PCR Detection of *Yersinia pestis* in Fleas: Comparison with Mouse Inoculation

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The “gold standard” for identifying *Yersinia pestis*-infected fleas has been inoculation of mice with pooled flea material. Inoculated mice are monitored for 21 days, and those that die are further analyzed for *Y. pestis* infection by fluorescent-antibody assay and/or culture. PCR may provide a more rapid and sensitive alternative for identifying *Y. pestis* in fleas. To compare these assays, samples were prepared from 381 field-collected fleas. Each flea was analyzed individually by both PCR and mouse inoculation. Sixty of the 381 flea samples were positive for *Y. pestis* by PCR; 48 of these PCR-positive samples caused death in mice (80.0% agreement). None of the 321 PCR-negative samples caused death. Among the 12 mice that survived inoculation with PCR-positive samples, 10 were later demonstrated by serology or culture to have been infected with *Y. pestis*. This suggests that death of inoculated mice is less reliable than PCR as an indicator of the presence of *Y. pestis* in flea samples. Mouse inoculation assays produce results that are comparable to PCR only when surviving as well as dead mice are analyzed for infection. The rapidity and sensitivity (10 to 100 CFU of *Y. pestis*) of PCR suggest that it could serve as a useful alternative to mouse inoculation for routine plague surveillance and outbreak investigations.

*Yersinia pestis*, the etiological agent of plague, is typically transmitted between rodent hosts and other mammals by the bites of infectious fleas. The risks of human infection are highest during periods of epizootic activity in local rodent populations (9). These risks are reduced by public education and the implementation of measures to limit the spread of plague, including the use of insecticides and rodent management techniques. Timely application of preventative measures is made possible by effective surveillance programs that are capable of rapidly identifying epizootics. For surveillance and outbreak investigation purposes, fleas are often easier and safer to collect and handle than animals potentially infected with *Y. pestis* or other pathogens (5). Data on which host and flea species are involved in a given epizootic also can provide valuable information for designing locally appropriate control programs.

The “gold standard” for testing fleas for *Y. pestis* infection has been the inoculation of mice with ground flea suspensions (14, 15). Typically, inoculated mice are monitored for 21 days, and tissues from mice that die are tested by fluorescent-antibody analysis for evidence of *Y. pestis* infection (8). Surviving mice are not routinely tested because mice are extremely susceptible to most wild-type strains of *Y. pestis* (50% lethal dose, 1 to 100 organisms) (14), and considerable time and personnel are required to process these additional samples. Mouse assays usually require at least 3 days, and often more, until infected mice succumb to *Y. pestis* infection; this creates a considerable delay in obtaining results from *Y. pestis*-infected flea samples. Also, false-negative results can occur when death is used as the assay endpoint, because some mice occasionally survive infection with *Y. pestis* (4).

Recently, molecular techniques have been proposed as a means of more rapidly identifying plague bacteria in fleas. DNA hybridization probes were developed but were unable to reliably detect fewer than 10⁷ plague bacteria (13, 17). More recently, several PCR assays have been developed for plague diagnosis (1, 7, 11). These assays provide much greater sensitivity than the above DNA probe techniques. Identification of *Y. pestis* in fleas by PCR has been described by Hinnebusch and Schwan (7). Although this assay decreases the time necessary to detect bacteria in fleas, it has not been compared to the standard mouse inoculation assay. We report here a modification of the PCR assay described by Hinnebusch and Schwan (7) and a comparison of this assay with mouse inoculation.

**MATERIALS AND METHODS**

*Y. pestis* strains. To test the sensitivity of the PCR assay, the virulent strain CO96-3188 was used. The avirulent A1122 strain was used as a positive control for all PCR assays involving field-collected fleas. *Y. pestis* strains were grown in brain heart infusion (BHI) broth at 28°C and harvested during log-phase growth. Serial 10-fold dilutions were then spread on blood-agar plates to determine the number of CFU, according to standard methods.

Flea samples. For comparison of mouse inoculation and PCR assays, 381 fleas of 12 different species were collected at various locations in Colorado and New Mexico from rodent burrows, domestic animals, captured animals, or animal carcasses (Table 1). Following collection, fleas were stored individually at 70°C or in pools in 2% saline held at 4°C. Epizootic plague activity was identified or suspected at each of the locations where fleas were collected. Fleas for the sensitivity assays were from a colony of *Oropsylla montana* which was derived from fleas originally collected in Bernalillo County, New Mexico, in 1992.

Preparation of fleas for PCR and mouse inoculation. Fleas for comparison of PCR and mouse inoculation assays were prepared by triturating individual fleas in 100 µl of BHI broth in 1.5-ml microcentrifuge tubes with sterile sand and disposable pestles. Fleas pooled and stored in saline at the time of collection were individually washed in sterile saline before trituration. Fifty microliters of the ground flea suspension was heated to 95°C for 10 min and then immediately centrifuged for 10 s at maximum speed (15,600 × g) to pellet flea tissue and sand. The supernatants were assayed by *pla* PCR within 10 min. Leftover supernatants were stored at −70°C.

Fleas used for testing the sensitivity of the PCR assay were triturated in six separate tubes containing 100 µl of BHI broth spiked with 10⁴, 10⁵, 10⁶, 10⁷, and 10⁸ CFU of *Y. pestis* CO96-3188. The triturated flea material was then
TABLE 1. Location, collection type, species, and number of fleas used in comparison between PCR and mouse inoculation

<table>
<thead>
<tr>
<th>Location and collection type</th>
<th>Species</th>
<th>No. tested</th>
</tr>
</thead>
<tbody>
<tr>
<td>Applewood Estates, Colo.</td>
<td>Oropsylla hirsuta</td>
<td>113</td>
</tr>
<tr>
<td>Rodent burrow</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lighthouse Church, Colo.</td>
<td>Oropsylla hirsuta</td>
<td>27</td>
</tr>
<tr>
<td>Rodent burrow</td>
<td>Oropsylla tuberculata cynomuris</td>
<td>21</td>
</tr>
<tr>
<td>Tribly Creek, Colo.</td>
<td>Oropsylla hirsuta</td>
<td>29</td>
</tr>
<tr>
<td>Rodent burrow</td>
<td>Oropsylla tuberculata cynomuris</td>
<td>7</td>
</tr>
<tr>
<td>Devil’s Backbone, Colo.</td>
<td>Oropsylla hirsuta</td>
<td>43</td>
</tr>
<tr>
<td>Cedar Grove, N. Mex.</td>
<td>Oropsylla montana</td>
<td>24</td>
</tr>
<tr>
<td>Rodent burrow</td>
<td>Hoplopalpus anomalus</td>
<td>6</td>
</tr>
<tr>
<td>Domestic animal</td>
<td>Hoplopalpus anomalus</td>
<td>1</td>
</tr>
<tr>
<td>Domestic animal</td>
<td>Cedilopysia inaequilis</td>
<td>4</td>
</tr>
<tr>
<td>Captured animal</td>
<td>Aetheca wagneri</td>
<td>1</td>
</tr>
<tr>
<td>Captured animal</td>
<td>Orchopeas leucopus</td>
<td>1</td>
</tr>
<tr>
<td>Abiquiu, N. Mex.</td>
<td>Aetheca wagneri</td>
<td>2</td>
</tr>
<tr>
<td>Captured animal</td>
<td>Orchopeas leucopus</td>
<td>2</td>
</tr>
<tr>
<td>Captured animal</td>
<td>Orchopeas sexdentatus</td>
<td>2</td>
</tr>
<tr>
<td>Captured animal</td>
<td>Malaneus simonius</td>
<td>1</td>
</tr>
<tr>
<td>Carson, N. F., N. Mex.</td>
<td>Aetheca wagneri</td>
<td>51</td>
</tr>
<tr>
<td>Captured animal</td>
<td>Cedilopysia inaequilis</td>
<td>4</td>
</tr>
<tr>
<td>Captured animal</td>
<td>Orchopeas leucopus</td>
<td>3</td>
</tr>
<tr>
<td>Captured animal</td>
<td>Malaneus simonius</td>
<td>1</td>
</tr>
<tr>
<td>Captured animal</td>
<td>Euthropalpus glaciatus</td>
<td>1</td>
</tr>
<tr>
<td>Captured animal</td>
<td>Epitelia sp.</td>
<td>1</td>
</tr>
<tr>
<td>C.F. Prairie, Colo.</td>
<td>Oropsylla hirsuta</td>
<td>6</td>
</tr>
<tr>
<td>Animal carcass</td>
<td>Oropsylla tuberculata cynomuris</td>
<td>30</td>
</tr>
</tbody>
</table>

heated and pelleted as described above. Pools of 0, 1, 5, 10, 20, and 25 fleas were used per sample.

PCR amplification targets. Primer sequences were the same as those described by Hinnebusch and Schwan (7) and were derived from the published sequence data for the plasminogen activator gene (pla) (16). These primers, Yp1 (5'-ATCTTACACTTCTCCGAGAA-3') and Yp2 (5'-CTTGGATGTTGAGCTTCCA-3'), correspond to nucleotides 971 to 990 and 1431 to 1450, respectively, and produce a 478-bp amplification product. All PCR tests with the pla primers will hereafter be referred to as pla PCR assays. A confirmatory PCR assay used primers targeting the caf1 gene of Y. pestis, as described by Chu et al. (2). These primers correspond to nucleotides 1 to 18 and 496 to 513 of the caf1 gene, which encodes the structural region of the F1 antigen (6), and produce a 513-bp amplification product. All PCR tests with the caf1 primers will hereafter be referred to as caf1 PCR assays.

PCR. The pla PCR protocol used in this study is a modification of the protocol described by Hinnebusch and Schwan (7). Briefly, for each PCR assay, 2.5 μl of the flea-BHI preparation was combined with 0.25 μl of each primer (Yp1 and Yp2, 30 pmol of primer/μl), 50 mM MgCl2, and 21.75 μl of denatured, distilled water in a 0.65-ml tube containing a Ready-To-Go PCR bead (Amersham Pharmacia Biotech, Piscataway, N.J.). The PCR bead contained 1.5 U of Taq DNA polymerase, 10 mM Tris HCl, 1.5 mM MgCl2, 200 μM (each) deoxynucleoside triphosphate (dNTP) and stabilizers, including bovine serum albumin. Positive controls were prepared by adding 2.5 μl of Y. pestis AT122 to the above reagents, and negative controls were prepared by adding 2.5 μl of sterile BHI to the above reagents. Two drops of sterile mineral oil were then added to the tubes before they were sealed and placed in a thermocycler (Minicycler; MJ Research, Watertown, Mass.) with the following amplification program: initial denaturation at 95°C for 5 min, followed by 35 cycles of denaturing at 95°C for 1 min, annealing at 51°C for 1 min, and primer extension at 72°C for 2 min. After the last cycle, primer extension was continued at 72°C for 10 min. The preparation of the caf1 PCR samples for the confirmatory PCR, with caf1 primers, was the same as described above, but the denaturation, annealing, and extension steps of the thermocycler program were done according to Chu et al. (2).

Mouse inoculation and sample collection. Three hundred microliters of each 400-μl flea-saline suspension was inoculated subcutaneously in the lower abdomen of individual white laboratory (Swiss Webster) mice (one mouse/flea suspension). Following inoculation, mice were labeled and held in cages and supplied with food and water ad libitum. Mice were checked twice daily for signs of morbidity or mortality. Mice that died within 21 days after inoculation were stored at −20°C prior to a postmortem examination, which included macroscopic observations, and collection of portions of these organs or nodes for bacterial examination. Surviving mice were held for 21 days before tests were canceled. Postmortem tissue samples were taken from all mice that were inoculated with pla PCR-positive fleas and survived to day 21. Blood samples also were collected from these mice and from a random sampling of 30 mice inoculated with PCR-negative fleas. All experiments with mice were done according to a protocol approved by the Division of Vector Borne Infectious Disease’s Animal Care and Use Committee (AUC no. 97-09-008-AM).

PIA. The passive hemagglutination assay (PHA) was used to examine mouse serum samples for anti-F1 antibodies. Samples with titers greater than 1:10 were considered to be positive. This methodology, which is described elsewhere (18), was selected because it is the standard serological assay used by the World Health Organization Collaborating Center on Plague at the Centers for Disease Control and Prevention.

FA testing. Impression smears of mouse tissues were analyzed by fluorescent-antibody (FA) assays as a presumptive test for the presence of Y. pestis cells. The FA tests were conducted as described elsewhere (3).

Bacterial culture. To obtain bacterial isolates from mouse tissue, small pieces of spleen, liver, and lymph node tissues were streaked on sheep blood (6%) agar plates. The suspension was also streaked on blood agar plates. All plates were incubated at 37°C for at least 48 h and examined for characteristic colonial morphology consistent with Y. pestis growth. Confirmation of identity of the isolate was by a positive FA test, biochemical profiles, and by evidence of lysis with Y. pestis-specific bacteriophage at both 25°C and 37°C (3). Bacteriophage lysis assays were done at both temperatures to eliminate false-positive reactions with Yersinia pseudotuberculosis, which can occur at 37°C but not at 25°C.

RESULTS

Sensitivity and pooling. Our modification of the pla PCR assay appeared to have sensitivity comparable to that of the original assay described by Hinnebusch and Schwan (7) and was able to detect as few as 10 CFU per sample, as determined by testing 10-fold serial dilutions of Y. pestis suspended in BHI broth containing ground, uninfected flea material (data not shown). The sensitivity of this assay did not noticeably change when up to 20 fleas were suspended in each serial dilution. Consistent results were not obtained, however, when 25 fleas per pool were used.

PCR-mouse inoculation comparison. Of the 381 field-collected flea samples tested by pla PCR, 60 were positive (i.e., contained a predicted 478-bp fragment that appeared identical in size to the one observed in the positive controls) (Table 2). The pla PCR-positive specimens included 28 of 208 (13.5%) Oropsylla hirsuta specimens and 32 of 58 (55.2%) O. tuberculata cynomuris specimens tested. None of the other flea species in our study were pla PCR positive. Figure 1 shows an example of the pla PCR assay results for flea samples collected in northern Colorado.

Of the 60 pla PCR-positive flea suspensions, 48 caused death in mice; all dead mice were positive by FA for Y. pestis infection. This resulted in 80% agreement between PCR and mouse
inoculation when death was used as an endpoint for the assay. Of the 12 mice that survived inoculation with \( \text{pla} \) PCR-positive flea suspensions, 10 had positive PHA serology titers (range, 1:32 to 1:8,192) at the end of the 21-day test period. When killed on day 21, 5 of these 10 mice also were determined by FA assay to have \( Y. \) \( \text{pestis} \) in the liver, spleen, or lymph nodes. Additionally, \( Y. \) \( \text{pestis} \) was isolated from the tissues of four of these five FA-positive mice. Overall, inoculation of mice with 58 of the 60 \( \text{pla} \) PCR-positive flea suspensions produced a positive FA assay, PHA serology, or \( Y. \) \( \text{pestis} \) isolate from mouse tissues (96.7% agreement). Both of the \( \text{pla} \) PCR-positive flea suspensions that did not produce a detectable infection in mice were positive when retested by \( \text{caf} \) PCR. \( Y. \) \( \text{pestis} \) also was isolated directly from one of these two flea suspensions. All PCR-negative flea suspensions were negative by mouse inoculation (100% agreement) when death of mouse was used as the endpoint. Additionally, each of 30 randomly selected serum samples from mice inoculated with \( \text{pla} \) PCR-negative flea suspensions was negative by PHA.

**DISCUSSION**

We have shown that our modification of the \( \text{pla} \) PCR assay has many advantages over mouse inoculation for identifying \( Y. \) \( \text{pestis} \) in fleas, including its sensitivity and rapidity. Hinnebusch and Schwan (7) demonstrated that the \( \text{pla} \) primers used in our study were suitable for amplifying target DNA from \( Y. \) \( \text{pestis} \) strains collected in Asia, Africa, and the Americas, suggesting that PCR assays based on these primers should have wide applicability for surveillance purposes and epidemiological investigations. These authors also indicated that the \( \text{pla} \) primers did not produce false positives when tested with samples of \( Y. \) \( \text{pseudotuberculosis} \), \( Y. \) \( \text{enterocolitica} \), and \( R. \) \( \text{typhi} \). The translated coding region of the \( \text{pla} \) gene has some degree of homology with the \( \text{ompT} \) gene of \( E. \) \( \text{coli} \) (16), but the \( \text{pla} \) primers used in this study target the 3' noncoding region of the plasminogen activator gene. A comparison of the \( \text{pla} \) primer sequences used in this study showed no homology with the \( \text{ompT} \) gene sequence (GenBank accession no. X06903).

The sensitivity of the \( \text{pla} \) PCR assay (10 to 100 CFU of \( Y. \) \( \text{pestis} \)) did not appear to diminish noticeably when assays were done with pools of as many as 20 fleas, which is only slightly less than the maximum of 25 fleas per pool usually recommended for the mouse inoculation assay (15). Testing to date has indicated that our \( \text{pla} \) PCR results are less reliable when the pool size is increased to 25 fleas. Although positives were occasionally obtained with samples that contained 25 fleas and were spiked with as few as 10 CFU, the 100 \( \mu \)l of BHI appeared to be insufficient to suspend that amount of flea material. This difficulty in suspending flea material may partially explain the inconsistent results. Consistent results may be obtainable by further modifying the procedures used to prepare the suspensions of flea material, but this type of modification was not evaluated during our study.

Our results suggest that the mouse inoculation assay can produce false-negative results when death is used as an endpoint, as indicated by the fact that only 80% of the mice that were inoculated with \( \text{pla} \) PCR-positive flea suspensions actually died. Among the 12 mice that survived inoculation with \( \text{pla} \) PCR-positive flea suspensions, 10 were later demonstrated by serology or bacterial isolation to have been infected with \( Y. \) \( \text{pestis} \). These 10 flea suspensions might have contained insufficient \( Y. \) \( \text{pestis} \) to produce a fatal infection in mice or might have contained bacteria of lower virulence than typical wild-type strains. Regardless of why some mice failed to die when inoculated with \( Y. \) \( \text{pestis} \)-infected flea suspensions, \( \text{pla} \) PCR reliably detected \( Y. \) \( \text{pestis} \) in these samples. The results of \( \text{pla} \) PCR and mouse inoculation, however, agreed closely (96.7%) when all mice, including those that survived to 21 days, were analyzed for evidence of \( Y. \) \( \text{pestis} \) infection. It is important that all the PCR-positive fleas were collected either from prairie dog burrows in areas of suspected plague activity or from a dead prairie dog known to have died from plague. Although \( Y. \) \( \text{pestis} \) can be isolated occasionally from fleas collected from apparently healthy animals, all such fleas examined during our study were PCR negative.

Additional testing of the two \( \text{pla} \) PCR-positive mouse inoculation-negative flea suspensions clearly demonstrated or strongly suggested that these suspensions did indeed contain \( Y. \) \( \text{pestis} \). Both flea suspensions were also positive by \( \text{caf} \) PCR.
Although culturing \textit{Y. pestis} directly from flea material is often ineffective because of contamination (15), we were able to isolate \textit{Y. pestis} from one of the \textit{pla} and \textit{caf1} PCR-positive, mouse inoculation-negative flea suspensions, providing clear proof that this flea was infected with \textit{Y. pestis}. We were unable to isolate \textit{Y. pestis} from the remaining \textit{pla} and \textit{caf1} PCR-positive flea suspension. Although, as with all PCR assays, the positive \textit{pla} and \textit{caf1} PCR results obtained from the culture-negative sample could have resulted from an external source of contamination, it is possible that this flea suspension contained dead bacteria with sufficient amounts of intact \textit{Y. pestis} DNA to be amplified by PCR. However, it is unlikely that the positive result for this last flea suspension was due to ampiclon contamination, because we obtained positive results with two different primer sets (\textit{pla} and \textit{caf1}), including one (\textit{caf1}) not previously used in our laboratory.

In addition to standard precautions taken to minimize possible sources of contamination in PCR assays (10), we took further steps to ensure sample quality. For example, field-collected fleas are often stored under various conditions, including on dry ice (−70°C), in 2% saline with Tween 80 detergent, or in 70% ethyl alcohol. Prior to this study, we found that the 2% saline-Tween 80 solutions used to store infected fleas occasionally tested positive for \textit{Y. pestis} by PCR and mouse inoculation (data not shown). Contamination of the 2% saline, therefore, is likely to result in the contamination of other fleas stored in the same container. These findings led us to wash fleas stored in saline solutions in fresh, sterile, 2% saline before triturating these samples for PCR, which appeared to eliminate the false-positive results occasionally obtained with unwashed flea material. Other methods, however, may prove superior for removing foreign DNA from the outside of fleas stored under this condition. Obviously, fleas stored in 70% alcohol cannot be tested by mouse inoculation but are suitable for PCR. It seems reasonable that fleas stored in alcohol also should be rinsed thoroughly prior to analysis by PCR to minimize cross-contamination.

Storage of fleas in alcohol is likely to be advantageous under certain circumstances, including those situations in which fleas are to be held without freezing for long periods (more than a few days) prior to analysis by PCR. We obtained good results when fleas were held dry on dry ice and at −70°C in an ultra-low freezer. Immediate freezing of samples reduces handling of the specimens, thereby reducing the risk of contamination. Our modified PCR protocol provided a simpler assay that required fewer steps to complete than the one described initially by Hinnebusch and Schwan (7). The PCR beads used with the mineral oil appeared to result in an assay whose sensitivity was comparable to that of these authors’ “hot start” method. When commercially prepared PCR beads are used, the likelihood of PCR reagent contamination is minimized, and the potential for contamination is further reduced because the number of times the tubes need to be opened is minimized. Sample preparation times also were decreased, by decreasing the number of preparation steps, without a noticeable change in performance.

On average, the sample preparation, PCR amplification, and electrophoresis of reaction products take about 4 to 5 h to complete. This is much faster than the mouse inoculation assay, which usually requires a minimum of 3 days to identify a positive sample and 21 days to confirm a negative one, not including the additional time required to perform serological or cultural assays on serum samples or tissues, respectively. We have not calculated a cost comparison, but the price of PCR reagents is likely to be offset by the additional person-hours needed to care for the mice and to conduct the bacteriological and serological testing involved with mouse inoculation assays. More importantly, the rapid turnaround time for samples ensures that surveillance data and results from epidemiological investigations will be available within hours after samples are received. This information can be invaluable in determining what control and prevention methods should be implemented to reduce risks posed by individual rodent and flea species found within the affected area.

Although this PCR assay should be adequate for most surveillance purposes requiring rapid identification of \textit{Y. pestis} in fleas, it cannot substitute for bacterial culture methods when additional data are needed, such as those pertaining to virulence, plasmid profile analysis, or antibiotic susceptibility. Because the PCR assay is unable to distinguish between \textit{Y. pestis} strains with various degrees of virulence or antibiotic susceptibility, we recommend that representative samples of the triturated flea material be kept at −70°C for future analysis.

Our results show that the death of inoculated mice is a less reliable indicator than PCR of the presence of \textit{Y. pestis} in flea samples. The results of the modified \textit{pla} PCR assay and the mouse inoculation assay were similar only when all surviving mice were tested for evidence of \textit{Y. pestis} infection at the end of the 21-day assay period. This is important, because under the current protocols, surviving mice are rarely tested after 21 days because of time and personnel constraints. It should be noted, however, that even follow-up testing of the surviving mice in our study failed to identify two \textit{Y. pestis}-infected flea pools. In conclusion, the rapidity of the \textit{pla} PCR test (~4 h), along with its sensitivity, makes it a suitable alternative to mouse inoculation assay for identifying infected fleas during surveillance activities and outbreak investigations.

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\textbf{REFERENCES}  

Sao Paulo 38:371–373.
laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor,
N.Y.
Identification of a Yersinia pestis-specific DNA probe with potential for use
In A. Barlows and W. J. Hausler, Jr. (ed.), Diagnostic procedures for bac-
terial, mycotic and parasitic infections. 6th ed. APHA, Inc., Washington, D.C.
maminogen activator gene of Yersinia pestis: relationship to ompT of Escherichia
hybridization probes for detection of the plague bacillus (Yersinia pestis) in
fleas (Siphonoptera: Pulicidae and Ceratophyllidae). J. Med. Entomol. 26:
342–348.