



COMPARING THE SENSITIVITY OF DETECTING VIRAL ANTIGEN IN DIFFERENT PARTS OF RABIES SUSPECTED BRAIN USING FLUORESCENT ANTIBODY TEST

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Abstract

Among 78 rabies suspected brain samples examined, 61 were found positive in direct fluorescent antibody test (FAT) and maximum percentage of positivity was observed (91.8 per cent) in impression smears from the brain stem. A thorough examination of various parts of brain tissue including the brain stem is needed before giving a concrete result for rabies diagnosis using FAT.

Key words: Rabies, direct fluorescent antibody test, brain stem

Only few diseases cause as much anxiety as does rabies. The laboratory diagnosis occupies a central role in meeting the threat of rabies because upon its verdict often depend the decision whether or not to proceed with a course of post exposure antirabies therapy. The choice of the parts of brain for taking impressions for FAT definitely affects the results of the test (Tepsumethanon *et al.*, 1997; Bingham and Merwe, 2002).

Clinical observation may only lead to a suspicion of rabies because signs of the disease are not characteristic and may vary greatly from one animal to another and no gross postmortem lesions can be considered pathognomonic. The only way to perform a reliable diagnosis of rabies is to identify the virus or viral antigens using laboratory tests (Hostnik *et al.*, 2001; David *et al.*, 2002).

The reliability of immunofluorescence depends on the section of the brain tissue taken because rabies virus does not infect uniformly and varies with species susceptibility (Dean *et al.*, 1996). So obviously the choice of the tissue for taking impressions for FAT definitely affects the sensitivity of the test. Hence the present study was undertaken to assess the reliability of detecting the viral antigens from various parts of the brain using FAT.

Materials and Methods

Brain samples were collected from 78 dogs suspected for rabies, brought for postmortem examination at the College of Veterinary and Animal Sciences, Mannuthy. Out of 78, 31 dogs were having definite history of dog bite. Impression smears were prepared from hippocampus (both right and left), brain stem and cerebellum and subjected to direct FAT as per CDC protocol (2003).

Impression smears were fixed by keeping in cold acetone (-20°C) for 30 min. The slides were taken out and allowed to dry. Diluted Fluorescien isothiocyanate conjugated antirabies antinucleo capsid antibody was added to the slides and incubated in a moist chamber at 37°C for 45 min. Slides were then washed in phosphate buffered saline (pH 7.4), two changes for 5 min. Slides were then

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examined under fluorescent microscope using 20% glycerol-Tris buffered saline as mountant.

Results and Discussion

Positive samples revealed foci of viral antigen as apple green fluorescence under ultraviolet illumination. Out of 78 brain samples examined 61 were positive by FAT (Table). When the positivity of impression smears from various parts of the brain were compared, brain stem showed maximum percentage of positivity (91.8 per cent). Higher sensitivity of impression smears from brain stem in diagnosing rabies by FAT was also reported by Tepsumethanon *et al.* (1997) and Bingham and Merwe (2002). Fifty samples (80.3 per cent) taken from hippocampus and 44 samples (72 per cent) from cerebellum showed good results equivalent to that of brain stem. The fluorescence varied in size and ranged from large oval to small sand-dust like particles. The

small sized fluorescing particles were observed in the brain stem, while large sized fluorescence was observed in the impression smears from hippocampus and cerebellum. In two cases impressions from brain stem were negative but hippocampus showed positive result. Samples from cerebellum showed positive result in three cases where brain stem were negative.

Thus, examination of various parts of brain for detection of rabies virus antigens using FAT showed maximum percentage positivity with impressions from brain stem. Hence examination of brain stem must be included in every specimen before giving a negative result. It was also concluded that a thorough examination of impression smears of various brain tissue is needed before giving a concrete result for rabies diagnosis using FAT.

Table. Comparison of different sampling sites in FAT for rabies diagnosis

Part of brain examined	Number positive	Percent positive
Brain stem	56	91.8
Hippocampus	50	80.3
Cerebellum	44	72.0

Total samples examined : 78

Number of samples positive for rabies : 61

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