RESEARCH ARTICLE

Comparison of various techniques used for diagnosis of rabies in cats

Mugale Madhav Nilakanth[1]*, Bhupinder Singh Sandhu[2], Beigh Akeel[2], Gupta Kuldeep[3], Sood Naresh Kumar[2], Charan Kamal Singh[2]

[1]Department of Veterinary Pathology, Madras Veterinary College, Vepary, Chennai, Tamilnadu, 600007, [2]Department of Veterinary Pathology, GADVASU, (PAU campus) Ludhiana, Punjab 141004, India
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*madhav.mugale@gmail.com

Özet

Mugale MN, Sandhu BS, Gupta K, Sood NK, Beigh A, Singh CK. Kedilerde kuduz diagnozunda farklı tekniklerin karşılaştırılması. **Eurasian J Vet Sci, 2013, 29, 2, 92-96**

Amaç: Mevcut araştırmanın amacı kedilerde kuduz teşhisinde en iyi diagnostik metottu belirlemek ve değerlendirmektir.

Gereç ve Yöntem: Kuduz süşhesi ile ölmüş 5 kedi değerlendirildi. Şüpheli kedilerin beyinleri toplandı. Direct Fluorescent Antibody Test (dFAT), Hematoxylin & Eosin (H&E) ve Immunohistochemistry (IHC) histopatolojik diagnostik testler uygulandı.

Bulgular: Kedilerin salya ve beyinlerine dFAT testi uygulandı ve 4 kedide kuduz virüsü açısından pozitif bulundu. Ölümden sonra Hematoxylin & Eosin (H&E) ve Immunohistochemistry (IHC) teknikleri uygulandı. Vakanın birinde meningitis ve Negri cisimciği gözlendi. Hipokampüs ve serebellumun her bölgesinde sayılan 100 hücrede H&E ve IHC açısından viral antijen varlığı gözlendi. Serebellumun Purkinje hücrelerinin %69'unda ve hipokampüs pramidial hücrelerinin %87'sinde kuduz viral antijeni belirlendi.

Öneri: IHC testi dFAT testinin yerine kullanılabilen değerli bir diagnostik testtir. IHC orta dereceli enfeksiyonlar pozitiftir ve retrospektif çalışmalar için oldukça değerlidir. Ayrıca kuduz pozitif beyin örneklerinin taşınmasında halk sağlığı açısından minimum risk taşımaktadır.

Anahtar kelimeler: Beyin, direct fluorescent antibody test, histopatoloji, immunohistochemistry, kuduz

Abstract

Mugale MN, Sandhu BS, Gupta K, Sood NK, Beigh A, Singh CK. Comparison of various techniques used for diagnosis of rabies in cats. **Eurasian J Vet Sci, 2013, 29, 2, 92-96**

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 & Eosin (H&E) and Immunohistochemistry (IHC) techniques were carried out. One case showed meningitis and remaining showed Negri bodies. Viral antigen depositions were observed by counting 100 cells each region of hippocampus and cerebellum by H&E and IHC. Hippocampus pyramidal cell showed 87% and cerebellum Purkinje cells showed 69% of rabies viral antigen deposition.

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 Axlund (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 animals 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. Intracytoplasmic 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 ^{0}C and another part in 10% neutral buffer formalin (10% NBF). Impression smear taken on clean and gleese free glass slide. Sections were dried. dFAT was employed by using lyophilize, 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 Anti mice monoclonal antisera 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 meningitis 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 ammons 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 histopathologically (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 haemorrhage were observed in 25% (1/4) samples cases, respectively.

Rabid positivity seen in 4 cases (100%) out of 5 cases were used monoclonal and polyclonal antiserum. The negative controls were kept for observation. A large amount of distinct, granu-

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

Symptoms	No of animals	Percent (%)
Off feed	4	100
Hyper salivation	2	50
Fever	4	100
History of biting	4	100
Not recognizing owner	2	50
Difficulty in standing	1	25
Behavioral change	3	75

[a, b, c]: Aynı satırdaki farklı harfler istatistiki açıdan önemlidir (p<0.05).

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

Histopathological alterations in cerebellum	Positive samples (n)	Percentage positive (%)	Histopathological alteration hippocampus	Positive samples (n)	Percentage positive (%)
Negri bodies	4	100	Negri bodies	4	100
Neuronal degeneration and Necrosis	3	75	Neuronal degeneration and necrosis	3	75
Satellitosis	3	75	Satellitosis	3	75
Gliosis	3	75	Gliosis	1	25
Neuronophagia	1	25	Neuronophagia	2	50
Hyperaemia	2	50	Hyperaemia	2	50
Hemorrhage	1	25	Hemorrhage	1	25
Perivascular cuffing	2	50	Perivascular cuffing	1	25
Meningitis	3	75	Meningitis	3	75
Oedema	2	50	Oedema	2	50

Figure 1. Rabies suspected cat showing aggressive behavior.

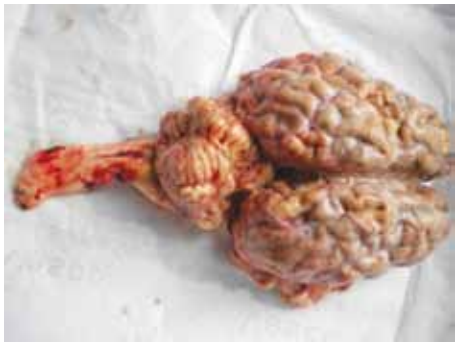

Figure 2. Brain of rabid cat showing congestion, hemorrhages & edema.

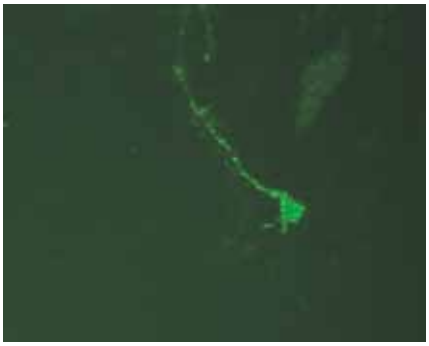

Figure 3. Impression smear drawn from hippocampus of a rabid cat showing apple green fluorescence in neurons.

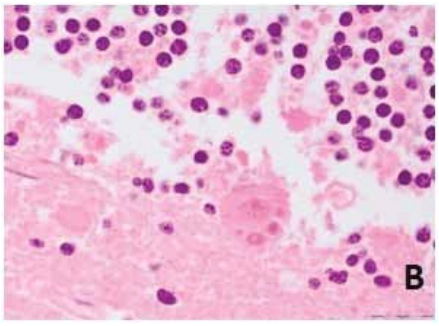

Figure 4. Section of cerebellum of rabid cat showing number of intracytoplasmic eosinophilic Negri body in purkinje cell by H&EX875.

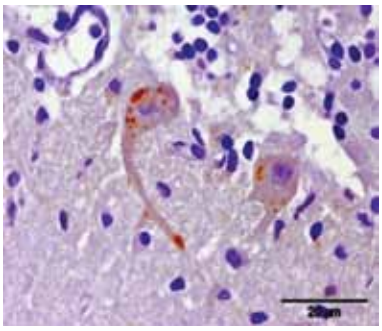

Figure 5. Section of cerebellum of rabid cat showing number of Negri body in purkinje cell by IHCX875.

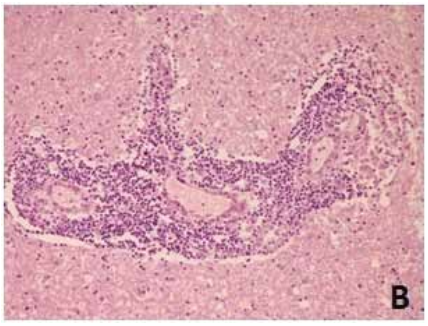

Figure 6. Section of hippocampus of rabid cat showing perivascular cuffing. H&EX875.

tion should be avoided and verified by other reliable technique such as dFAT or IHC. Although dFAT is reliable and quick diagnostic method; but 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 or 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 antiperoxidase 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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