SHORT REPORT: SERODIAGNOSIS OF PLAGUE IN HUMANS AND RATS USING A RAPID TEST

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Abstract. Serodiagnosis of plague is very useful for its retrospective confirmation and for epidemiologic studies in humans and in rodents, since rats constitute the main natural reservoir of Yersinia pestis. We have developed a rapid test for the detection of IgG antibodies to fraction 1 (F1) based on immunochromatography and protein A to detect both human and rat IgG. When tested with reference human sera (35 positive and 37 negative), this assay showed a sensitivity of 94.3% and a specificity of 89.2%. When Rattus rattus and R. norvegicus reference sera (22 positive and 24 negative) were used, the sensitivity was 100% and the specificity was 91.7%. This simple serodiagnostic tool is of great potential value in the surveillance of plague. As far as we know, this test is the first of its kind designed for diagnosis of both humans and animals.

Plague has been one of the most feared diseases in history, and the third pandemic can be regarded as still ongoing since Yersinia pestis sporadically re-emerges from its reservoir of wild rodents. Plague is a flea-borne disease that may cause large epizootics among rodent population (‘rat fall’ phenomenon), and humans living in close contact with these rodents may acquire bubonic plague following infective flea bites. Most of the time, plague is transmitted without noticeable signs among the natural reservoir in which antibodies to fraction 1 (F1) can be detected. During the last 15 years, more than 20 countries have reported plague cases to the World Health Organization. Yersinia pestis is considered one of the bacteriologic agents that could be used for biological warfare, causing pneumonic plague if spread by aerosol.

Because plague is a fulminating disease and the clinical diagnosis is non-specific, the treatment of suspected cases must be started without delay. A retrospective laboratory confirmation of plague can be obtained by detecting antibodies directed against a capsular protein (the F1 antigen). A simple assay to detect antibodies to the F1 fraction will be useful for epidemiologic surveys and for monitoring the transmission of Y. pestis among the animal reservoir in natural plague foci. We have developed a rapid assay in a half-dipstick format that is able to detect both human and rat IgG antibodies to the F1 fraction.

The test is based on the principle of vertical flow immunochromatography using colloidal gold particles conjugated to protein A. The format of the test is a strip made of specially formulated nitrocellulose (membrane test platform; Whatman, Maidstone, United Kingdom) backed by a protective sheet and flanked at its top by an absorption pad (3MMChr; Whatman). Purified F1 antigen (Institut Pasteur de Madagascar, Antananarivo, Madagascar) at a concentration of 500 μg/mL in phosphate-buffered saline (PBS) was filtered through a 0.22-μm pore size membrane and spread as a thin capture line (Biojet Quant-3000; Biodot Ltd., Cambridge, United Kingdom) at a concentration of 1 μL/cm 10 mm above the bottom of the nitrocellulose strip. The composite was trimmed into 5-mm strips and stored in hermetically closed tubes in the presence of a desiccant (silica gel; Sigma, L’Isle d’Abeau, France) at room temperature. Ten microliters of each serum diluted 1:100 in PBS were added to 15 μL (absorbance at 540 nm = 10) of protein A immunogold conjugate (British Biocell International, Cardiff, United Kingdom) and 45 μl of migration buffer (PBS, 0.5% Tween, 0.5% bovine serum albumin) in the wells of a flat-bottomed enzyme-linked immunosorbent assay (ELISA) microplate (HTS Polysorb; Nunc, Roskilde, Denmark). The strip was dropped into the well and the test could be visually followed as the upward wetting of the strip up to the absorbent pad. After 20 minutes, the assay result was scored positive when a distinct pink line was observed on the F1 capture line. The different parameters of the test were optimized step-by-step using a panel of anti-F1 positive and negative human and rat sera. The anti-F1 IgG indirect ELISA was used as the reference test to assess the sensitivity and specificity of the rapid test.

The sensitivity of the anti-F1 dipstick test with human sera was assessed using 35 ELISA-positive control sera from convalescent plague patients. These sera were collected in Mahajanga harbor in Madagascar during the outbreak of plague in September 1997. The anti-F1 IgG ELISA titers ranged from 50 to 6,400. The specificity was assessed with 37 anti-F1 ELISA-negative control sera collected from healthy subjects in the same city and at the same period of time.

Rats were trapped alive in Antananarivo, Madagascar and individual rat sera (22 ELISA-positive and 24 ELISA-negative sera) were collected from Rattus rattus and R. norvegicus, the two species of rats that serve as reservoirs of Y. pestis in Madagascar. Sera of trapped rats testing positive in anti-F1 ELISA were pooled. The titer of the pool was 104,000. The positive individual controls (ELISA titers ranging from 400 to 416,000) were obtained from rats immunized with purified F1 antigen injected intramuscularly once a week for six weeks (1 mg/mL of F1 antigen in Freund’s complete adjuvant for the first injection and in Freund’s incomplete adjuvant for the subsequent injections). All sera were kept frozen (-20°C) before being tested.

The anti-F1 dipstick assay showed positive results for 33 of 35 positive human sera (sensitivity = 94.3%) and for 22 of 22 positive rat sera plus the pool (sensitivity = 100%). Negative results were obtained for 33 of 37 negative human sera (specificity = 89.2%) and 22 of 24 negative rat sera (specificity = 91.7%). The 95% confidence intervals for these results are shown in Table 1.

The clinical diagnosis of plague can be misleading and laboratory tests are time-consuming and technically demanding. Although rare wild F1-negative strains have been reported, the detection of this antigen in the diagnosis of plague during...
the acute phase of the disease is quite relevant.\textsuperscript{5,7} A very robust dipstick assay for the detection of F1 antigen was recently developed and evaluated in the field in Madagascar.\textsuperscript{8} The detection of antibodies to F1 is of considerable importance in the retrospective confirmation of plague in convalescent patients and in post-outbreak epidemiologic surveys.\textsuperscript{9} IgG seroconversion in patients was generally observed one week after the onset of the disease, or on the sixth day post-inoculation in the murine model of infection.\textsuperscript{3,10} Because IgG persist much longer than IgM, we have designed a rapid assay for the detection of specific IgG. Protein A was chosen as a ligand because of its affinity for IgG of many different mammals.\textsuperscript{11}

As far as we know, this is the first rapid test of its kind that is applicable with both human and animal sera. Due to its good sensitivity and specificity, this dipstick test may be used as a convenient and rapid tool in the surveillance of plague.

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table 1

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<th>Humans</th>
<th>Rats</th>
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<tr>
<td>Sensitivity</td>
<td>0.808–0.993</td>
<td>0.846–1.0</td>
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<tr>
<td>Specificity</td>
<td>0.746–0.970</td>
<td>0.73–0.99</td>
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