. 2014). As we found no evidence of *Y. pestis* in the fleas we collected from burrowing owls, if and how effectively *P. irritans* can carry or transmit *Y. pestis* to burrowing owls, and the response of burrowing owls to such infection, remain to be determined. We suggest that future studies similar to ours conducted during plague epizootics in the western U.S. would clarify the potential relationships we describe or help confirm our negative results.

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Figure Legends

FIG. 1. Trail camera image taken 31 May 2012 of a burrowing owl (*Athene cunicularia*) nest in southwestern Idaho showing adults (flying) and nestlings near a nest burrow. Adult and juvenile burrowing owls were captured at nest burrows, fleas collected from their plumage, and owl blood obtained for analysis of potential exposure to *Yersinia pestis*, which is the organism that causes plague.

FIG. 2. Geographic coordinates and locations of 10 study sites in five western states (white) in which potential relationships between burrowing owls, fleas, and *Yersinia pestis* were examined. See text for explanations of study site abbreviations.

FIG. 3. Micrographs (10x) of (a) male and (b) female *Pulex irritans* collected from burrowing owls. Fleas vary in actual size from approximately 1.5 – 3.0 mm.

Figure 1.



Figure 2.

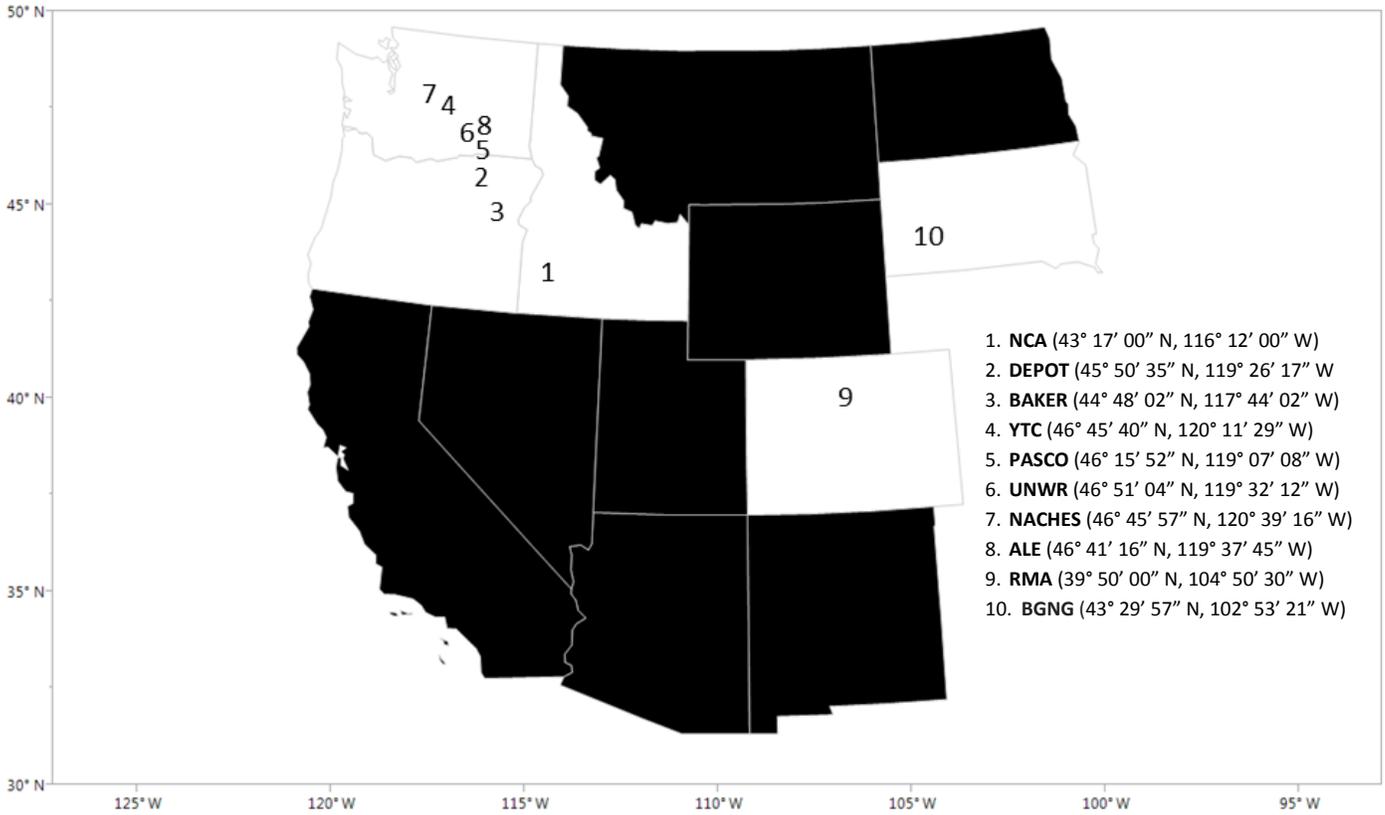


Figure 3.

(a)



(b)



Table 1. PCR primer sequences for amplification of *Y. pestis* in burrowing owl blood and fleas.

Sequence	Gene	Band Size (bp)	Reference
5' CCGGATGCTGAGTGGAAAG 3' 5' TCATGTGCCCGAACCCAGTC 3'	<i>pla</i> primary	553	This study
5' TCAGCCAGTTAGACTGGAAG 3' 5' GGGAAGTTTCCGGTATAAGC 3'	<i>pla</i> nested	361	This study
5' GCAGGTGGTGGCAAAGTGAG 3' 5' CTACCCGAGGATGCCATTC 3'	<i>lcrV</i> primary	585	This study
5' CCACCTGAATGGTGGTTTG 3' 5' TCGCCGAATACACAATGGG 3'	<i>lcrV</i> nested	358	This study
5' TACGGTTACGGTTACAGCAT 3' 5' GGTGATCCCATGTACTTAACA 3'	<i>caf</i>	240	Tomaso et al. (2003)
5' GATCAGGAGCCATGCACAA 3' 5' ACATTTGGCCTGAGAGATGTA 3'	<i>yop</i>	330	Tomaso et al. (2003)
5' AGGATCTCCCAAACATTCTGGC 3' 5' GAATCTGGACTAGATGACCTCC 3'	Owl1	335	Faircloth et al. (2010)

Table 2. Fleas collected from adult and nestling burrowing owls to evaluate prevalence of plague-causing *Yersinia pestis*.

Study Site ¹	State	Year	Owl Nest Sites	Total <i>Pulex irritans</i>	<i>Pulex irritans</i> Males	<i>Pulex irritans</i> Females	Non- <i>Pulex</i> Individuals	Total Fleas Collected
1. NCA	ID	2012	24	773	385	388	1	774
		2013	29	1679	869	810	7	1686
2. DEPOT	OR	2012	9	1037	562	475	16	1053
		2013	10	548	256	292	1	549
3. BAKER³	OR	2012	-	-	-	-	-	-
		2013	1	1	1	0	0	1
4. YTC²	WA	2012	3	32	19	13	0	32
		2013	-	-	-	-	-	-
5. PASCO³	WA	2012	-	-	-	-	-	-
		2013	4	106	31	75	0	106
6. UNWR^{3,4}	WA	2012	-	-	-	-	-	-
		2013	0	0	0	0	0	0
7. NACHES²	WA	2012	2	243	110	133	0	243
		2013	-	-	-	-	-	-
8. ALE	WA	2012	2	296	183	113	3	299
		2013	1	48	10	38	0	48
9. RMA³	CO	2012	-	-	-	-	-	-
		2013	1	1	0	1	0	1
10. BGNG^{3,5}	SD	2012	-	-	-	-	-	-
		2013	0	0	0	0	0	0
All Study Sites		2012-2013	86	4764	2426	2338	28	4792

¹See text for study site locations and abbreviations.

²Study site visited only in 2012.

³Study site visited only in 2013.

⁴Only owl blood was collected from this study site (See Table 3).

⁵Adult burrowing owls trapped in South Dakota (BGNG, n = 55) were devoid of fleas at the time of capture.

Table 3. Blood samples collected from adult (Male and Female) and nestling (pooled at each nest site) burrowing owls to evaluate prevalence of plague-causing *Yersinia pestis*.

Study Site ¹	State	Year	No. of Nest Sites Providing Blood Samples	No. Adult Males for PCR	No. Adult Females for PCR	No. of Pooled Nestling Samples For PCR ⁴	Number of Adults for Serology
1. NCA	ID	2012	37	4	28	37	-
		2013	38	23	29	26	44
2. DEPOT	OR	2012	9	1	1	8	-
		2013	15	11	10	-	24
3. BAKER³	OR	2012	-	-	-	-	-
		2013	8	5	4	-	10
4. YTC²	WA	2012	-	-	-	-	-
		2013	-	-	-	-	-
5. PASCO³	WA	2012	-	-	-	-	-
		2013	6	2	5	-	7
6. UNWR³	WA	2012	-	-	-	-	-
		2013	2	2	-	-	2
7. NACHES²	WA	2012	1	-	-	1	-
		2013	-	-	-	-	-
8. ALE	WA	2012	2	-	-	2	-
		2013	2	1	2	-	3
9. RMA³	CO	-	-	-	-	-	-
		2013	12	10	9	-	20
10. BGNG³	SD	2012	-	-	-	-	-
		2013	11	7	10	-	18
All Study Sites		2012-2013	143	66	98	74	128

¹See text for study site locations and abbreviations.

²Study site visited only in 2012.

³Study site visited only in 2013.

⁴Blood contributing to pooled sample was from 1 – 11 burrowing owl nestlings per site (mean = 6.2 ± 2.7 SD nestlings per pool).