Validity of Spinal Cord Examination as a Substitute Procedure for Routine Rabies Diagnosis

TSUN K. LEE AND MAURICE E. BECKER
Division of Virology, Bureau of Laboratories, Michigan Department of Public Health, Lansing, Michigan 48914

Received for publication 12 July 1972

A significant portion of specimens received by this laboratory for rabies diagnosis is unsatisfactory for testing due to decomposition of the brain, or severe mutilation of the head when the animal was killed. Examination of the spinal cord was therefore explored as a possible alternative method when standard brain examination was not possible. In this study, both brain and spinal cord of 248 rabies-suspect animals were examined to assess the reliability of the spinal cord method. Brain and spinal cord of the 248 animals were examined by fluorescent antibody (FA) method, and mouse inoculation tests were performed on 247 brain specimens and 13 spinal cord specimens. By using both brain and spinal cord, 30 animals representing 8 species were diagnosed as rabid by FA, and 218, representing 11 species, were negative. There was 100% agreement between two procedures with FA as the criterion. This study showed that in cases where the usual examination is precluded due to brain destruction, the spinal cord procedure offers an equally reliable alternate method of diagnosis.

The Virology Division of the Michigan Department of Public Health annually receives around 2,000 animals for rabies diagnosis. Although most of the specimens present no diagnostic problem, there is a small but significant portion varying from 40 to 80 per year which are unsatisfactory for testing. Although some specimens are too decomposed to yield reliable results, the majority are unsatisfactory because of extensive destruction of the brain resulting from physical trauma. These unsatisfactory specimens present a serious problem because physicians rely heavily upon the laboratory results as a guide in determining the necessity for prophylactic treatment of exposed individuals. An alternative diagnostic procedure is therefore highly desirable when brain material is not available.

Since the cervical portion of the spinal cord usually remains intact even when the brain has been completely destroyed, the present study was undertaken to determine whether examination of this tissue would yield results identical to that of the brain. Rabies antigen has been demonstrated in the spinal cord of rabid animals by standard histopathological techniques (14), but to the authors' knowledge, no comparative study has been made to determine the validity of the spinal cord examination for routine diagnosis, either by histopathological techniques or by the more accurate fluorescent antibody (FA) method. The suitability of spinal cord for demonstrating rabies antigen by FA technique has been suggested in several experimental studies (6, 7, 13).

MATERIALS AND METHODS

Preparation of tissues for FA examination. In this study, only heads or cords of suspect species were examined, i.e., species in which rabies is most likely to occur in Michigan. Heads of large animals were generally severed near the second or third cervical vertebra when shipped to the laboratory. Small animals were submitted whole. To avoid the possibility of cross-contamination, the spinal cord was processed first. Cross-sectional impressions were made from the excised cervical section of spinal cord. The calvarium was then removed and, by using separate sets of instruments, impressions were made from excised sections of Ammon's horn, cerebral cortex, and cerebellum by the procedure of Tierkel and Neff (14).

FA test. The FA procedure was essentially that of Goldwasser, et al. (9). Lyophilized fluorescein-labeled antirabies globulin of equine origin (Baltimore Biological Laboratories, lot no. 9041906) was reconstituted in distilled water, titrated, and used at a working dilution of 1:25. The labeled globulin was diluted in either 20% normal mouse brain or 20%
rabies-infected mouse brain homogenized in 20% horse serum-distilled water.

A Bausch & Lomb binocular microscope was fitted with a cardoid condenser, 40× Fluorite oil immersion objective lens, and 10× broad-field eyepieces. The light source was a Reichert “Fluores” unit with a high-pressure mercury lamp (OSRAM, HBO-200). The primary filter was Corning no. 5970, and the secondary filter was Wratten 2A. The mounting medium was 90% glycerine in buffered saline solution at pH 8.1. The immersion oil was Cargille, type B, low fluorescence.

**Animal inoculation test.** The procedure was that described by Tierkel and Neff (14). Pooled brain tissue or spinal cord was ground in a mortar with sterile Alundum. An approximate 10% suspension in phosphate-buffered saline (pH 7.2 to 7.4) was centrifuged at 2,000 rev/min for 10 min. Nine-tenths milliliter of the supernatant fluid was pipetted into a sterile test tube containing antibiotic solution so that the final concentration was 1,000 units of penicillin and 1,000 µg of streptomycin per ml. The suspension was left at room temperature for at least 0.5 hr before inoculation of 0.03 ml intracerebrally into each of five weanling mice. Any mouse which became ill or died during the 21-day observation period was checked for rabies by FA examination of the brain.

**RESULTS**

The results of FA testing of 248 rabies-suspect animals can be seen in Table 1. By using both brain and spinal cord, 30 animals representing 8 species were diagnosed as rabid by FA, and 218 representing 11 species were negative. The agreement of brain and cord results on both negative and positive specimens was complete with FA used as the criterion. No FA-negative animal was subsequently diagnosed as being rabid by animal inoculation of brain.

The results of the mouse inoculation test on the 30 animals with FA-positive brain and spinal cord are shown in Table 2. The brain gave a higher percentage of positive results by the mouse test than did the spinal cord. Twenty-seven out of 29 specimens were positive in mice when brain was tested, whereas only 10 out of 13 specimens were positive in mice when spinal cord was tested. The brain of one FA-positive bat (72-421) could not be tested in mice. However, it was positive in mice with spinal cord material. Hence, a total of 28 out of 30 FA-positive specimens was confirmed by animal inoculation. Two bat specimens (72-325 and 72-1225) were negative.

A comparison of the stained slides from both types of nervous tissue indicated that Negri bodies tended to be more numerous in the brain impressions. In both cases, however, the minute but diagnostically significant sandlike particles often seen in rabies infection (5) were present in

### Table 1. Results of brain and spinal cord examinations on 248 animals

| Species | FA test | Animal test |
|---------|---------|-------------|
|         | Brain   | Spinal cord | Brain | Spinal cord |
| Bat     | 8/125*  | 5/124*      | 3/6   |
| Bobcat  | 1/2     | 1/2         | 1/1   |
| Cat     | 1/12    | 1/12        | 1/1   |
| Cow     | 3/11    | 3/11        | 1/1   |
| Coyote  | 0/1     | 0/1         | 0/1   |
| Dog     | 1/19    | 1/19        | 1/1   |
| Horse   | 7/36    | 7/36        | NT    |
| Pig     | 0/2     | 0/2         | NT    |
| Raccoon | 0/1     | 0/1         | NT    |
| Skunk   | 6/33    | 6/33        | 1/1   |

* Number positive/number examined.

```
| Species | Mouse inoculation test* |
|---------|-------------------------|
|         | Brain | Spinal cord |
| Fox (71-1500) | +     | NT         |
| Horse (71-1515) | +     | NT         |
| Fox (71-1537) | +     | NT         |
| Fox (71-1546) | +     | NT         |
| Fox (71-1547) | +     | NT         |
| Skunk (71-1666) | +     | NT         |
| Cow (71-1670) | +     | NT         |
| Fox (71-1682) | +     | NT         |
| Cow (71-1684) | +     | NT         |
| Skunk (71-1711) | +     | NT         |
| Fox (71-1932) | +     | NT         |
| Bat (71-2010) | +     | NT         |
| Skunk (71-2022) | +     | NT         |
| Skunk (71-2202) | +     | NT         |
| Fox (72-49) | +     | NT         |
| Bat (72-108) | +     | +          |
| Skunk (72-134) | +     | NT         |
| Bat (72-325) | -     | NT         |
| Bat (72-421) | NT   | +          |
| Bat (72-434) | +     | -          |
| Horse (72-623) | +     | +          |
| Bat (72-811) | +     | +          |
| Horse (72-828) | +     | +          |
| Bobcat (72-947) | +     | +          |
| Bat (72-1029) | +     | +          |
| Skunk (72-1082) | +     | +          |
| Cat (72-1183) | +     | +          |
| Bat (72-1225) | -     | -          |
| Cow (72-1765) | +     | +          |
| Dog (72-2040) | +     | +          |
```

*+, Rabies positive; NT, not tested.
sufficient quantity to be readily detectable.

**DISCUSSION**

The diagnosis of rabies by FA staining of brain impressions was first reported by Goldwasser and Kissling (8). Later, Goldwasser et al. (9) and Beauregard and Casey (2) described FA procedures on salivary glands, and recently Schneider suggested the FA staining of corneal impressions (12). Both of these methods are purportedly useful in determining if virus excretion has occurred, but negative findings do not rule out rabies because virus may be present in concentration too low to be detectable, or because the centrifugal spread of the virus from the brain has not yet occurred. The corneal method would be useful in selected cases, but the routine examination of 700 to 800 corneal epithelial cells from every suspect animal would be too time-consuming. At one time, both the salivary glands and brain of suspect bats were examined, because virus was thought to occur in salivary glands without concurrent brain infection. More recent evidence, however, obtained with the very reliable FA technique has demonstrated that, even in bats, brain examination alone is entirely adequate for establishing a diagnosis (4).

The possible use of spinal cord for diagnosis of rabies was suggested by the experiments of Serokowa et al. (13) and Dean et al. (6). Both groups showed that the FA test could detect rabies antigen days earlier in spinal cord material than in brain material when mice were infected by the peripheral route with street virus. Dean (5) has similarly reported that examination of brain stem is highly reliable and may yield positive results when other portions of the brain are negative. In our study, however, we did not encounter any specimen in which rabies antigen was found only in spinal cord. It is true that two of the bat spinal cord specimens (72-434 and 72-1020) were not confirmed when spinal cord was inoculated into mice, but the brain was positive by both the FA and mouse tests. There were, however, two bats (72-325 and 72-1225) which were negative in mice when brain was used as inoculum. Discrepancy in results between FA and mouse inoculation has been reported by others (1, 3), and the failure to isolate virus may be due to limitations of the animal test (10, 11). On the other hand, some cases of rabies have been detected only by mouse inoculation. Our laboratory has always tested all rabies-suspect animals by both FA and mouse inoculation because of occasional discrepancies between the two tests. In fact, over the past 10 years, some 16 positive specimens would have been missed if we had used FA alone, and 14 if we had used mouse inoculation only. Further to improve our diagnostic acumen in this laboratory, we have now instituted the spinal cord examination described herein for all specimens that are unsatisfactory for testing by the usual brain examination.

**ACKNOWLEDGMENT**

We gratefully acknowledge the excellent technical assistance of Elaine C. Davis in this study.

**LITERATURE CITED**

1. Beauregard, M., P. Boulanger, and W. A. Webster. 1965. The use of fluorescent antibody staining in the diagnosis of rabies. Can. J. Comp. Med. 29:141-147.
2. Beauregard, M., and G. A. Casey. 1969. Demonstration of rabies antigen in salivary glands of rabies suspected animals. Can. J. Comp. Med. 33:55-58.
3. Carski, T. R., R. E. Wilsnack, and R. K. Sikes. 1962. Pathogenesis of rabies in wildlife. II. Fluorescent antibody studies. Amer. J. Vet. Res. 23:1048-1052.
4. Center for Disease Control Zoonoses Surveillance. 1971. Monthly summary, July 1971.
5. Dean, D. J. 1966. The fluorescent antibody test, p. 59-68. In P. Atanasius et al. (ed.), Laboratory techniques in rabies, 2nd ed. World Health Organization, Geneva.
6. Dean, D. J., W. M. Evans, and R. C. McClure. 1963. Pathogenesis of rabies. Bull. W.H.O. 29:803-811.
7. Fischman, H. R. 1969. Fluorescent antibody staining of rabies-infected tissues embedded in paraffin. Amer. J. Vet. Res. 30:1213-1221.
8. Goldwasser, R. A., and R. E. Kissling. 1958. Fluorescent antibody staining of street and fixed rabies virus antigens. Proc. Soc. Exp. Biol. Med. 98:219-223.
9. Goldwasser, R. A., R. E. Kissling, and T. R. Carski. 1959. Fluorescent antibody staining of rabies virus antigens in the salivary glands of rabid animals. Bull. W.H.O. 20:573-588.
10. Johnson, H. N. 1969. Rabiesvirus, p. 321-353. In E. H. Lenette and N. J. Schmidt (ed.), Diagnostic procedures for viral and rickettsial infections, 4th ed. American Public Health Association, Inc., New York.
11. Parker, R. L., and R. K. Sikes. 1966. Development of rabies inhibiting substance in skunks infected with rabies virus. Pub. Health Rep. 81:941-944.
12. Schneider, L. G. 1969. The cornea test; a new method for the intravitam diagnosis of rabies. Zentralbl. Veterinarmed. Reihe B 16:24-31.
13. Serokowa, D., K. Krawcznski, and W. Brzosko. 1967. The use of immunofluorescence for the detection of street rabies virus in the central nervous system of mice in the incubation period of the disease. Exp. Med. Microbiol. 19:204-216.
14. Tierkel, E. S., and H. O. Neff. 1957. Rabies. Methods in laboratory diagnosis, p. 1-42. Communicable Disease Center, Atlanta.