Indirect Hemagglutination Test for Pseudorabies Antibody Detection in Swine†

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An indirect hemagglutination test for the detection of antibodies in swine serum specific for pseudorabies virus is described. The indirect hemagglutination test was less time consuming than the standardized virus neutralization test while being highly sensitive and accurate. Serum samples that were toxic to virus neutralization indicator cells were readily tested in the indirect hemagglutination test. The indirect hemagglutination test may also be more sensitive than the virus neutralization test for determining early titers in pseudorabies virus infections. Complete methodology is described.

Pseudorabies virus (PrV) infection of swine has produced major economic losses to swine producers in the United States in recent years due to the increased incidence and severity of this herpetic disease (2, 4, 6).

The screening of sera for PrV-specific antibodies by the virus neutralization (VN) test is presently being used (4). Although the VN test is accurate, it requires several days for completion, expertise to conduct it, and a laboratory equipped for tissue culture (3). Also, serum samples may be toxic to the indicator cells in the test, and a second serum sample must be obtained to determine PrV serodiagnosis.

Indirect hemagglutination (IHA) tests have been used for serodiagnosis of other herpesvirus infections (7, 9, 10). IHA tests can be performed with common laboratory equipment and with average technical skill in 1 to 2 h. The tests are accurate and are not affected by cytotoxic sera. The IHA test also measures serum immunoglobulin M levels reliably in herpesvirus infections (1). Immunoglobulin M levels indicate early infections and may not be detected with other tests (10).

This paper describes the adaptation of the IHA test (9) for the detection of PrV antibodies in swine serum. The IHA test was demonstrated to be more accurate and less time consuming than the VN test and was also able to effectively test samples which were toxic in the VN system.

MATERIALS AND METHODS

Serum samples. Samples were obtained from PrV-infected swine, isolated controls, and field submissions to the Animal Disease Diagnostic Laboratory, School of Veterinary Medicine, Purdue University. All sera were heat inactivated at 56°C for 30 min, and 1.0 ml of each was absorbed with 0.1 ml of a 50% suspension of washed sheep erythrocytes.

VN tests. All sera were heat inactivated and tested for PrV antibodies with a microtitration VN test (4).

PrV antigen. The Sullivant strain of PrV (50% tissue culture infective dose, 10⁶/ml) was propagated and irradiated with ³⁵Co as described elsewhere (8). The antigen preparation was pelleted at 50,000 × g for 30 min and resuspended to its original volume in 0.15 M phosphate-buffered saline (PBS), pH 6.7.

Preparation of tanned sheep erythrocytes. Sheep blood was collected in Alsever solution and aged 1 to 3 weeks before use. After the buffy coat was removed, erythrocytes were washed three times in 0.15 M PBS (pH 7.2) and resuspended in the same buffer to a 2.5% suspension. An equal volume of a 1:20,000 dilution of tannic acid was added, and the mixture was incubated at 37°C for 15 min. The cells were centrifuged at 800 × g for 10 min, washed once with PBS, (pH 7.2), and resuspended to a 2.5% suspension in 0.15 M PBS (pH 6.7).

Sensitization of tanned erythrocytes. One volume of PrV antigen, suspended in PBS (pH 6.7), was added to one volume of tanned erythrocytes and incubated at 37°C for 30 min (the optimal antigen concentration was previously determined to be 5 × 10⁷ 50% tissue culture infective doses of PrV antigen for 10 ml of 2.5% tanned erythrocytes). Cells were washed twice in PBS (7.2) and resuspended to a 1% suspension in PBS (pH 7.2) containing 1% normal rabbit serum, which had previously been heat inactivated and absorbed with sheep erythrocytes.

IHA test. Twofold dilutions of serum in 0.025 ml, with 1% normal rabbit serum in PBS as the diluent, were made in U-bottomed Linbro microtitration plates (Flow Laboratories, Hamden, Conn.). Sensitized erythrocytes (0.025 ml) were added to each dilution. Plates were sealed with plastic tape, shaken, and incubated at room temperature for 2 h and then read or refrigerated until read. Erythrocyte settling patterns were read as positive if agglutination of cells was 80% or more of the bottom of the well. Smaller agglutination patterns were read as plus-minus, and buttoning of erythrocytes was read as negative. The IHA titer of
a serum was the highest dilution which caused positive agglutination. Known PrV-positive and -negative controls were run with each test, as were erythrocyte controls and serum dilutions with tanned nonsensitized erythrocytes.

RESULTS

Diagnostic laboratories commonly use procedures to screen swine sera for neutralizing antibodies to PrV at dilutions of 1:4 to 1:32. Any serum that neutralizes virus, inclusive of these dilutions, is designated PrV positive, although further titrations may be utilized to ascertain specific titers. The IHA test for PrV was standardized with test sera so that positive agglutination at a 1:4 serum dilution or above was also considered as positive for PrV antibodies.

All samples that were positive in the VN test were also positive in the IHA test. However, the IHA test indicated that a small number of samples were PrV positive which were negative by the VN test (Table 1).

Samples that were toxic to the indicator cells in the VN test did not affect readability of the IHA test. However, IHA results had to be compared with retests on nontoxic VN serum from these swine, submitted 2 to 10 weeks later. On the second or third retest of these swine, all were negative in the VN test. Sera were not available for IHA retesting, but of the original toxic samples many were positive (Table 2) and had titers of 1:32 or 1:64.

An optimum antigen concentration for sensitizing erythrocytes was essential for the sensitivity and reproducibility of the IHA test. In our experiments, irradiated and live virus worked equally well at a concentration of $5 \times 10^7$ 50% tissue culture infective doses to 10 ml of tanned 2.5% erythrocytes.

DISCUSSION

Serodiagnosis is essential for detection and control of PrV infections. Diagnostic tests for PrV include a microimmunodiffusion test (3), which has sensitivity of low order, and a VN test, which is presently being used (4). The VN test is time consuming, requires specialized laboratory conditions, and cannot test samples which are toxic to the indicator test cells.

The IHA test for detection of PrV antibodies was compared with the VN test for sensitivity and accuracy on serum samples obtained from experimental and private swine populations. The IHA test results correlated 100% with sera which were positive in the VN test (Table 1). The IHA test also readily tested sera which were toxic in VN tests. The IHA test, however, detected positive samples which were considered negative by the VN test. These discrepancies in test results for these samples may be due to greater sensitivity for certain antibody classes in the IHA test than is possible in the VN test. The sensitivity of the IHA test for detecting small amounts of immunoglobulin M classes of immunoglobulins in herpesvirus infections has been demonstrated previously (1).

The accuracy of the IHA tests depends on the absorption of test sera with sheep erythrocytes to remove all antibodies against sheep-specific antigens. The testing of absorbed serum dilutions against nonsensitized erythrocytes should not show the presence of any agglutination. If agglutination occurs above the undiluted serum level, the serum should be further absorbed with more erythrocytes.

The IHA test is a convenient and readily utilisable diagnostic tool for detection of PrV-specific antibodies. Although we used fresh erythrocytes, cells may be glutaraldehyde fixed and stored frozen until needed (5, 10). The adaptation of the IHA test for detecting PrV or reinforcing VN test results, and for testing toxic sera, is encouraged.

ACKNOWLEDGMENT

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LITERATURE CITED


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**Table 1. Comparisons between the VN and IHA tests for detection of PrV-specific antibodies in swine serum**

<table>
<thead>
<tr>
<th>Serum sample</th>
<th>No. PrV positive ( % positive )</th>
<th>VN test</th>
<th>IHA test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nontoxic in VN test</td>
<td>61/232 (26.29)</td>
<td>68/232 (29.31)</td>
<td></td>
</tr>
<tr>
<td>Toxic in VN test</td>
<td>0/64 (0.0)</td>
<td>10/64 (15.63)</td>
<td></td>
</tr>
</tbody>
</table>

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**Table 2. Titers of sera positive by IHA and negative by VN**

<table>
<thead>
<tr>
<th>Serum sample</th>
<th>No. of sera at dilution:</th>
<th>1:4</th>
<th>1:8</th>
<th>1:16</th>
<th>1:32</th>
<th>1:64</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nontoxic</td>
<td></td>
<td>2</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Toxic</td>
<td></td>
<td>4</td>
<td>2</td>
<td>1</td>
<td>3</td>
<td></td>
</tr>
</tbody>
</table>

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* a Sera were obtained from experimental and private herds.
* b Sixty-one of sixty-one (100%) of VN-positive sera were also positive by IHA.
* c Toxic to VN indicator cells.
* d Determined from the second or third serum submissions from these swine 2 to 10 weeks after serum toxicity was determined.
IHA FOR PSEUDORABIES ANTIBODY IN SWINE


