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The Fluorescent Antibody Test (FAT) in the Diagnosis of Rabies in Sri Lanka

by

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SUMMARY The fluorescent antibody test in the diagnosis of rabies infection in naturally infected animals was introduced for the first time in Sri Lanka. The result of the FAT gives a favourable comparison with the results of the biological test, the agreement being 95.5% in relation to both positive and negative instances.

INTRODUCTION

Although fluorescent microscopy is not a new technique, its use to detect antigen antibody reactions by immunofluorescence stems from the work of Coons and Kaplan (1950); their techniques were later adopted for the diagnosis of rabies by Goldwasser and Kissling (1958).

The fluorescent antibody test (FAT) is said to be the most accurate test available at present for the diagnosis of rabies. We set out to evaluate the efficiency of this test under the conditions in Sri Lanka by comparing the results of the FAT with those of the standard biological test.

MATERIAL AND METHODS

Only fresh brain specimens found to be negative in the "routine" direct microscopic test, were examined by the fluorescent antibody technique. Most of the brains examined were that of the dog, however, occasionally brains of cats, goats, monkeys and humans were tested.

In the early stages of our study, a French antirabies fluorescein labelled globulin (conjugate) supplied by the Institute Pasteur in Paris was used in a dilution of 1 in 8 ; later a commercial conjugate obtained from the Baltimore Biological Laboratories was used in a dilution of 1 in 20.

A Reichert Zetopan fluorescent microscope was used in our work. An HBO 200 Watt mercury vapour lamp served as the light source. A 40% high power dry objective with a N. A. of 0.65 and an 8% ocular were used. This microscope was also fitted with UV exciter filters and barrier filters.

In this laboratory the direct method of FAT was used. Two smears, one from brain tissue taken from the hippocampus (ammon's horn) and the other from the brain stem, were prepared from each specimen on two different slides. After these smears have been dried in air, they were fixed in cold acetone at -20° C for 4 to 24 hours. Two circles about one inch in diameter were made with a Chinagraph pencil on each smear. Onto one circle was placed one drop of conjugate diluted with a 20% suspension of normal mouse brain and on the other was placed the same conjugate diluted with a 20% suspension of mouse brain containing CVS rabies fixed virus. After 30 minutes incubation at 37°C, the slides were rinsed according to a standard procedure (Johnson, 1964). The circles were then covered by cover slips using 33% glycerol in buffered physiological saline solution of pH 7.3. After a further two hours incubation at 37°C the slides were ready for examination.

The amount of fluorescent material visualised was graded 1 + to 4 +. The presence of fluorescent particles together with disk ring forms were graded as 3 + or 4 +. Positive and negative controls were included in all tests. Only specimens graded 3 + and 4 + were immediately reported. All specimens were simultaneously inoculated intracerebrally into white mice.

Specimens found to be negative in the FAT and positive in the biological test were titrated intracerebrally in young white mice using eight mice per dilution and the mice observed for a period of 21 days. The LD 50 of the virus was calculated by the method of Reed and Muench (1938).

RESULTS

In the period December 1967 to November 1968, a total of 246 specimens were examined by the fluorescent technique.

TABLE 1

Comparison of the results of Biological and Fluorescent antibody tests in the diagnosis of rabies.

Technique	Result	No. of Specimens	% of total tested	Evaluation
Biological NEG / FAT NEG		137	55.7	95.5% complete agreement
Biological POS / FAT POS		98	39.8	
Biological POS / FAT NEG		3	1.2	Missed by FAT
Biological NEG / FAT POS		8	3.3	False positive
Total		246	100.0	

TABLE 2

Some details of specimens missed by the fluorescent antibody test.

Laboratory No. of specimen	Titre (LD50) of virus in neg Log.	Reading in repeated FAT tests 1 2 3 4 5 6 7 8
375	2.4	— — — —
667	Less than 1.0	— — — —
764	3.0	— — — + — + + —

Of these 137 were negative both in the FAT and biological test and 98 were positive in both tests. Thus there was 95.5% agreement between the FAT and biological test.

In 3 cases the FAT was negative but the biological test gave positive results. The latter figures show that in 1.2% of all tested specimens, rabies infection was missed by the fluorescent technique.

In 8 cases the FAT was found to be positive but the biological test gave negative results. This amounted to 3.3% of all tested specimens and could be considered as "false positives".

A titration to determine the amount of virus present was carried out in the three cases missed by the FAT. These specimens were also rechecked by FAT. The results of the titration are shown in Table 2. The LD 50 of the virus was $10^{-2.4}$, less than 10^{-1} and $10^{-3.0}$ respectively. In the first two cases the rechecking by FAT again gave negative results, in the third case, however, three out of eight smears were found to be positive.

In the eight "false positive" cases, the amount of fluorescence was graded five times as 2 + and three times as 1 + only.

DISCUSSION

In this laboratory only specimens found to be microscopically negative in the "routine" direct examination of brains were examined by FAT. However, earlier studies of FAT by other workers in this field also included microscopically positive specimens. Microscopically positive specimens were not included in our study for economic reasons.

We find that the 95.5% agreement of the FAT with the positive and negative results of the biological test is very favourable and is in keeping with the results of other laboratories abroad.

It is important to stress that the advantage of the fluorescent antibody test in the diagnosis of rabies is that results are obtained in a few hours as compared to the prolonged period of observation, namely 21 days needed in the case of the biological test. This advantage is particularly outstanding in cases where the direct microscopic examination gave negative results as in the past all persons exposed to the risk of infection in such cases had to undergo a full course of antirabies treatment.

In 1.2% of all tested specimens (2.97% of all positive biological test), rabies infection was not diagnosed by FAT. The missing of rabies infection in some cases by FAT is to be expected since it is known from FAT studies in other virus infections that this test has a threshold of sensitivity. The three missing cases in this laboratory also gave an indication of this fact in rabies infection in animals. The results of virus titrations done in this laboratory indicate that the threshold of sensitivity of FAT in rabies infection in naturally infected animals is around $10^{-2.4}$ LD 50 of virus.

On the results of our study we are of opinion that post exposure treatment in FAT negative cases should be decided on the basis of clinical and epidemiological evidence pertaining to the animal concerned. In the face of strong clinical and/or epidemiological evidence in favour of rabies infection in the animal, the specimen should be rechecked by FAT in at least six smears and a biological test also carried out simultaneously.

The "false positives" are said to be due to the presence of dead virus which will thus give a negative result in the biological test (Carski and Wilsnack, 1966). However, in this laboratory there was neither clinical nor epidemiological evidence of rabies infection in the above mentioned eight cases. In these eight cases as the amount of fluorescence was low i.e. 1 + or 2 + we are of opinion that these eight cases are true "false positives".

After one year's experience with the fluorescent antibody technique in the diagnosis of rabies, we are of the opinion at the present moment that cases showing 3 + or 4 + of fluorescence could be reported as positive for rabies infection. Also in those cases read as negative the FAT could be reported as negative for rabies infection except in cases mentioned above. The remaining cases graded as 1 + or 2 + should not be reported until examined by the biological test.

The advantages derived by the introduction of FAT in the diagnosis of rabies infection in Sri Lanka could be stated as follows :—

In our laboratory, there are annually about 145 specimens which are microscopically negative but turn out to be positive in the biological test. In the past the persons exposed to the risk of rabies infection with regards these cases were nevertheless advised to take antirabies treatment. Most of them were however reluctant to do so in view of the negative direct microscopic examination. After the introduction of the fluorescent antibody test in diagnosis, however, 101 of these 145 specimens were also tested by the FAT in this laboratory. The persons exposed to rabies infection in the 98 of these cases which were FAT positive were now readily willing to undergo post exposure treatment.

Two hundred and sixty five (265) specimens were both negative in the direct examination and in the biological test. However, in the past, all persons in contact with these cases were recommended antirabies treatment as the results of the biological test would take a long time. However, in the future, those persons in contact with the FAT negative cases in this group need not undergo antirabies treatment except in the cases where there is clinical and/or epidemiological evidence in favour of rabies infection in the animal concerned.

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