Comparison of the Reliability and Sensitivity of Three Serological Procedures in Detecting Antibody to *Yersinia pestis* (*Pasteurella pestis*)

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Three serological procedures, the agar-gel precipitin inhibition, the complement fixation, and the indirect hemagglutination tests, were used to detect and measure antibody to *Yersinia pestis* in the sera from 383 individuals. Although all three tests were useful in detecting plague antibody, the most reliable and sensitive test procedure was indirect hemagglutination.

Various serological tests for the detection of antibody to *Yersinia pestis* (*Pasteurella pestis*) have been employed as diagnostic procedures, in epidemiological surveys, and in studies to determine the efficacy of plague vaccines (6-9, 15, 16). The agglutination test has been found to be nonspecific; plague bacilli are agglutinated by sera containing antibodies against *Yersinia pseudotuberculosis*, which limits its value in rodent surveys. The mouse protective antibody indexes (MPI) test (12), although probably the truest indirect measure of immunity, requires large numbers of mice, extensive animal holding facilities, and cannot be used to evaluate sera containing preservatives such as sodium azide.

Three additional tests have frequently been used, the indirect hemagglutination (IHA) test (4), the complement fixation (CF) test (5), and the agar-gel precipitin inhibition (AGPI) test (13). Proponents of each test have presented evidence for the specificity, sensitivity, and simplicity of their method. To compare the relative merits of the three tests, a series of 400 sera were tested, and the results were compared.

**MATERIALS AND METHODS**

**Serological procedures.** AGPI tests were performed as described by Ray and Kadull (13). The titer was taken as the greatest dilution of unknown serum that completely inhibited the formation of a visible precipitin line. CF tests were carried out according to the method of Chen and Meyer (5) as modified for microtiter technique by Cavanaugh et al. (3). The IHA test of Chen and Meyer (4) was modified by employing pyruvate-stabilized sheep red blood cells (SRBC) (14) sensitized by coupling *Y. pestis* fraction 1 antigen to the SRBC with chronic chloride.

**Test subjects.** Sera from 383 individuals were tested. Single serum samples were obtained from 307 individuals with a well documented history of from 1 to 48 plague immunizations and from 75 subjects who had never received an inoculation of plague vaccine. Eighteen sera from one confirmed pneumonic plague patient were obtained prior to, during, and subsequent to the clinical episode (1). Complete immunization histories including the number of inoculations, total volume of vaccine received, and the dates of immunization and bleedings were available for each person.

**RESULTS**

The results of AGPI, IHA, and CF tests for the detection of *Y. pestis* antibody in the sera of 307 immunized and 75 nonimmunized individuals are shown in Table 1. The CF test, although highly specific, failed to detect antibody in the majority of immunized persons. The AGPI test, whereas three times as sensitive as the CF test in demonstrating antibody in the immunized group, was relatively nonspecific as demonstrated by 26.7% positive reactions among nonimmunized individuals. In addition to a high degree of specificity, the IHA test was the most sensitive for detection of *Y. pestis* antibody produced in response to plague immunization.

When data were analyzed according to parameters of the number of inoculations of plague vaccine administered and time since...
last inoculation (Tables 2 and 3), the efficiency of the IHA procedure was evident. The 58 (19.9%) immunized individuals with no detectable IHA antibody had received an average of less than two inoculations of plague vaccine. Of this group, the AGPI test detected antibody in 19 (32.8%), but failed to detect it in 39 (21.7%) other persons who had received an average of 9.5 inoculations and who had IHA antibody.

In the study of actual infection with *Y. pestis*, all three tests show a dramatic rise in titer in sera obtained during the acute phase of the disease. Figure 1 represents the antibody titers of an individual with laboratory-acquired pneumonic plague. The preinfection IHA titer was due to four plague vaccine inoculations. Antibody detected by the IHA test persisted at high titer for at least 1 year, whereas antibody detectable by the other two tests had disappeared.

### TABLE 1. Comparison of three serological tests for detection of *Yersinia pestis* antibody in sera of 382 individuals

<table>
<thead>
<tr>
<th>Plague immunization</th>
<th>No. persons</th>
<th>AGPI* positive</th>
<th>IHA* positive</th>
<th>CF* positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>307</td>
<td>185</td>
<td>60.3</td>
<td>249</td>
</tr>
<tr>
<td>−</td>
<td>75</td>
<td>20</td>
<td>26.7</td>
<td>1</td>
</tr>
</tbody>
</table>

* Agar-gel precipitin inhibition.

* Indirect hemagglutination.

* Complement fixation.

### TABLE 2. Efficiency of serological procedures for detection of *Yersinia pestis* antibody in sera from 307 individuals inoculated with plague vaccine

<table>
<thead>
<tr>
<th>Positive tests*</th>
<th>No. individuals</th>
<th>Avg. no. inoculations</th>
<th>Avg. no. months since last inoculation</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>39</td>
<td>1.8</td>
<td>14.9</td>
</tr>
<tr>
<td>AGPI</td>
<td>19</td>
<td>1.7</td>
<td>16.6</td>
</tr>
<tr>
<td>IHA</td>
<td>76</td>
<td>9.5</td>
<td>16.9</td>
</tr>
<tr>
<td>AGPI</td>
<td>115</td>
<td>16.5</td>
<td>18.0</td>
</tr>
<tr>
<td>IHA AGPI</td>
<td>51</td>
<td>17.0</td>
<td>18.9</td>
</tr>
<tr>
<td>IHA CF</td>
<td>7</td>
<td>9.0</td>
<td>28.7</td>
</tr>
</tbody>
</table>

* AGPI, agar-gel precipitin inhibition; IHA, indirect hemagglutination; CF, complement fixation.

### DISCUSSION

All three serological test procedures were of value in determining the antibody response to acute infection with *Y. pestis*. However, with sera obtained from a population of vaccinated and nonvaccinated individuals, the IHA test was a superior procedure for the detection of plague antibody.

Past experience with the IHA test has shown that the degree of specificity was directly correlated to the purity and concentration of the fraction 1 antigen employed in sensitizing the SRBC (14), i.e., when the 75 sera from the nonvaccinated individuals were tested with nonstandardized fraction 1, sensitized, nonstabilized tannic acid-treated cells, 12 sera gave false-positive reactions due to the presence of a minor protein contaminant found in all lots of fraction 1 prepared from several strains of
Y. pestis. Since outdated, whole-cell Formalin-killed plague vaccine was the antigen used in the AGPI test, the false positive reactions may have resulted from antibody reactions with antigens other than the fraction 1 component, possibly one or more of the 22 known antigens common to Y. pestis and Y. pseudotuberculosis (10).

The IHA and CF procedures have been routinely used to test sera from a great variety of wild and domestic mammals for the presence of plague antibody. Considerable difficulties have been encountered with the CF test due to nonreactivity with the sera of certain species (11), and anticomplementary activity of many sera collected from the field. Conversely, with the exception of approximately 20% of the sera obtained from Herpestes (mongoose), no technical difficulties have been encountered with the IHA test (2). The AGPI test has not been used to test animal sera from field studies and therefore no comparisons can be made concerning the reliability of this test. However, the large percentage of nonspecific positive results obtained with human sera indicate that this test would be of questionable value for field studies. Although all three tests were useful in detecting plague antibody, the most reliable and sensitive test procedure was IHA hemagglutination.

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LITERATURE CITED


2. Cavanaugh, D. C., P. J. Deoras, D. H. Hunter, J. D. Marshall, Jr., D. V. Quy, J. H. Rust, Jr., S. Purnaveja, and P. E. Winter. 1970. Some observations on the necessity for serological testing of rodent sera for Pasteurella pestis antibody in a plague control pro-


