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Comparative study of DFAT, cytology and histopathology in diagnosis of rabies

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Abstract
Rabies was a fatal disease which was diagnosed by various diagnostic methods. In this study, samples were collected from brain of dogs suspected for rabies. Brain impression smear were subjected to direct fluorescent antibody technique. Cytological study was done for identification of Negribodies. Formalin fixed samples were processed and studied for histopathological lesions. Out of 10 samples collected, 7 samples showed positive for rabies. On comparison of above diagnostic techniques, dFAT and Cytology were 100% specificity with each other whereas histopathology had only 43.5%.

Keywords: Rabies, direct, fluorescent antibody, Cytology, Histopathology

Introduction
Rabies is one of the oldest and most feared viral zoonotic diseases caused by highly neurotrpoic, single stranded RNA lyssa virus of *Rhabdoviridae* family. It causes fatal encephalomyelitis with the highest case fatality rate as compared to any other infectious disease (Sharma *et al*., 2014) [1]. Rabies is endemic in India and accounts for about 36% of global rabies burden. India is the hotbed of human rabies as about 20,000 persons are known to die annually that accounts to about one-third of the annual burden of an estimated 59,000 rabies deaths globally (Hampson *et al*, 2015) [2]. Stray dogs were the main source of infection in animals and vaccination is the only way to combat the disease before and after exposure or infection as there is no treatment available once the symptoms have appeared (Nandi and Kumar, 2010) [3].

Materials and Method
Samples were collected during postmortem examination for diagnosis of rabies by direct fluorescent antibody technique (dFAT), cytology and histopathological studies. Gross examination of brain was done for macroscopic changes. Impression smear were taken in the cut section of hippocampus major after blotted with blotting paper to remove blood. Direct fluorescent antibody technique (dFAT) was done after fixing the slides in acetone at -20 ℃ with polyclonal antibody as per WHO protocol (Rupprecht *et al.*, 2018) [4]. Impression smears were fixed in absolute methanol and stained with William's modification of Van Geison's stain for detection of Negri bodies as (Arulanandam *et al*., 2020) [5]. Part of brain constituting cerebrum and cerebellum were collected for histopathological examination using Hemotoxilin and Eosin stain.

Result and Discussion
Totally eleven brain samples were collected during postmortem conducted in the Department of Veterinary Pathology, Madras Veterinary College, in which one sample collected as negative control from animal which came for general postmortem. Another 10 samples were collected from partial necropsy conducted for rabies. These animals were suspected for rabies based on clinical signs and were in the ward Under Observation for Rabies (UOR) till death. 70% of positivity to rabies noticed in animals suspected for rabies, whereas overall incidence of rabies was 52% in suspected cases as per Mugale *et al*., (2013) [6]. Remaining animals may be died due to some other causes.
Grossly, no changes (Fig. 1) noticed in 70% (7/10) of cases (Sumedha, 2010) [7]. But in 30% (3/10) of cases congestion was (Fig. 2) noticed over the meninges giving reddish discoloration to the whole brain as noticed by Nilakanth *et al*., (2013) [8]

On cytological examination of impression smear stained with William's modification of Van Geison's stain, amo yr ng 10 samples suspected for rabies, 7 samples were positive for rabies by identifying the intracytoplasmic inclusion bodies, namely Negri bodies in the neuronal cells (Fig. 3) as seen by Praveena *et al*,. (2003) [9]. Negri bodies are magenta red colored intracytoplasmic inclusion bodies present in the neuronal cells, when stained with William's modification of Van Geison's stain as already reported by Arulanandam *et al*., (2020) [5]

Samples were subjected to direct fluorescent antibody technique (dFAT). Among the 10 samples 7 samples showed fluorescence under fluorescent microscope indicated that rabies viral (RBV) antigens (Fig. 4) were present in the affected cells. In positive samples, apple green fluorescence was observed when focused under fluorescent microscope with specific wave length as reported earlier by Praveena *et al*.(2003) [9] and Wahan *et al*. (2011) [10]

On histopathological examination, of the 11 samples (Table-1) except 1 sample which was collected from a normal animal, various changes were noticed in the rabies affected animals such as congestion and mild spongiosis (Fig. 5) in 100% (7/7) of positive cases. Negribodies were seen in 43.5% (3/7) of cases. Chromatolysis, Satellitosis, Perivascular cuffing (Fig. 6) and neuronal degeneration were recorded in 43.5% (3/7) of the positive cases. Various histopathological changes such as Purkinje cell degeneration, astrogliosis were also noticed in 14.3% (1/7) of cases.

In histopathological examination, Negri bodies were usually seen in the hippocampus in dogs and was observed in 60% of cases by Wahan *et al*., (2011) [10] whereas, we observed in the cerebrum and cerebellum at the level of 43.5% (3/7).

On comparative study of the diagnostic techniques, dFAT is the golden standard technique with 100% specificity to cytology, whereas only 43.5% sensitivity in presence of Negri bodies under histopathological examination. Based on this study, dFAT is the standard technique and cytology may be used as supportive technique (Praveena *et al*., 2003) [9].Histopathology may be used for differential diagnosis and for general screening for various pathological changes. Since only 70% of the cases were positive for rabies which were suspected for rabies, antemortem diagnostic techniques to be used to confirm the rabies so that unnecessary post-exposure vaccination may be avoided and the animals with neurological signs may be screened for diseases other than rabies and treated accordingly.

Table 1: Histopathological examination

S. No	Sample No.	Impression smear Cytology	(dFAT)	Histopathological changes in cerebrum and cerebellum
1	NC	Negative	Negative	No abnormalities detected
2	R1	Positive	Positive	Congestion Mild spongiosis Satellitosis with neuronophagia
3	R2	Positive	Positive	Congestion Mild spongiosis Neuronal degeneration Purkinje cell degeneration Mild internal granular cell necrosis Negribodies present
4	R3	Positive	Positive	Cerebral congestion Perivascular cuffing with few cells Spongiosis
5	R4	Positive	Positive	Congestion Spongiosis Neuronal degeneration and chromatolysis Astrogliosis Neuronophagia Negribodies present
6	R5	Negative	Negative	Mild degeneration and congestion
7	R6	Positive	Positive	Degenerative changes in purkinje cells Spongiosis Perivascular cuffing
8	R7	Positive	Positive	Minimal mononuclear cell infiltration Satellitosis Astrocytosis Spongiosis Vacuolation of cytoplasm of neurons Negribodies present
9	R8	Positive	Positive	Mononuclear cell infiltration Spongiosis Neuronal degeneration Astrocytosis with mononuclear infiltration
10	R9	Negative	Negative	Congestion and spongiosis in cerebellum
11	R10	Negative	Negative	Neuronal degeneration noticed

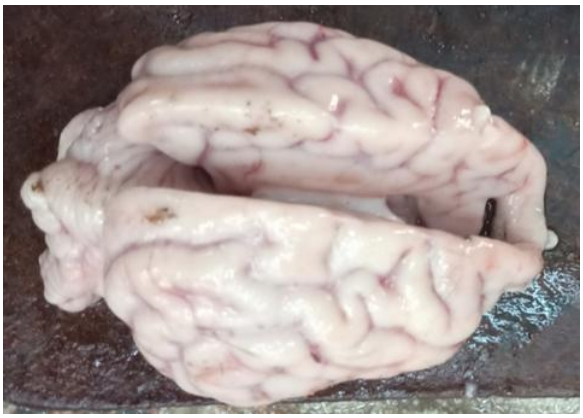

Fig 1: Normal Brain

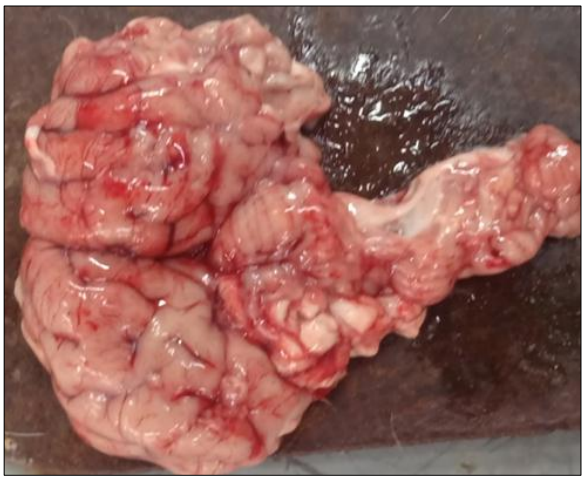

Fig 2: Brain Hemorrage and congestion

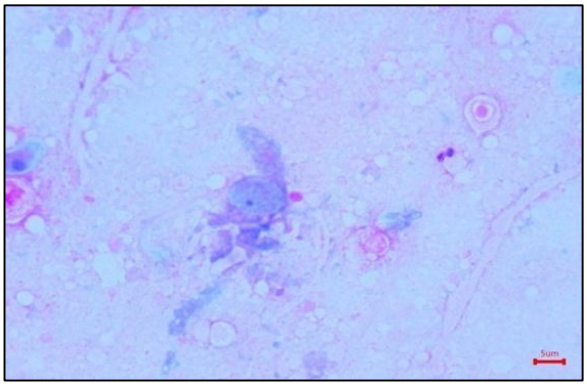

Fig 3: Negri bodies in cytology

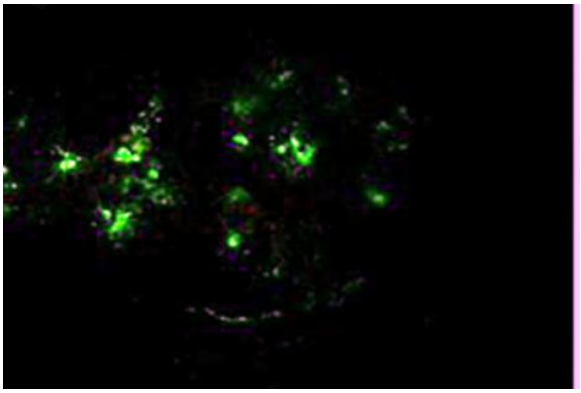

Fig 4: Apple green flourescence of conjugated antirabies antibodies in impression smear of hippocampus in dFAT

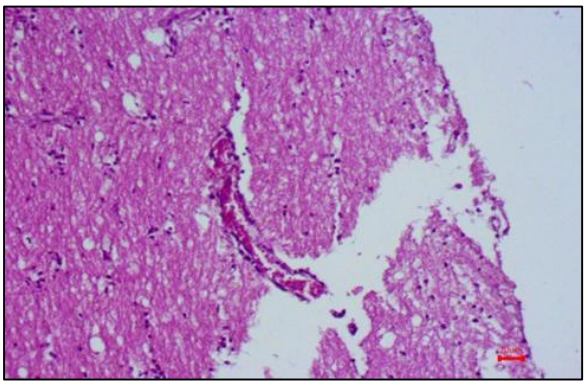

Fig 5: Congestion and mild perivascular cuffing in cerebrum

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