COMPARATIVE STUDY OF FOUR TECHNIQUES
FOR SERODIAGNOSIS OF PARAINFLUENZA VIRUS TYPE 1
IN PIGS

GAZIM BIŽANOV AND IRENA JONAUSKIENĖ *

Institute of Immunology, Vilnius University,
LT-2021 Vilnius, Lithuania
e-mail: Rene@imi.lt

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Sera obtained from 777 pigs were tested for the presence of Sendai virus-immunospecific antibodies by the agar gel immunodiffussion (AGID), counterimmunoelectrophoresis (CIE), haemagglutination inhibition (HI) and serum neutralisation (SN) tests. As many as 598 sera contained Sendai virus antibodies as shown by the SN test, 552 sera were HI positive, 536 sera were CIE positive, and only 444 ones were AGID positive. When the SN test was applied as the reference one, the sensitivity and specificity of the HI, CIE, and AGID tests were found to be 90.96% and 95.53%, 88.29% and 95.53%, 67.75% and 77.65%, respectively. The analytical accuracy of the HI and CIE tests relatively to the SN test was similar. The good consistency between the HI and CIE tests (κ=0.98) suggests that the two methods are interchangeable.

Key words: pig, Sendai virus, antibodies, serological techniques.

Sendai virus (SV) is a type 1 of parainfluenza virus (paramyxovirus family), which is a natural respiratory pathogen in rodents (13, 24). It has been demonstrated serologically that this infection is widespread among humans (1, 9, 15) and occurs fairly often in pigs (11, 12). Recently, paramyxovirus infection in pigs have been reported (3, 5, 14, 22).

The haemagglutination inhibition (HI) test has been widely accepted for many years as the standard method for assessment of vaccine responses in clinical trials (29) as well as in serological surveys (4, 6, 16, 23). Sera from mammals and birds often contain non-specific inhibiting factors that induce false reaction in the HI test and, in order to avoid these reactions, sera ought to be pretreated with some compounds, for example by using kaolin, sodium periodate, receptor-destroying enzyme, chicken red blood cells or by heat inactivation (26).

Immunoprecipitation techniques, including counterimmunoelectrophoresis (CIE) and agar gel immunodiffusion (AGID), continue to play a considerable role in virological surveillance and in immunodiagnostics of various infectious diseases, although, in contrast with the CIE, the AGID is relatively low sensitive (7, 8, 10, 19).
On the other hand, the serum neutralization (SN) test is often used as confirmatory assay for the detection of antibodies to various viruses (16, 25). It is important that immunodiagnostic efficacy of a serological test may be practically evaluated by different techniques including the ability to detect low levels of antibodies at any stage of infection (18, 25).

The aims of the present study were, firstly, the serodiagnosis of SV infection with four distinctive techniques in pigs, and secondly, the estimation of the specificity and sensitivity, including the analytical accuracy as well as the correlation and consistency of the three different tests (HI, CIE and AGID) in comparison with the SN assay.

**Material and Methods**

**Pig sera.** Totally, 777 pig sera were sent from different pig farms (North and South areas of Lithuania) for testing. No clinical signs of a disease were observed in any of the tested pigs. SV-negative reference serum (control) were obtained from the Lithuanian National Veterinary Laboratory (Vilnius).

**Pretreatment of the sera.** 1) Proteolytic enzymes: the aliquots containing 8 mg/ml of total protein each were prepared from one serum sample and these were used for pre-treatment with one of the three enzymes: α-chymotrypsin (Calbiochem, San Diego, CA, USA), bromelain (Sigma) and pronasa (Sigma). Each enzyme was added in an amount of 2 mg to 500 μl of the aliquot and this mixture was incubated at 37°C overnight. Afterwards, the mixture was cooled to 1-3°C for 5 min and then it was tested by the HI. Control serum aliquot was heat inactivated at 56°C for 30 min. 2) The three-step procedure (TSP) was employed for the pre-treatment of sera as described earlier (21) for four of the tests.

**Preparation of viral antigen.** Fushimi strain of SV was grown and prepared as described previously (2).

**Agar gel immunodiffusion.** The AGID test was performed as described elsewhere (10). Positive responses were expressed as the reciprocal of the highest dilution of serum at which a precipitation line against SV was seen. Dilutions of 1:4 or more were considered as positive.

**Counterimmunoelectrophoresis.** The CIE test was performed according to Dias and Myers (8). The endpoint titres were calculated as described for AGID test.

**Haemagglutination inhibition.** HI was performed as described previously (2). The antibody titres were expressed as the reciprocal of the highest dilution of serum at which complete inhibition of haemagglutination was seen. Titres of 8 or more were regarded as positive (the titres in the reference were less than 4).

**Serum neutralization.** SN was carried out as described previously (25). The antibody titres were expressed as the reciprocal of the highest dilution of serum at which 50% neutralization of the SV was seen. Titres of 4 or more were considered as positive.

**Total protein estimation.** The protein content was calculated as described earlier (17).

**Statistical analysis.** The mean antibody titres of the pig sera were compared using Student’s t-test. All values were expressed as mean ± standard deviation and were considered to be statistically significant at P<0.05. Relative sensitivity, specificity and analytical accuracy were determined as described formerly (16). Pearson’s correlation...
coefficients (r) were calculated in order to measure the strenght of the association of the SN titres, the HI titres, the CIE titres and the AGID titres, using the Yates correction (28). Kappa (κ) was evaluated to measure the degree of the consistency between two methods (20).

Results

Pretreatment of the sera. In the test, the pig serum samples pretreated with proteolytic enzymes showed lower titres than the control ones (statistically significant, P<0.01), while pattern pre-treatment by TSP resulted in the lowest titres (statistically significant, P<0.01), when compared both with the control and the enzyme pretreated samples (Table 1).

Serodiagnosis of SV with four tests. The prevalence of antibodies to SV in pig sera as obtained by four assays is presented in Table 2. The seropositive reactions of the SN, the HI, the CIE, and the AGID for 777 pig sera were 76.96%, 71.04%, 68.98% and 57.14%, respectively.

Comparison of serological tests. The HI was the most sensitive test (90.96%) when the HI, CIE, and AGID tests were compared to the SN test (the reference test), while the specificity of the HI and CIE tests was of the similar value (95.53% in both cases) (Table 3). However, the sensitivity, specificity, and predictive accuracy values of the CIE test with respect to the HI test were higher than of the others.

The predictive accuracy values of the HI and CIE tests were higher than those of the AGID test when these were compared with the SN test.

A close correlation (r=0.80) was found between the HI and SN titres of the same sera. Similar correlations (r=0.76) were obtained when the CIE and the SN titres were compared, whereas the correlation (r=0.92) between the CIE and HI titres was the highest.

There was a good consistency between the HI and SN tests (κ=0.79), the CIE and SN tests (κ=0.74) as well as the CIE and HI tests (κ=0.98) (Table 3).

Table 1
Influence of pig serum pre-treatment on the haemagglutination inhibition test values

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>Mean geometric anti-Sendai virus titre (log₂)</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-chymotrypsin</td>
<td>5.3 ± 0.5</td>
</tr>
<tr>
<td>Bromelain</td>
<td>5.0 ± 0.9</td>
</tr>
<tr>
<td>Pronasa</td>
<td>5.0 ± 0.8</td>
</tr>
<tr>
<td>Heat inactivation + kaolin+ packed chicken erythrocytes (TSP)</td>
<td>3.7 ± 0.5</td>
</tr>
<tr>
<td>Heat inactivated serum (control)</td>
<td>7.1 ± 0.4</td>
</tr>
</tbody>
</table>
### Table 2
Comparative results of the examination of 777 pig sera by serum neutralization (SN), haemagglutination inhibition (HI), counterimmunoelectrophoresis (CIE) and agar gel immunodiffusion tests (AGID)

<table>
<thead>
<tr>
<th></th>
<th>HI</th>
<th>SN</th>
<th>AGID</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>positive</td>
<td>negative</td>
<td>positive</td>
</tr>
<tr>
<td>CIE</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>positive</td>
<td>532</td>
<td>4</td>
<td>528</td>
</tr>
<tr>
<td>negative</td>
<td>20</td>
<td>221</td>
<td>241</td>
</tr>
<tr>
<td>total</td>
<td>552</td>
<td>225</td>
<td></td>
</tr>
<tr>
<td>AGID</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>positive</td>
<td>437</td>
<td>7</td>
<td>444</td>
</tr>
<tr>
<td>negative</td>
<td>115</td>
<td>218</td>
<td>333</td>
</tr>
<tr>
<td>total</td>
<td>552</td>
<td>225</td>
<td></td>
</tr>
<tr>
<td>SN</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>positive</td>
<td>544</td>
<td>54</td>
<td>598</td>
</tr>
<tr>
<td>negative</td>
<td>8</td>
<td>171</td>
<td>179</td>
</tr>
<tr>
<td>total</td>
<td>552</td>
<td>225</td>
<td></td>
</tr>
</tbody>
</table>

### Table 3
Relative sensitivity, specificity, predictive accuracy, correlation coefficient and kappa of the haemagglutination inhibition (HI), counterimmunoelectrophoresis (CIE) and agar gel immunodiffusion (AGID) tests

<table>
<thead>
<tr>
<th></th>
<th>HI compared to SN</th>
<th>CIE compared to SN</th>
<th>AGID compared to SN</th>
<th>CIE compared to HI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity (%)</td>
<td>90.96</td>
<td>88.29</td>
<td>67.75</td>
<td>96.37</td>
</tr>
<tr>
<td>Specificity (%)</td>
<td>95.53</td>
<td>95.53</td>
<td>77.65</td>
<td>98.22</td>
</tr>
<tr>
<td>Positive predictive value</td>
<td>0.98</td>
<td>0.98</td>
<td>0.91</td>
<td>0.99</td>
</tr>
<tr>
<td>Negative predictive value</td>
<td>0.76</td>
<td>0.71</td>
<td>0.42</td>
<td>0.92</td>
</tr>
<tr>
<td>Correlation coefficient</td>
<td>0.80</td>
<td>0.76</td>
<td>0.38</td>
<td>0.92</td>
</tr>
<tr>
<td>Kappa</td>
<td>0.79</td>
<td>0.74</td>
<td>0.17</td>
<td>0.98</td>
</tr>
</tbody>
</table>
Discussion

The presence of non-specific inhibitors in the HI sera still remains a problem. In order to reduce appearance of the false positive reactions, pre-treatment of the same serum with the proteolytic enzymes, as well as by TSP was applied in this study. Our findings revealed that pre-treatment of the sera by TSP was more successful. Similar results was demonstrated using heparin/magnesium chloride in combination with guinea pig erythrocytes (21).

It was shown previously that the pig sera gave 30% seropositive reactions against two strains of SV (11). Besides, there were observed specific antibodies to viruses of the parainfluenza group in pigs with clinical signs and pathological changes, typical of respiratory infections (27). Interestingly, 92% seropositive reactions to SV have been observed in human sera (9). The recent studies also demonstrated that the paramyxovirus infection in pigs was marked by biochemical (3, 5, 22) or histopathologic (14) findings. In the present study, the prevalence of antibodies to SV among pigs in Lithuania was detected using the four distinct tests. The seropositive reactions of the SN, HI, CIE and AGID assays were found to be 76.96%, 71.04%, 68.98% and 57.14%, respectively. Therefore, these data suggest that the pigs would be infected with paramyxovirus.

The sensitivity, specificity and the predictive accuracy of the HI and CIE tests used in this study as well as the close relationship between the results of the HI and CIE (κ=0.98) suggest that the two methods are interchangeable and that both can be used for the determination of SV-specific antibodies, while the AGID test is less applicable. The similar results were found, when the AGID test applied in bovine leukemia virus diagnostic studies (10). There were detected from 47.1% to 60.8% positive sera as well as from 14.3% to 16.5% weak positive sera and this test also showed a lower sensitivity when compared with HI.

The presence of SV-serologically positive animals demonstrates that this virus can circulate within the pig population in Lithuania.

References