Evaluation of the Corneal Test as a Laboratory Method for Rabies Diagnosis

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The corneal test (CT) for rabies diagnosis was evaluated in samples from 313 subjects of different species. Some of the subjects were inoculated experimentally and others were naturally infected. When the CT was compared with immunofluorescence staining and mouse inoculation tests on brains of the same subjects, a sensitivity of 41.7% and a specificity of 100% were found. The authors conclude that a positive CT result would confirm the diagnosis of rabies, but a negative one would not exclude the possibility of disease.

Most laboratory methods (6, 17, 19) for rabies diagnosis require brain samples and cannot be performed before the patient’s death. Previously, serologic techniques such as serum-neutralization (1) or indirect immunofluorescence (6) were the only tests which could be performed while the patient was still alive, provided there had been no prior vaccination. Subsequently, Vallone et al. (18) described the premortem diagnosis of rabies by immunofluorescence staining (FA) of smears made with the sediment of saliva samples in two children who died of the disease in Uruguay.

More recently, Schneider (16) described the corneal test (CT), which seemed promising for the diagnosis of the disease without the need of a necropsy. He was able to detect rabies antigen by FA staining of corneal impression smears from 41 to 43 mice inoculated with street rabies virus. The test became positive either before or during the clinical stage of the disease; once positive it remained so until death.

The present study was designed to evaluate the sensitivity and specificity of the CT, comparing it with the classical methods for rabies diagnosis. Animals inoculated with different rabies virus strains and naturally infected individuals were used.

A preliminary report on this study was presented at the II Jornadas Argentinas de Microbiología, 22–26 November 1970, Buenos Aires, Argentina.

MATERIALS AND METHODS

Cornea samples. Three hundred and thirteen samples were obtained by the technique described by Schneider (16). A microscope slide was pressed against the eyeball of the following species several times: 205 albino mice inoculated with fixed rabies virus strains or brain suspensions of samples received at this laboratory for rabies diagnosis; 16 cattle challenged with a virus strain isolated from a Desmodus rotundus (5); 72 dogs, 14 cats, 3 human beings, 1 horse, 1 rabbit, and 1 rat, all suspected of being rabid. Most of the samples were obtained at the time of death.

FA test. The technique described by Goldwasser et al. (7) was used for staining the corneal and brain impressions. The characteristics of the conjugate and equipment used have been described elsewhere (10).

Mouse inoculation test. The technique described by Koprowski (9) was used to inoculate mice intracerebrally with 20% suspensions of brain specimens.

RESULTS

In the description of his technique, Schneider (16) indicated that 700 to 800 cells should be obtained on the impression smear. Throughout this study we found it difficult to obtain that many cells and, more frequently, 100 to 200 cells adhered to each slide.

Of the 313 specimens studied, there was complete agreement between the CT and FA test of the corresponding brain in 68 positive and 150 negative cases (Table 1). On the other hand, 95 CT-negative samples belonged to subjects whose brains were positive for rabies.
Table 1. Results of the corneal test in rabid and nonrabid subjects of various species

<table>
<thead>
<tr>
<th>Species</th>
<th>Rabies status*</th>
<th>Corneal test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>Mice*</td>
<td>Rabid 45</td>
<td>62</td>
</tr>
<tr>
<td></td>
<td>Not Rabid 0</td>
<td>98</td>
</tr>
<tr>
<td>Cattle*</td>
<td>Rabid 1</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Not Rabid 13</td>
<td>13</td>
</tr>
<tr>
<td>Dogs</td>
<td>Rabid 20</td>
<td>26</td>
</tr>
<tr>
<td></td>
<td>Not Rabid 0</td>
<td>26</td>
</tr>
<tr>
<td>Cats</td>
<td>Rabid 1</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Not Rabid 0</td>
<td>10</td>
</tr>
<tr>
<td>Humans</td>
<td>Rabid 1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Not Rabid 1</td>
<td>1</td>
</tr>
<tr>
<td>Other</td>
<td>Rabid 0</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Not Rabid 2</td>
<td>2</td>
</tr>
<tr>
<td>Totals</td>
<td>Rabid 68 (41.7%)</td>
<td>95 (58.3%)</td>
</tr>
<tr>
<td></td>
<td>Not Rabid 0</td>
<td>150 (100%)</td>
</tr>
</tbody>
</table>

* Based on results of FA and mouse inoculation tests on brain postmortem.

Of mice and cattle postmortem brain infected with rabies were tested, the sensitivity for mice was 41.7% and for cattle 41.5%.

The FA and mouse inoculation tests agreed for all of the brains examined.

The sensitivity and specificity for the CT obtained in the present study were nearly 100%, respectively. The sensitivity for the test was 41.8% for experimentally infected animals and 41.5% for naturally infected individuals.

**DISCUSSION**

We are aware of four previous reports on the use of the CT in a limited number of naturally infected subjects: four foxes (16) and three human beings (4, 11, 12). In the present study, a large number of naturally infected subjects and experimentally infected mice and cattle were studied.

The sensitivity obtained for the CT was similar for both experimentally and naturally infected individuals. This sensitivity (41.7%) was lower than that obtained by Schneider in mice inoculated with a single strain (16). This difference may have resulted from the wider variety of species and virus strains involved in our study or from the smaller number of cells, compared to Schneider's results, that remained on the slides after the corneal impressions were made. The low sensitivity for the CT obtained with mice (Table 1) may be accounted for by the fact that some of these animals were inoculated with fixed rabies virus strains which do not infect salivary glands. Schneider found a correlation between the demonstration of virus in the cornea and the salivary gland. Atanasiu et al. (2) experimentally infected seven foxes, three of which subsequently were found positive to the CT (sensitivity, 42.8%).

In a preliminary, unpublished study, some false-positive CT results were obtained. As further experience was acquired with the technique, the present study was carried out, and a specificity of 100% was obtained. Nonspecific fluorescence, most likely crystals, may be seen occasionally on corneal cells and could confuse the beginer.

The CT has been described as an "ante-mortem" rabies diagnosis method (16). The recent report of Ishii (8) on investigations of complement-fixing activity in saliva samples done before 1955 might open a new possibility for the early diagnosis of the disease.

Early diagnosis of rabies in the biting animal is of extreme importance to physicians in the management of potential human exposures. Moreover, early diagnosis of cases in man may be important in prolonging the illness period (13-15) until recovery of the patient (3) by intensive therapy and constant respiratory assistance. A positive CT result could be useful in early diagnosis and would be of value for laboratory confirmation of rabies cases when the classical methods cannot be performed. According to the present experience, a negative result would not exclude the possibility of the disease.

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**LITERATURE CITED**