

Comparison between fluorescent antibody technique and cytological and histopathological examinations in the diagnosis of rabies

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Rabies is a highly fatal infection to all warm blooded animals and is transmitted by the bite of rabid animals. A rapid, readily available and accurate diagnostic technique is the keystone for prevention of rabies. Despite, the dramatic clinical signs in rabies, laboratory diagnosis is the only way to confirm the disease. In this study, an attempt was made to ascertain the efficacy of the commonly used methods in diagnosing rabies like fluorescent antibody technique (FAT) and cytological and histopathological examinations.

Rabies suspected carcasses (80) referred to the Department of Pathology, were subjected to this study. Brain samples were taken from hippocampus, cerebrum and cerebellum. Impression smears taken from hippocampus were fixed in methanol and stained by Seller's and William's modification of Van Gieson's stain. For FAT, the smears were fixed in cold acetone and stained by lyophilized adsorbed antinucleocapsid antibody conjugated with fluorescein isothiocyanate (FITC).

For histopathological studies the tissues were fixed in 10% formal saline and Zenker's fixative and were stained by routine haematoxylin and eosin, phloxine and fastochrome and Mann's stain. The case declared positive by FAT were as used standard positive cases for the purpose of comparison with other techniques used in this study.

Of the 80 carcasses studied, 52 were found positive for rabies by examination of impression smears by FAT. The remaining 28 animals died due to causes other than rabies. The species-wise details of total carcasses examined and confirmed positive by employing various tests are given in Table 1. Of the 52 cases confirmed positive by FAT, 49 cases revealed the presence of Negri body (Figs 1, 2).

Cytological examination of brain for Negri bodies by means of impression smear remained the least sensitive when compared to FAT and histopathological examination. Similar findings were obtained by Mitchell and Monlux (1962), and

Table 1. Comparison between cytology, FAT and histopathology

Species	No. of samples screened	No. of samples positive based on FAT	Comparison between different diagnostic techniques					
			Cytology		FAT		Histopathology	
			No.	Per cent	No.	Per cent	No.	Per cent
Canine	64	37	36	97.3	37	100.0	36	97.3
Bovine	5	5	5	100.0	5	100.0	5	100.0
Caprine	8	8	6	75.0	8	100.0	7	87.5
Feline	2	1	1	100.0	1	100.0	1	100.0
Equine	1	1	1	100.0	1	100.0	1	100.0
Total	80	52	49	94.23	52	100.0	50	96.5

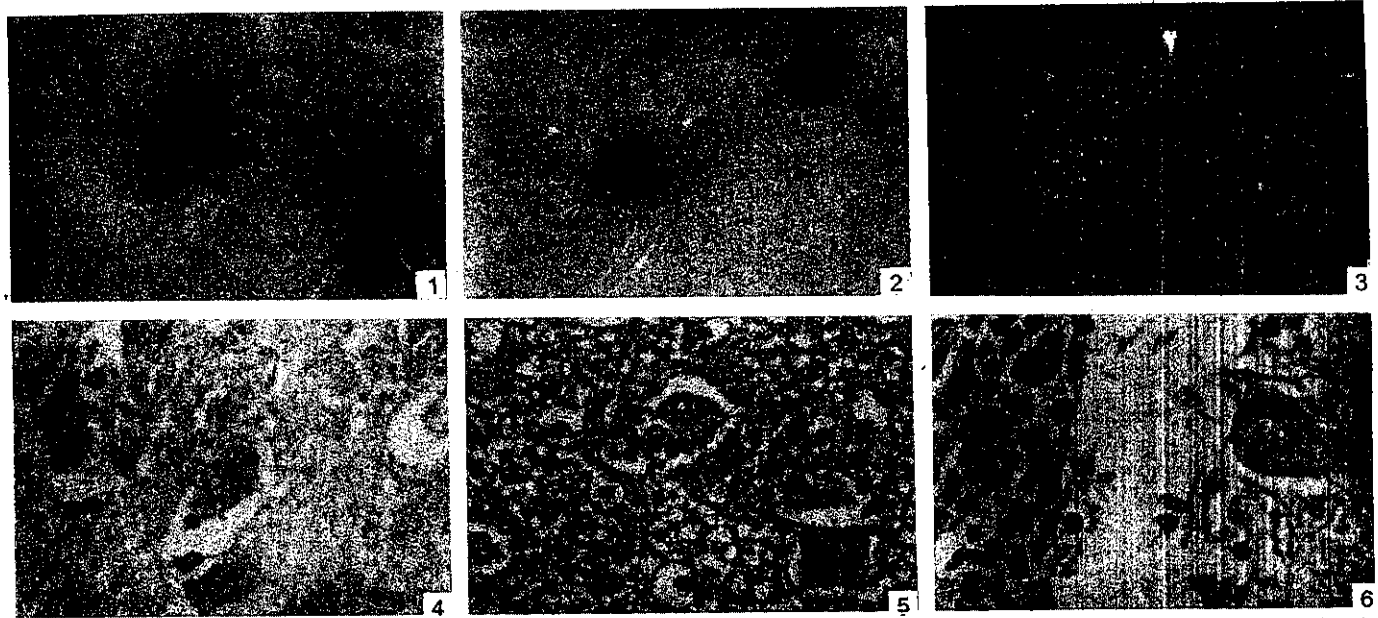
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Greene and Dreesen (1998). However, Davies and Lowings (2000) concluded that the impression smear examination for Negri bodies was rapid, inexpensive and continued to remain as the standard diagnostic techniques in some developing countries.

Fluorescent antibody technique revealed brilliantly



Figs 1-6. 1. Dog-Negri bodies-magenta coloured oval inclusion bodies-seller's $\times 800$. 2. Calf-Negri bodies-magenta red colour oval and spherical-Willam's modification of van Gieson's- $\times 800$. 3. Dog-hippocampus showing brilliant apple green fluorescence-FAT- $\times 1000$. 4. Dog-hippocampus showing Negri body-Phloxine and Tartrazine- $\times 1000$. 5. Dog-hippocampus showing Negri bodies-Mann's- $\times 1000$. 6. Goat- cerebellum showing inclusion bodies in pyriform cells-H&E- $\times 1000$.

fluorescing apple green or greenish yellow structures varying in size from tiny bodies like dust or sand particles in all the 52 positive samples (Fig.3). Findings corroborate with the observations of Wilsnack (1960) and Carski *et al.* (1962). Zimmer *et al.* (1990) stated that FAT and peroxidase-antiperoxidase technique detected 98% and while histopathology could detect only 53% of rabies positive cases.

Histopathological examination of cerebrum, cerebellum and hippocampus was carried out in all the 52 positive cases confirmed by FAT. Tissues fixed by formal saline were superior for serial sections when compared to Zenker's fixative. Out of 52 positive cases, histopathological studies proved to be effective in diagnosing rabies only in 50 cases. The inclusion bodies stood out prominently by phloxine and tartrazine staining (Fig. 4). Similarly by man's staining technique, the Negri bodies appeared as red coloured bodies against faint bluish cytoplasm (Fig. 5). The routine haematoxylin and eosin staining could also stain the Negri bodies that appeared inside the cytosol of neurons as reddish bodies (Figs 6, 7) with basophilic stippling in a few. In general, microscopic lesion of brain varied from mild congestion and degenerative changes in the neurons to perivascular cuffing (Figs 8, 10, 11) and multifocal, non-suppurative inflammation (Fig. 9). The findings observed in this study were in agreement with that of Charlton (1988) and Greene and Dreesen (1998).

In the present study, Negri bodies were observed in the neuronal cells of cerebrum, cerebellum and hippocampus with minimal inflammatory response. Jubb and Huxtable (1993) reported mild inflammatory response in the brain, which revealed presence of multiple Negri bodies. Greene and

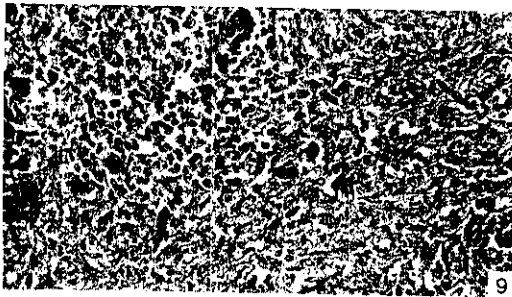
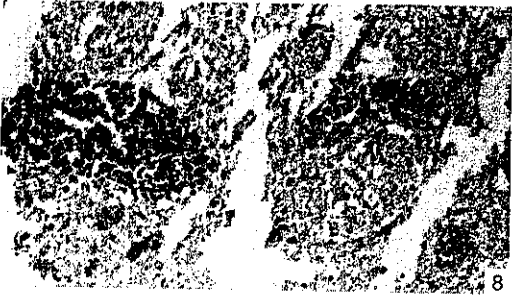
Dreesen (1998) stated that non-suppurative inflammatory response was more pronounced in animals, which had long course of illness.

SUMMARY

The efficacy of fluorescent antibody technique (FAT), cytological examination and histopathological examination in diagnosing rabies was compared. Eighty brain samples were screened for this purpose. FAT was highly sensitive. The positive cases identified by FAT were used as standard positive cases for the purpose of comparison with other techniques employed in the study. Cytological examination remained the least sensitive followed by histopathological examination.

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Figs 7-11. 7. Cow-cerebellum showing Negri bodies in Purkinji cells-H&E- \times 1000. 8. Dog-Cerebrum showing multilayered perivascular cuffing-H&E- \times 400. 9. Dog-cerebrum showing multifocal infiltration of mononuclear cells consisting of lymphocytes, plasma cells and macrophages-H&E- \times 400. 10. Dog-cerebrum showing perivascular cuffing-H&E- \times 1000. 11. Donkey-cerebrum showing multifocal infiltration of lymphocytes, plasma cells and macrophages in grey matter-H&E- \times 800.

