DOI: 10.7589/2013-07-174 *Journal of Wildlife Diseases*, 50(2), 2014, pp. 384–388

A Rapid Field Test for Sylvatic Plague Exposure in Wild Animals

Rachel C. Abbott,[1] Robert Hudak,[2] Roy Mondesire,[3] Laurie A. Baeten,[4] Robin E. Russell,[1] and Tonie E. Rocke[1,5] [1]USGS National Wildlife Health Center, 6006 Schroeder Rd., Madison, Wisconsin 53711, USA; [2]Merlin Labs, Inc., 6082 Corte Del Cedro, Carlsbad, California 92011, USA; [3]RoMonics, LLC, 5343 Pinehurst Ct., Boulder, Colorado 80301, USA; [4]Veterinary Diagnostic Laboratory, Colorado State University, 1644 Campus Delivery, Fort Collins, Colorado 80523, USA; [5]Corresponding author (email: trocke@usgs.gov)

ABSTRACT: Plague surveillance is routinely conducted to predict future epizootics in wildlife and exposure risk for humans. The most common surveillance method for sylvatic plague is detection of antibodies to *Yersinia pestis* F1 capsular antigen in sentinel animals, such as coyotes (*Canis latrans*). Current serologic tests for *Y. pestis*, hemagglutination (HA) test and enzyme-linked immunosorbent assay (ELISA), are expensive and labor intensive. To address this need, we developed a complete lateral flow device for the detection of specific antibodies to *Y. pestis* F1 and V antigens. Our test detected anti-F1 and anti-V antibodies in serum and Nobuto filter paper samples from coyotes, and in serum samples from prairie dogs (*Cynomys ludovicianus*), lynx (*Lynx canadensis*), and black-footed ferrets (*Mustela nigripes*). Comparison of cassette results for anti-F1 and anti-V antibodies with results of ELISA or HA tests showed correlations ranging from 0.68 to 0.98. This device provides an affordable, user-friendly tool that may be useful in plague surveillance programs and as a research tool.

Key words: Coyote, lateral flow test, plague surveillance, prairie dog, *Yersinia pestis*.

Since its introduction to the US in 1900, *Yersinia pestis*, the causative agent of plague, has become established within wild rodents in the western states. The ecology of plague is complex and not completely understood. Epizootics occur unpredictably and cause high mortality in some rodent species, including prairie dogs (*Cynomys* spp.). Although human plague is rare in the US, humans are at greater risk of infection during rodent epizootics because of increased risk of bites from infected fleas (Gage and Kosoy 2005).

Plague surveillance is routinely conducted to predict future epizootics in wildlife and exposure risk for humans (Gage 1999). Carnivores, which can be infected with *Y. pestis* by flea bites or consuming infected prey, are often employed as sentinel animals and used in serosurveys to detect antibodies to *Y. pestis*. Sampling one carnivore is equivalent to sampling hundreds of rodents, making this a sensitive and cost-effective method of surveillance (Gage 1999). The coyote (*Canis latrans*), a species commonly used in serosurveys, is a wide-ranging omnivore that contacts various rodent species and their fleas during scavenging and predation. In general, coyotes are resistant to clinical disease following exposure to *Y. pestis* (Baeten et al. 2013). However, they develop anti–*Y. pestis* antibodies, providing a way to monitor plague activity in a region.

Currently carnivore surveillance programs for sylvatic plague rely on serologic analyses to measure antibodies to *Y. pestis* F1 capsular proteins (Chu 2000). The current hemagglutination (HA) test and enzyme-linked immunosorbent assay (ELISA) are labor intensive and must be conducted in laboratories by trained personnel. Results from these tests are often not available until months later. To better determine areas where plague is active in a timelier manner, a simple field serology test for plague antibodies is highly desired.

We are developing a rapid screening test for antibodies to *Y. pestis* F1 and V proteins, based on lateral flow technologies. We present results of our lateral flow test on serum and Nobuto strip samples from coyotes and serum from prairie dogs, lynx (*Lynx canadensis*), and black-footed ferrets (*Mustela nigripes*).

In the construction of our cassette (Fig. 1), recombinant F1 capsular protein

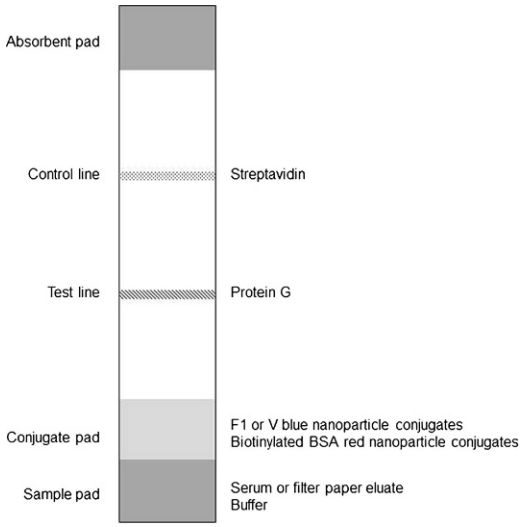

FIGURE 1. Schematic of the lateral flow cassette. *Yersinia pestis* antigens conjugated to blue nanoparticles and biotinylated bovine serum albumin (BSA) conjugated to red nanoparticles are impregnated onto the conjugate pad. Sample and buffer solution are added to the sample pad and flow through the nitrocellulose membrane. Anti-F1 or anti-V antibodies bind to the corresponding antigen nanoparticles, and the complexes are captured at the test line by protein G. Biotinylated BSA nanoparticles are captured at the control line by the immobilized streptavidin.

and recombinant virulence-associated V antigen were prepared as described previously (Heath et al., 1998; Little et al., 2008). Purified F1 and V proteins were each conjugated to blue carboxylate-modified nanoparticles (CMLNPs) using a two-step 1,ethyl-3-(3-dimethylaminopropyl) carbodiimide/N-hydroxysuccinimide coupling.

Biotin was conjugated to bovine serum albumin and coupled to red CMLNPs, following the protocol described above. Coupled nanoparticles were impregnated onto Whatman 8975 pads. The pads were dried at 37 C and stored desiccated. Protein G was deposited at the test line region. Streptavidin was deposited at the control line region. The membranes were dried at 37 C and stored desiccated. Strips were cut in 4-mm widths, assembled into plastic housings, placed in poly-foil pouches with desiccants, and heat-sealed.

Serum samples were obtained from eight captive coyotes one to four times before and after experimental infection with *Y. pestis* (Baeten et al. 2013). Advantec Nobuto blood filter strips (Cole-Parmer, Vernon Hills, Illinois, USA) of coyote whole blood were obtained (one strip/coyote from duplicate samples collected) from the archives of the National Wildlife Disease Program; the samples were collected from adult coyotes during carnivore surveillance activities in three western states (Montana, Colorado, and New Mexico, USA) in 2010. All serum and duplicate filter paper samples had been tested by HA test for detection of anti-F1 antibodies by the source laboratories.

Serum samples were obtained from 26 captive black-tailed prairie dogs (*Cynomys ludovicianus*) after vaccination with an experimental plague vaccine (Rocke et al. 2010) and surviving challenge with virulent *Y. pestis*; six animals had only one sample. Serum samples were obtained from 27 captive Canada lynx (Wolfe et al. 2011) before and after vaccination with the F1-V fusion protein vaccine (Powell et al. 2005) and from 23 wild-caught vaccinated black-footed ferrets. Antibody titers against *Y. pestis* F1 and V antigens were measured previously by ELISA for prairie dog, lynx, and ferret serum samples.

Nobuto strips were eluted by cutting the absorbent segments into five or six pieces directly into 1.5 mL microcentrifuge tubes using clean scissors. We added 400 μL phosphate buffered saline to each tube. Tubes were agitated at 4 C overnight for complete elution. Eluates were pipetted into new 1-mL microcentrifuge tubes and stored at −20 C.

We added 5 μL of serum or eluate to the sample well and five drops of buffer to the buffer well and read results after 15 min and again at 30 min. Results were recorded on a subjective scale: Negative = no blue sample line; 1+ = faint blue line; 2+ = light blue line; 3+ = medium blue line; 4+ = dark blue line. Tests were

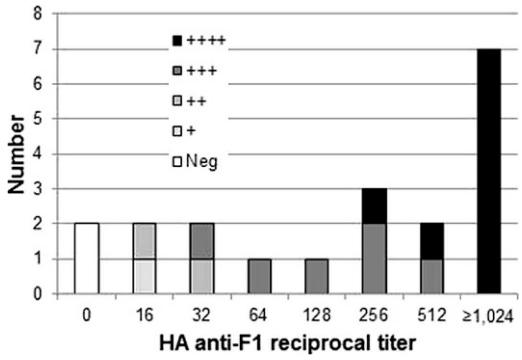

FIGURE 2. Correlation between lateral flow cassette results and hemagglutination (HA) test titers of antibodies against *Yersinia pestis* F1 antigen in sera ($n=20$) taken from coyotes (*Canis latrans*) experimentally infected with *Y. pestis*. The number of samples with corresponding cassette result and titer appears on the *y* axis. The quantitative relationship between cassette results and HA titer levels was strong (0.98±0.01 SE by polyserial correlation).

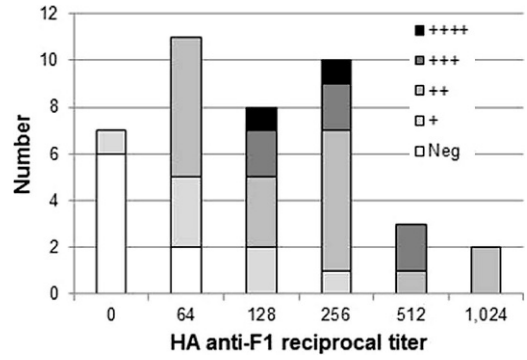

FIGURE 3. Correlation between lateral flow cassette results and hemagglutination (HA) test titers of antibodies against *Yersinia pestis* F1 antigen in Nobuto strip eluates ($n=41$) taken from coyotes (*Canis latrans*) sampled during plague surveillance activities. The number of samples with corresponding cassette result and titer appears on the *y* axis. The quantitative relationship between cassette results and HA titer levels was moderate (0.75±0.07 SE by polyserial correlation).

considered invalid if no pink control line appeared in the cassette.

Cassette results for F1 antigen and V antigen were compared with results of ELISA or HA tests using the function polyserial in the R library "polycor" (Fox 2010). This function allowed us to determine correlations between the ordinal cassette results and the titer concentrations determined by ELISA or HA tests. We log transformed the titer levels (log2) prior to analysis.

The cassettes detected anti-F1 antibodies in coyote serum and eluates. For serum samples, the test scoring system reflected the antibody titers obtained by HA testing (Fig. 2). The cassettes detected anti-F1 HA titers as low as 1:16, and the polyserial correlations were high (0.98±0.01 SE) indicating the quantitative relationship between the cassette results and titer levels was strong. For eluates, there were two equivocal samples that had negative results on the lateral flow test but HA titers of 1:64 (titers≥1:32 are considered positive). The cassette results for eluates did not reflect antibody titers as clearly as those for serum samples (Fig. 3), and the polyserial correlations were lower (0.75±0.07 SE). The cassettes also detected anti-V antibodies in serum and eluates from coyotes (Table 1). Unfortunately no ELISA results were available for comparison.

The cassettes detected anti-F1 and anti-V antibodies in prairie dog, lynx, and black-footed ferret sera. In prairie dogs, the cassettes detected anti-F1 and anti-V ELISA titers ≥1:160, the lowest dilution tested (Fig. 4), and the polyserial correlations were 0.82±0.06 SE and 0.68±0.10 SE, respectively. For lynx and ferrets, the cassettes detected titers ≥1:2,560, and the polyserial correlations were high (>0.98) for tests on both species.

Lateral flow devices for measurement of antibodies offer an affordable and rapid alternative to current methodologies (Zhang et al. 2009). We developed a simple, rapid, and affordable screening test for plague antibodies in animal sera and filter paper eluates based on lateral flow technologies using two *Y. pestis* antigens, F1 and V proteins. Our lateral flow test detected anti-F1 and anti-V antibodies in samples from coyotes, prairie dogs, black-footed ferrets, and lynx, and the correlation of cassette results to standard serologic tests is fairly strong

TABLE 1. Results of lateral flow cassettes in detecting antibodies to *Yersinia pestis* V-antigen in sera and Nobuto filter paper strip eluates from experimentally and naturally infected coyotes (*Canis latrans*), respectively. No enzyme-linked immunosorbent assay antibody titers were available for comparison.

Lateral flow cassette result	No. coyote sera[a]	No. coyote Nobuto strip eluates[a]
Negative	4	29
+	10	7
++	5	3
+++	0	0
++++	0	1

[a] One serum sample and one Nobuto strip eluate had invalid results on the cassettes and are not included in this table.

for these species. The ease and timeliness with which the test can be performed make it applicable for field use or as a screening test prior to antibody titration by HA or ELISA.

Nobuto strips offer several advantages over serum samples; they provide a simple method of blood collection that requires no refrigeration, is easily transported, and involves minimal personnel training. Other studies have demonstrated good agreement between serum and Nobuto strip eluate anti–*Y. pestis* antibody titers (Wolff and Hudson 1974). However, elution of Nobuto strips requires a 1:10 dilution of the sample. Thus, animals with low titers may have negative results. Nevertheless, the advantages of Nobuto strips may outweigh this loss depending on the situation.

Another rapid immunochromatography-based test has been developed for detection of *Y. pestis* anti-F1 antibodies in several species, including dogs (*Canis lupus familiaris*), rats (*Rattus* spp., *Psammomys* spp.), mice (*Mus* spp., *Lemniscomys* spp.), shrews (*Suncus* spp.), hedgehogs (*Atelerix* spp.), and humans (Rajerison et al. 2009). The use of both F1 and V antigens in our device should provide a higher predictive value than other field tests for plague exposure in animals. Detection of antibodies to *Y. pestis* proteins other than F1 may be useful in determining antibody prevalence in wild animals and avoiding false negative results. Vernati et al. (2011) showed that 65% of coyote serum samples from Wyoming that were antibody-positive by ELISA using whole-cell extracts were negative for anti-F1 antibodies by Western blot. However, 90% of these samples were reactive with at least one other *Y. pestis* antigen, LcrV, YpkA, YopD, and Pla. Use of additional antigens, in addition to F1, in serologic tests may limit false-negative results, especially for cases involving *Y. pestis* strains that do not express F1 antigen (Friedlander et al. 1995).

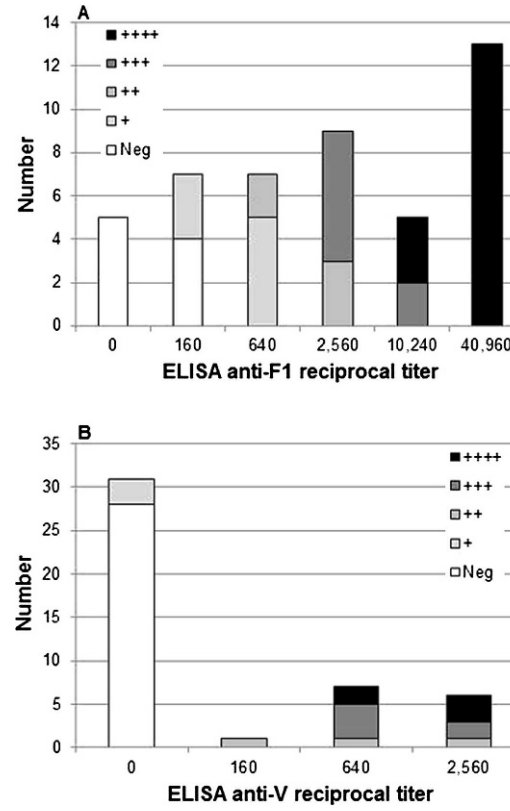

FIGURE 4. Correlation between lateral flow cassette results and enzyme-linked immunosorbent assay (ELISA) titers of antibodies against *Yersinia pestis* F1 (A; $n=46$) and V (B; $n=45$) antigens in sera taken from prairie dogs (*Cynomys ludovicianus*) vaccinated against and experimentally infected with *Y. pestis*. The number of samples with corresponding cassette result and titer appears on the *y* axis. The quantitative relationship between cassette results and ELISA titer levels was moderate (0.82 ± 0.06 SE and 0.68 ± 0.10, respectively, by polyserial correlation).

Lateral flow cassettes may allow for more intensive sampling in plague surveillance programs due to faster results and ease of use. More timely access to increased prevalence data would enable field personnel, managers, and public health authorities to predict areas of increased plague risk with more accuracy and, thus, better target limited resources to prevent human and wildlife plague exposures. Our cassettes detected anti-F1 and anti-V antibodies in several species including two carnivores and a rodent. The ability to detect plague antibodies in multiple species will be useful in plague surveillance when a variety of species are sampled, as well as in research applications.

The cassettes are currently being used as a screening test to detect anti-F1 and anti-V antibodies in Nobuto strip samples collected from prairie dogs in a large field study; ELISA will be used to confirm positive samples. Evaluation of the cassettes using whole blood is also planned.

We thank Tom DeLiberto and John Baroch, USDA Wildlife Services, for sending coyote Nobuto strips from the USDA archives and sharing HA results and Judy Williamson for ELISA assays. Ferret samples were sent by Travis Livieri of Prairie Wildlife Research, and lynx samples came from Lisa Wolfe of Colorado Parks and Wildlife. Use of trade, product, or firm names does not imply endorsement by the US government. Editorial comments of Christopher Brand, Christopher Johnson, and two anonymous reviewers were greatly appreciated.

LITERATURE CITED

Baeten LA, Young J, Pappert R, Schriefer ME, Gidlewski T, Kohler D, Bowen RA. 2013. Immunological and clinical response of coyotes (*Canis latrans*) to experimental inoculation with *Yersinia pestis*. *J Wildl Dis* 39:932–939.

Chu M. 2000. *Laboratory manual of plague diagnostic tests*. US Dept. of Health and Human Services, Washington, DC, 129 pp.

Fox J. 2010. *Polycor: Polychoric and polyserial correlations*. R package version 0.7-8. http://CRAN.R-project.org/package=polycor. Accessed December 2013.

Friedlander AM, Welkos SL, Worsham PL, Andrews GP, Heath DG, Anderson GW Jr., Pitt MLM, Estep J, Davis K. 1995. Relationship between virulence and immunity as revealed in recent studies of the F1 capsule of *Yersinia pestis*. *Clin Infect Dis* 21(Suppl): S178–S181.

Gage KL. 1999. Plague surveillance. In: *Plague manual: Epidemiology, distribution, surveillance and control*. World Health Organization, Geneva, Switzerland, pp. 135–166. http://www.who.int/csr/resources/publications/plague/WHO_CDS_CSR_EDC_99_2_EN/en/index.html. Accessed June 2014.

Gage KL, Kosoy MY. 2005. Natural history of plague: Perspectives from more than a century of research. *Annu Rev Entomol* 50:505–528.

Heath DG, Anderson GW Jr., Mauro JM, Welkos SL, Andrews GP, Adamovicz J, Friedlander AM. 1998. Protection against experimental bubonic and pneumonic plague by a recombinant capsular F1–V antigen fusion protein vaccine. *Vaccine* 16:1131–1137.

Little SF, Webster WM, Wilhelm H, Powell B, Enama J, Adamovicz JJ. 2008. Evaluation of quantitative anti-F1 IgG and anti-V IgG ELISAs for use as an in vitro-based potency assay of plague vaccine in mice. *Biologicals* 36:287–295.

Powell BS, Andrews GP, Enama JT, Jendrek S, Bolt C, Worsham P, Pullen JK, Ribot W, Hines H, Smith L, et al. 2005. Design and testing for a non-tagged F1-V fusion protein as vaccine antigen against bubonic and pneumonic plague. *Biotechnol Prog* 21:1490–1510.

Rajerison M, Dartevelle S, Ralafiarisoa LA, Bitam I, Tuyet DTN, Andrianaivoarimanana V, Nato F, Rahalison L. 2009. Development and evaluation of two simple, rapid immunochromatographic tests for the detection of *Yersinia pestis* antibodies in humans and reservoirs. *PLoS Negl Trop Dis* 3:e421.

Rocke TE, Pussini N, Smith SR, Williamson J, Powell B, Osorio JE. 2010. Consumption of baits containing raccoon pox-based plague vaccines protects black-tailed prairie dogs (*Cynomys ludovicianus*). *Vector Borne Zoonot Dis* 10:53–58.

Vernati G, Edwards WH, Rocke TE, Little SF, Andrews GP. 2011. Antigenic profiling of *Yersinia pestis* infection in the Wyoming coyote (*Canis latrans*). *J Wildl Dis* 47:21–29.

Wolfe LL, Shenk TM, Powell B, Rocke TE. 2011. Assessment of a recombinant F1-V fusion protein vaccine intended to protect Canada lynx (*Lynx canadensis*) from plague. *J Wildl Dis* 47:888–892.

Wolff KL, Hudson BW. 1974. Paper-strip blood-sampling technique for the detection of antibody to the plague organism *Yersinia pestis*. *Appl Microbiol* 28:323–325.

Zhang G, Guo J, Wang X. 2009. Immunochromatographic lateral flow strip tests. *Methods Mol Biol* 504:169–183.

Submitted for publication 18 July 2013.
Accepted 31 October 2013.