Short Communication

ANTEMORTEM DIAGNOSIS OF RABIES IN A DOG BY FLUORESCENT ANTIBODY TECHNIQUE AND CONFIRMATION BY WILLIAM'S MODIFIED VAN GIESON'S STAIN ON AUTOPSY

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ABSTRACT

Rabies in dogs can be diagnosed by ante-mortem examination that plays an important role in early identification of infection in dogs. An unvaccinated 4 month old male dog was admitted in Teaching Veterinary Clinical Complex at Madras Veterinary College with the clinical symptoms of respiratory distress, paresis, anorectic hyperesthesia, relaxed rectal sphincter, hypothermia, congested mucous membrane, frothy salivation, brownish diarrhoea. It was suspected for Rabies and was admitted in the rabies ward unit. The dog was screened for rabies viral antigen in salivary smear and corneal impression smears and confirmed for rabies by FAT in ante-mortem examination. After 2 days, death of animal was confirmed for rabies by post-mortem examination by Williams Modified Van Gieson's Staining method. The results confirmed the presence of rabies in that animal

Key words: Ante-mortem diagnosis, rabies, saliva, FAT, William's modified van Gieson's stain

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INTRODUCTION

Rabies is a fatal and zoonotic disease in mammals (Jackson and Wunner, 2007). It was primarily transmitted through bite of rabies infected animals. Canine rabies is threat to more than 3.3 billion people as per record of WHO (WHO,2010). Rabies is a dreadful and untreatable disease causing death of 59,000 people annually in Asia and Africa (Hampson *et al.*, 2011). In India, Sudarshan *et al.* (2007) reported that around 20,000 people die annually due to rabies (Sudarshan *et al.,* 2007). Dogs are the important reservoir host for rabies and cause 97 % of human death. In India rabies control in practiced through control of stray dog population. Post mortem brain testing by FAT, is the gold standard method of rabies diagnosis. (Jemberu *et al.,* 2013).

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The aim of this study was to find out the ante-mortem diagnosis of rabies in a suspected dog having clinical signs, compared and confirmed with post-mortem examination.

MATERIALS AND METHODS

The study was carried out at infectious disease unit of Veterinary Teaching Hospital, Madras Veterinary College, Chennai. A dog suspected with clinical symptoms of respiratory distress, paresis, anorectic hyperesthesia, relaxed rectal sphincter, hypothermia, congested mucous membrane, frothy salivation, brownish diarrhoea was admitted in Under Observation for Rabies (UOR) unit (Fig 1 & 5). Impression smears were taken from saliva and cornea using gloves. Collected impression smears were dried and kept in Coplin jar contain acetone as a fixative. The following methods of study were used: a) microscopic examination of Williams'-modified van Gieson's stained impressions (WMVGS) and b) microscopic examination of impressions stained by the fluorescent antibody technique (FAT) (Fig 2&3). The same was applied on the brain tissue sample after post-mortem. Before sample collection, preventive measures like Anti Rabies Vaccine and protective items like gloves, apron, masks, goggle and face shield were used to prevent exposure to rabies virus.

Direct Fluorescent Antibody test (DFAT)

Lyophilised, adsorbed anti- rabies nucleocapsid antibody conjugate with fluorescein isothiocyenate (Biorad) was used in FAT (Meslin *et al.,* 1996).

*Prepared from rabid suspected dog brain was placed in Coplin jar containing acetone and fixed at 4°C for one hour.

*The slides were air-dried and anti-rabies nucleocapsid conjugate was added. It was incubated (Bio-Rad, France) for 45 minutes at 37°C in a humid chamber.

*The slides were washed three times with PBS for 5 min and air-dried.

*The dried slides were applied with glycerol saline and visualised under fluorescent microscope.

*Presence of apple-green round to oval intracellular accumulations of viral particles was observed.

Williams modified Van Gieson's staining procedure

The smears were fixed in absolute methanol and stained with William's modification of Van Gieson's stain for detection of Negri bodies as described by Arulanandam *et al.,* 2020

Stock solution: Saturated alcoholic solutions of basic fuchsin and methylene blue. Working solution was freshly prepared by mixing 3 drops of basic fuchsin, 7 drops of methylene blue in 10 mL of distilled water.

Post-Mortem examination of dog

Impression smear was taken from hippocampus and fixed in acetone. Direct FAT and Williams Modified Van Gieson's Stain method was performed in impression smears and confirmed for rabies.

RESULTS AND DISCUSSION

The dog aged 2 years suspected for rabies showed clinical sigs of respiratory distress, paresis, anorectic, hyperesthesia, relaxed rectal sphincter, hypothermia, congested mucous membrane, frothy salivation, brownish diarrhoea. It was unvaccinated. Saliva smear and corneal impression smear showed presence of viral inclusion bodies by direct FAT. Hippocampus of impression smear collected from post-mortemexamination and subjected to cytological examination of Negri bodies identification by using Williams Modified Van Gieson's staining method for the confirmation of Rabies.

Kaw *et al.* (2014) reported that saliva and nuchal skin biopsy are proved to be ante-mortem diagnostic in animals. Singh and Ahmad, (2018) suggested there was difficulty and risk in collecting samples in live rabid animals and hence only limited studies available in ante-mortem diagnostic in animals.

Direct Fluorescent antibody test is the easy, quick reliable method for the diagnosis of rabies. WHO recommended the FAT test as a gold standard test for the rabies antigen demonstration in the brain samples. Umoh *et al.* (1985) reported that FAT could detect 90 per cent of cases which would have been missed by Negri body staining and mouse inoculation. The specificity of this test was 100%, even with clinical samples, a positive result with this test will help the physician to establish a definitive diagnosis of rabies ante-mortem and help in the appropriate management of the patient as well as those with close contacts.

Hemachuda and Mitrabhakdi, (2000) reported that most of the animal bites in developing countries like India occur in peripheral districts not having laboratory facilities for the confirmation of the rabies status. Brains of these animals were sent to central laboratories for diagnosis and post-exposure treatment instituted pending the test report, which may take more time and post-exposure treatments could be avoided if the rabid status of the animal could be ruled out by the use of simple tests that could be carried out at small peripheral laboratories Ante-mortem diagnosis including the much-publicized corneal test and frozen section skin biopsy.

Hemachuda and Mitrabhakdi, (2000) suggested that direct FAT provides 100 per cent sensitivity in post-mortem diagnosis of rabies samples in human and animals and in the case of ante-mortem diagnosis of rabies from non-nervous tissue like saliva and corneal impression smear insignificant results or poor sensitivity were revealed.

In autopsy the impression smear was confirmed by the presence of intracytoplasmic inclusion bodies of negri bodies by William's method (Fig. 4). Hence, William's modification of Van Geison's stain is the easy, quick reliable stain for the diagnosis of Negri bodies in the impression smear of brain. Samples were positive for

rabies by identifying the intracytoplasmic inclusion bodies, namely Negri bodies in the neuronal cell. Negri bodies are magenta red colored intracytoplasmic inclusion bodies present in the neuronal cells (Arulanandam *et al*., 2020)

The present paper concludes that the presence of rabies in the dog was confirmed by clinical signs and ante-mortem examination of saliva and corneal impression smear with FAT. The same was confirmed with presence of Negri bodies in the impression smear made from hippocampus an post-mortem. William's Modified van Gieson's stain is the easy, quick reliable stain for the diagnosis of negri bodies in the impression smear of brain (Arulanandam *et al.,* 2020). In conclusion, our findings showed saliva and corneal impression smear also to be a clinical sample for ante-mortem diagnosis of rabies in dog.

Fig 1: Dog with salivation

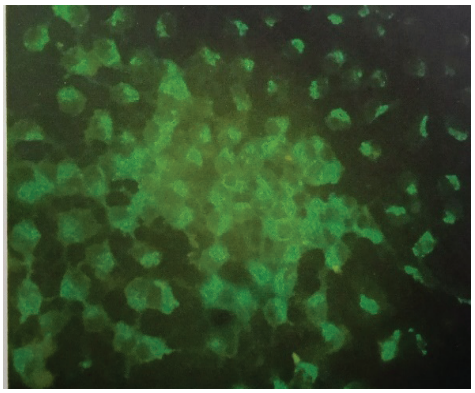

Fig 2: Saliva impression smear -FAT

Apple green fluorescence of conjugated antirabies antibodies in impression smear of hippocampus

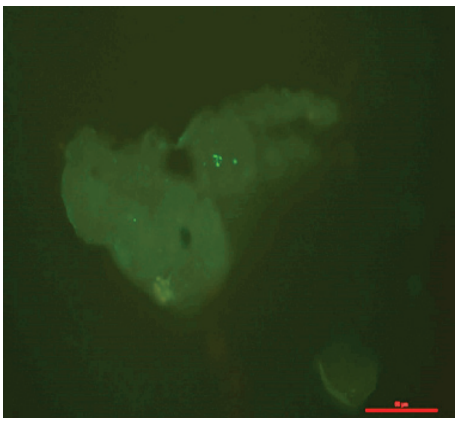

Fig 3: Corneal impression smear -FAT

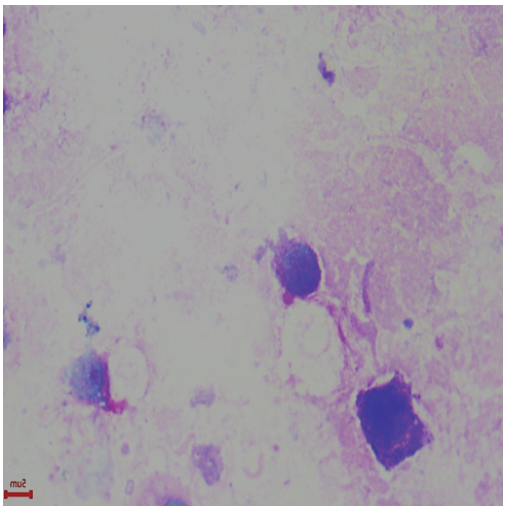

Fig 4: Negri bodies – William modified van Gieson's stain - PM Negri bodies in cytology

Fig 5: Dog in Under observation Unit

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