Comparison of various techniques used for diagnosis of rabies in cats

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Aim: Objectives of this study was to compare and evaluate the best method for diagnosis of rabies in cats.

Materials and Methods: Antemortem examination of 5 suspected cats were evaluated. Brains were collected from the suspected cats. Direct Fluorescent Antibody Test (dFAT), Hematoxylin and Eosin (H&E) and Immunohistochemistry (IHC) histopathological diagnostic tests were applied.

Results: The dFAT test was conducted on the saliva and fresh brain impression smear of all cats, among that 4 cats showed positive for rabies virus. After death of cats, Hematoxylin and Eosin (H&E) and Immunohistochemistry (IHC) histopathological diagnostic tests were applied.

Conclusion: The IHC can be used as reliable diagnostic technique in addition to dFAT. IHC shows positivity in mildly infected cases and having immense value for retrospective studies. It also minimizes the risk of public health hazard during shipping of rabid positive brain samples.

Keywords: Brain, direct fluorescent antibody test, histopathology, immunohistochemistry, rabies
Introduction

Rabies virus infection leads to magnanimous fatal disease condition, by affecting the brain in all warm blooded animals. In case of cats, clinical signs are presage the prognosis. A rabid cat foreshadows following clinical signs anorexia, pyrexia and hyper salivation, alteration of behaviour, startled look and aggressiveness. Krebs et al (1995) reported that rabies animals not only suspected on recent history of a dog bite or exposure to a rabid animal but also direct or indirect contact with infected animals. O’Brien and Aslund (2005) noted that the tendency of bite may be the consequence loss of inhibitory control by cortical neurons over the subcortical bite reflex. Dogs and cats change in behavioral pattern. They turn and snap at anything that touches them and around the mouth. Generally rabid cats show 3 forms as furious, dumb and paralytic forms. The furious phase is more consistently developed in cats showing behavior abnormalities observed by Fogelman et al (1993). The paralytic phase (generalized paralysis/paraparesis, incoordination, coma and death) usually begins after five days of starting first clinical signs. The paralytic phase usually develops within 2 to 4 days after the first signs are noted. Nerves affecting the head and throat are the first involved and aimals may begin to salivate as a result of their inability to swallow. Due to respiratory, facial and diaphragm muscles paralysis cat shows deep laboured breathing and a dropped jaw. Animals may make a choking sound and many owners think that there is something lodged in the cat’s throat. The animal will get weaker and eventually go into respiratory failure and die. Intercytoplasmic eosinophilic inclusion bodies in pyramidal cells of hippocampus or Purkinje cells of cerebellum of cats (Negri bodies) are considered as pathognomonic finding of rabies. Among the cats, histopathological changes due to rabies virus in nervous tissues have been qualitatively reported but have been seldom analysed quantitatively by both IHC and histopathologically. Among the cats, histopathological changes due to rabies virus in nervous tissues have been qualitatively reported but have been seldom analysed quantitatively by both IHC and histopathologically. Also, fewer attempts have been done to quantify the histopathological changes in brains of 5 rabid suspected cats.

Hence, present study was undertaken to compare and record quantitatively the histopathological alterations in various parts of brain of naturally infected rabid felines.

Material and Methods

A total 5 cases of naturally infected and rabies suspected cases studied and clinical observations were noted. Brains of all dead cats were meticulously collected. Tissue samples of hippocampus and cerebellum collected from rabid cats. Sections were divided in 2 parts one is kept in PBS at -20 °C and another part in 10% neutral bufer formalin (10% NBF). Impression smear taken on clean and gleese free glass slide. Sections were dried. dFAT was employed by using lyophilized, adsorbed Antirabies nucleocapsid Fluorescein Isothiocyanate (FITC) conjugate which acquired from Bio-rad Marnes-La-Coquette, France. dFAT was employed as diagnostic technique because of its sensitivity, accuracy and speed as recommended by World Health Organization Meslin et al (1996). The slides were examined using an AHBT3 - RFC reflected light fluorescence attachment (Olympus, Japan). Histopathology of brain, spinal cord was done by H and E staining method given by Luna (1968). IHC was done by using Antirabies monoclonal antiserum and the kit Advanced SS™ One step polymer Horseradish Peroxidase (HRPO) Immunohistochemical detection system (BioGenex Laboratories Inc., San Ramon, California, USA). Sections counterstained with GIll's haematoxylin were used. Immunohistochemistry was done as recommended by procedure of manufacturer and Pedroso et al (2008). Total 100 pyramidal cell in hippocampus and 100 purkinje cells were observed for inclusion body.

Results

Rabies was diagnosed in 4 cases and one case having meningitis (total 5 suspected rabies cases). Out of total five cases, three suspected cases were euthanized and two cases are naturally died. Suspected rabid cats presage prominent symptoms such as anorexia, aggressiveness, strange look (Figure 1), and pyrexia were observed in all (4/4) cases, followed by hyper salivation and not recognizing to owner (2/4) (Table 1).

Diffused congestion and meninges were observed in different areas of brain in all cases (Figure 2). Degree of hemorrhage varied widely sometimes on meninges and extensively seen on hippocampus and cerebellum. Out of 5 cases FAT was positive for 4 cases in both hippocampus and cerebellum region (Figure 3). Histopathological observation in hippocampus of rabid cat revealed that presence of Negri bodies of variable size and number. Some of pyramidal cells of amberm horn were infected with Negri body. Neuronal degeneration with necrosis and gliosis were present in 75% (3/4) cases (Table 2). However, perivascular cuffing was observed in 25% (1/4) cases (Figure 6), satellitosis in 75% (3/4) cases and meningitis in 25% case (1/4) samples. Hyperemia and edema was shown by 50% (2/4) cases. Whereas, neuronophagia in 50% cases and hemorrhage were observed in 25% (1/4) cases. It is that rabies virus infects the various neuronal cells and presence of inclusion bodies in the cytoplasm of neuronal cells and the remaining cells non-suppurative encephalitis and necrosis observed. Cerebellum is positive for Negri bodies in all cases (4/4) but only 64% purkinje cells of cerebellum region infected with Negri bodies histopathologicaly (Table 2, Figure 4). Neuronal degeneration with necrosis and gliosis were present in 75% (3/4) cases. However, perivascular cuffing was observed in 50% (2/4) cases (Fig.6), satellitosis and meningitis in 75% (3/4) samples. Hyperaemia and edema was shown by 50% (2/4) cases. Whereas, neuronophagia and hemorrhage were observed in 25% (1/4) samples, respectively.

Rabid positivity seen in 4 cases (100%) out of 5 cases were used monoconal and polynodal antisemur. The negative controls were kept for observation. A large amount of distinct, granulo-
lar rabies viral antigen deposits stained as sharply demarcated brown precipitates of variable sizes were found within the pyramidal cells of hippocampus, some of neurons of the hippocampus and in the processes of neurons and Purkinje cells of cerebellum (Figure 5), by counting of 100 cells of each region shows, 87% of pyramidal cell and 69% of Purkinje cells of cerebellum having viral antigen deposition.

Discussion

Rapid and precise diagnosis of rabies is essential in rabies endemic area for administration of post exposure prophylaxis. Suspected rabid cat foreshadows clinical symptoms such as altered behaviour, startled look, aggressiveness, hind limb paralysis and biting with provocation. Eng and Fishbein (1990) reported that rabid cats showed more aggressive behavior than dogs (55% in cats and 31% in dogs). Bernard (1985) and Fekadu (1991) noticed isolated reports of survival after a confirmed clinical disease in cats, dogs and humans. Cats often die within 3-4 days, similar observation noted by Rupprecht and Childs (1996). Rosevare et al (2009) reported that 25% of deaths occur within 4 days after initiation of clinical signs and among rabid stray cats were the most commonly reported 47.4%. dFAT is considered as an accurate and fame method for diagnosis of rabies by Miranda and Robles (1991), Rudd et al (2005), Lembo et al (2006). FAT provides a reliable diagnosis in 95% to 99% of rabies in cat cases for all genotypes and in fresh samples shown by Bourhy et al (1989), Birgham and Van der Merwe (2002). Non-rabid tissues having intracytoplasmic inclusions which are indistinguishable from Negri bodies Maxie and Youssef (2007). Faizee et al (2012) observed that rabies virus infects the various neuronal cells and presence of inclusion bodies in the cytoplasm of neuronal cells and the remaining cells non-suppurative encephalitis and necrosis observed. Neuronophagia and haemorrhage were observed rabies infected brain Similar histopathological alterations have been qualitatively reported by Murphy et al (1980). Deborah et al (1991) given that IHC technique improves diagnostic accuracy. By counting of 100 cells of each region showed that 87% of pyramidal cell and 69% of Purkinje cells of cerebellum having viral antigen deposition. These findings are similar as reported by Gunawardena and Blakemore (2007), Pedroso et al (2009) and Sumedha (2010). IHC gives better result than any other technique given by Suja et al (2001). In the present study, 87% neuronal infected cells were detected with help of IHC method as compared to routine histopathological method in which 64-75% neuronal infected cells detected. Inconsistent Negri bodies formation along with presence of nonrelated proteinous intracytoplasmic eosinophilic inclusion may lead to false positive diagnosis of rabies by normal H & E method by Jubb and Huxtable (1996). Generally, intracytoplasmic eosinophilic inclusion bodies (Lyssa bodies) are seen in brain of non-rabid cats, cattle, moose, woodchucks, and skunks as noted by researchers (Nietfeld et al (1989), Maxie and Yossef (2007). Therefore rabies diagnosis based on presence or absence of inclusion bodies; especially in cases without inflamma-

### Table 1. Clinical signs in rabid cats (Total positive = 4).

<table>
<thead>
<tr>
<th>Symptoms</th>
<th>No of animals</th>
<th>Percent (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Off feed</td>
<td>4</td>
<td>100</td>
</tr>
<tr>
<td>Hyper salivation</td>
<td>2</td>
<td>50</td>
</tr>
<tr>
<td>Fever</td>
<td>4</td>
<td>100</td>
</tr>
<tr>
<td>History of biting</td>
<td>4</td>
<td>100</td>
</tr>
<tr>
<td>Not recognizing owner</td>
<td>2</td>
<td>50</td>
</tr>
<tr>
<td>Difficulty in standing</td>
<td>1</td>
<td>25</td>
</tr>
<tr>
<td>Behavioral change</td>
<td>3</td>
<td>75</td>
</tr>
</tbody>
</table>

*Table 1: Clinical signs in rabid cats (Total positive = 4).*

### Table 2. Histopathological alterations in brain of rabid cats (Total positive cases=4).

<table>
<thead>
<tr>
<th>Histopathological alterations in cerebellum</th>
<th>Positive samples (n)</th>
<th>Percentage positive (%)</th>
<th>Histopathological alteration in hippocampus</th>
<th>Positive samples (n)</th>
<th>Percentage positive (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negri bodies</td>
<td>4</td>
<td>100</td>
<td>Neuronal degeneration and necrosis</td>
<td>4</td>
<td>100</td>
</tr>
<tr>
<td>Neuronal degeneration and Necrosis</td>
<td>3</td>
<td>75</td>
<td>Satellitosis</td>
<td>3</td>
<td>75</td>
</tr>
<tr>
<td>Satellitosis</td>
<td>3</td>
<td>75</td>
<td>Gliosis</td>
<td>3</td>
<td>75</td>
</tr>
<tr>
<td>Gliosis</td>
<td>3</td>
<td>75</td>
<td>Neurophagia</td>
<td>2</td>
<td>50</td>
</tr>
<tr>
<td>Neurophagia</td>
<td>1</td>
<td>25</td>
<td>Hyperaemia</td>
<td>2</td>
<td>50</td>
</tr>
<tr>
<td>Hyperaemia</td>
<td>2</td>
<td>50</td>
<td>Hemorrhage</td>
<td>1</td>
<td>25</td>
</tr>
<tr>
<td>Hemorrhage</td>
<td>1</td>
<td>25</td>
<td>Perivascular cuffing</td>
<td>1</td>
<td>25</td>
</tr>
<tr>
<td>Perivascular cuffing</td>
<td>2</td>
<td>50</td>
<td>Meningitis</td>
<td>3</td>
<td>75</td>
</tr>
<tr>
<td>Meningitis</td>
<td>3</td>
<td>75</td>
<td>Oedema</td>
<td>2</td>
<td>50</td>
</tr>
</tbody>
</table>

*Table 2: Histopathological alterations in brain of rabid cats (Total positive cases=4).*
tion should be avoided and verified by other reliable technique such as dFAT or IHC. Although dFAT is reliable and quick diagnostic method; it has certain drawbacks such as requirement of costly UV Light microscope for observation Lembo et al (2006) and fresh brain impression smear sample which contain live rabies virus which is hazardous to public health reported by Woldehiwet (2005). Transporting fresh samples is a problem in countries where diagnostic laboratories are not well established or where lack of refrigeration and high ambient temperatures can interfere with the dFAT given by Birgham and Van Der Merwe (2002). Field collection of brain samples often occurs far from diagnostic laboratories. Delays in sample collection and/or shipping in some countries may add 3 or more days from the death of the animal to the point of laboratory testing; the resulting autolysis further hinders diagnostic accuracy. In the formalin-fixed specimens used in IHC, the rabies virus is rapidly inactivated by formaldehyde, making the transport and laboratory processing of specimens much safer reported by Last et al (1994). There is no autolysis in formalin preserved and fixed brain tissue. In addition to these; IHC technique is useful for detection of suspected and mildly infected cases (Sinchaisri et al 1992), wherein conventional H and E and dFAT fails to detect lesion viral antigen in formalin preserved tissue. IHC technique has been shown to increase diagnostic accuracy by improving visualization of infectious agent in the same histological lesion and section reported by Deborah et al (1991). However, newer and more sensitive methods for diagnosis of rabies in brain tissue by IHC technique have been attempted by various researchers Palmer et al (1985), Jogai et al (2001), and Suja et al (2004) and results of these methods are encouraging. Among the various methods, Avidin–Biotin peroxidase and peroxidase anti-peroxidase systems gives better results by using monoclonal/polyclonal antisera in formalin fixed paraffin embedded tissue sections similarly reported by Gunavardena and Blakemore (2007), Faiden et al (1988), and Metze (1991) for diagnosis of rabies antigen/Negri bodies. IHC enables pathologists to know the specific cells involved in spread of rabies virus in brain of infected hosts.

Conclusions

IHC for rabies detection using targeted sections of brain could improve accurate diagnosis in various species. The public health implications of this disease warrant continued efforts to develop more accurate sampling and testing modalities. This IHC protocol provides an alternative to FAT and can be used safely, even in tropical and remote areas.

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References


