COMPARISON OF DIRECT AND INDIRECT FLUORESCENT ANTIBODY
TECHNIQUES FOR DETECTION OF MYCOPLASMA HYPONEMONIAE IN SWINE LUNGS

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Specific diagnosis of mycoplasmal pneumonia of swine (MPS) has long been
hampered by the slow and fastidious growth of M. hyponemaiae as well as by the frequent
overgrowth of the organism by other myco-
plasms and bacteria. Rapid diagnosis has
been achieved by use of direct (L’Ecuyer and
Boulanguer, 1971; Amanfu, 1972; Mayo-
ing, 1971; Amanfu et al., 1980) and indirect (Gois et al., 1975)
fluorescent antibody techniques. The relative
usefulness of these techniques in comparison
to culture and other diagnostic procedures
has not been thoroughly evaluated. In the
work reported here we evaluated the useful-
ness of DFA and IFA for detection of M. hyp-
onemaiae in swine lungs.

Methods: Pigs utilized in this study were
described in a companion report (Piffer and
Ross, 1982). All pigs were exposed by
contact to other swine infected with M. hyp-
onemaiae and examined by necropsy at 48
days (Experiment A) or 49-50 days (Exper-
iment B) after contact-exposure began.

Tissues were collected, embedded in OCT,
cut with a cryostat and the DFA procedure
was done as described by Amanfu et al. (1980).
The IFA procedure was similar to that de-
scribed by Amanfu et al. (1980), but with
washing and timing as described by Rosendal
and Black (1972). Commercial goat anti IGP
and antiserum to M. hyponemaiae prepared in
rabbits (Ross and Karmon, 1970) were used in the IFA test.

Discussion: Since no differences were
observed between the immunofluorescence
techniques utilized, it appears that IFA is
more suitable than DFA for less-equipped laboratories because the fluorescein-tagged
globulin can be bought commercially. In
addition, the antiserum can be used at a
higher dilution than the direct conjugate.
The azo-dye counterstain reduced nonspecific
fluorescence and provided a better contrast
with no evidence of reduced sensitivity.

Results obtained by means of PA test
corresponded closely to those obtained by
culture procedure and macroscopic and micro-
scopic evaluation for MPS lesions. These
results are in agreement with those obtained
by l’Ecuyer and Boulanguer (1970), and Amanfu
et al. (1980). In spite of this fact, a few
lobes that were culture-positive were
negative by PA while in one case the reverse
occurred.

Table 1.

<table>
<thead>
<tr>
<th>Procedure</th>
<th>No. of lobes in graded PA-pos. categories of cases</th>
<th>No. of pos. fluorescence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experiment A (18 pigs)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DFA with counterstain</td>
<td>15</td>
<td>1</td>
</tr>
<tr>
<td>IFA without counterstain</td>
<td>15</td>
<td>2</td>
</tr>
<tr>
<td>IFA with counterstain</td>
<td>14</td>
<td>3</td>
</tr>
<tr>
<td>Experiment B (19 pigs)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DFA with counterstain</td>
<td>15</td>
<td>2</td>
</tr>
<tr>
<td>IFA without counterstain</td>
<td>15</td>
<td>0</td>
</tr>
<tr>
<td>IFA with counterstain</td>
<td>16</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>aBoth cardiac lobes from each pig examined.</td>
<td></td>
<td></td>
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<tr>
<td>bIntensity of fluorescence was graded</td>
<td></td>
<td></td>
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<tr>
<td>1 = least intense to 4 = most intense.</td>
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</table>

Table 2.

<table>
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<th>No. of No. of No. of No. of lobes</th>
<th>lobes</th>
<th>lobes</th>
<th>lobes</th>
<th>lobes</th>
<th>eval.</th>
<th>culture</th>
<th>DFA-pos.</th>
<th>with</th>
<th>pos.</th>
<th>for</th>
<th>macro.</th>
<th>micro.</th>
<th>lesions</th>
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<td>24</td>
<td>16</td>
<td>12</td>
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<td>12</td>
<td>14</td>
<td>20</td>
<td>12</td>
<td>14</td>
<td>12</td>
<td>12</td>
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</tr>
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