Indian Journal of Animal Sciences **82** (1): 3–8, January 2012

Comparison of clinico-pathological, immunohistochemical and immunofluorescent techniques for diagnosis of rabies in animals

S A WAHAN[1], B S SANDHU[2], C K SINGH[3], K GUPTA[4], N K SOOD[5] and A KAW[6]

Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab 141 004 India

Received: 3 March 2011; Accepted: 26 July 2011

ABSTRACT

Rabies is a fatal zoonotic disease, affecting animal species and human beings. In present investigation, sensitivity of immunohistochemistry and histopathology of brain tissues of 38 rabies-suspected animals was compared. Out of 38 rabies-suspected cases, 18 and 16 cases were found positive by immunohistochemistry and histopathology respectively. Sensitivity of immunohistochemistry and histopathology in comparison to FAT on brain tissue smears was found to be 94.74 and 84.21% respectively. Hundred neurons per case were observed for negri bodies and number of negri bodies in positive neurons. Average number of negri bodies detected per neuron by IHC and histopathology was 2.71 and 1.94 respectively. With IHC, 58.83% neurons were positive for Negri bodies and 47.94% with H and E staining. The amount of rabies viral antigen/number of Negri bodies detected with IHC was much more abundant than could be expected from the corresponding H and E stained sections. In cattle and buffaloes, important clinical signs of rabies were bellowing, anorexia, pyrexia and hypersalivation whereas, in dogs change in behaviour, aggressiveness and anorexia were the most common clinical signs. Immunohistochemistry in formalin-fixed paraffin embedded tissue sections was found more sensitive than histopathology for detection of negri bodies/rabies antigen and therefore, of immense value for retrospective studies.

Key words: Animals, FAT, Histopathology, Immunohistochemistry, Rabies

Rabies, caused by highly neurotropic negative sense single stranded RNA (ssRNA) virus, results in acute encephalitis in warm-blooded animals (Pringle 1991). For routine histopathological diagnosis of rabies, the tissue sections of brain are examined for negri bodies. These however, are not present in all cases and all locations. Hence, there is a need for a better method of diagnosis of rabies using formalin-fixed paraffin-embedded tissue. The sensitivity of the immunoperoxidase method is much higher than the diagnostic method using conventional H and E stained sections as it detects many more negri bodies (Jogai *et al.* 2001). Rabies detection by immunohistochemistry (IHC) using light microscopy is 10 times cost effective than fluorescence microscopy (Lembo *et al.* 2006). Further, use of fluorescent antibody test is limited due to costs of acquiring and maintaining a fluorescent microscope. Immunohistochemistry is a fast method that can be used in routine procedures. Formalin fixation of tissues simplifies collection, storage and transport of samples, and eliminates hazards and the need for biological containment of the highly pathogenic live virus. It also facilitates the retrospective study analysis especially in already fixed tissues. In the present investigation, sensitivity of immunohistochemistry and histopathology of brain tissues of rabies suspected animals was compared.

Present address: [1,6]M.V.Sc. Student ([1]sumedhaawahan @gmail.com; [6]preeti_kaw@yahoo.com), [2]Associate Professor (bhupisandhu@rediffmail.com), [3,5]Senior Veterinary Pathologist ([3]rabiesck@gmail.com,[5]kgupta1968 @yahoo.com), [4]Assistant Professor (nareshsood47@yahoo.com), Department of Veterinary Pathology, College of Veterinary Science.

MATERIALS AND METHODS

Collection of case history: Rabies-suspected animals (38) i.e. dogs, buffaloes and cows presented at Rabies Research-cum-Diagnostic laboratory, of the University were used. The data regarding age, sex, history of bite, date of bite, source of bite and clinical signs was acquired as per the questionnaire prepared for the purpose from owners of the animals.

Sample collection: The samples were collected from rabies-suspected cases that were received for postmortem detection of rabies in the Department of Veterinary Pathology, of the University, and the animals under observation when died at Veterinary Clinics, GADVASU. The samples collected were whole brain. Three pieces of each tissue sample were stored; in deep freezer at –20°C, in 50% glycerol saline

solution, and in 10% neutral buffered formalin solution respectively.

Direct fluorescent antibody technique (dFAT) of brain tissue impression smears: The dFAT was employed as diagnostic technique because of its sensitivity, accuracy and speed as recommended by World Health Organization (Meslin *et al*. 1996). Lyophilized, adsorbed antirabies nucleocapsid fluorescein isothiocyanate (FITC) antibody conjugate was acquired from France. Each vial of lyophilized, adsorbed antirabies nucleocapsid conjugate was reconstituted with 3 ml of distilled water as recommended by manufacturer and centrifuged at 1500 rpm for 5 min for clarification. The clarified conjugate (0.1 ml) was added on the duplicate impression smears on every slide for each tissue samples. Control positive slides from infected mouse brain and control negative from normal mouse brain were prepared along with the test smear and 0.1 ml conjugate was also added on positive and negative control slides. The smears were covered with cover slips and slides incubated at 37°C for 30 min by placing in a humidified chamber. The slides were twice washed in 0.01 M phosphate buffered saline (PBS) pH 7.5 for 5 min each. Thereafter, air-dried and mounted in 90% buffered glycerol (pH 8.5). The slides were examined using an $AHBT_3$ - RFC reflected light fluorescence attachment.

Antirabies polyclonal antiserum: Anti rabies polyclonal antisera raised in Rabies research-cum-diagnostic laboratory of the department was used as the primary antibody in immunohistochemistry study. The reactivity of the polyclonal antisera was tested by agar-gel precipitation test.

Antirabies monoclonal antibody: Antirabies monoclonal antibody was obtained from US Biological having isotype IgG2a clone no. 1.B.456 (reacts with glycoprotein of more than 20 different strains rabies virus from 4 serogroups: CVS, lagosbat, Makola, Duwenhage were positive in neutralization reaction)**.**

Immunohistochemistry technique: One step polymer horseradish peroxidase (HRPO) immunohistochemical detection system counterstained with Gill's haematoxylin was used. Immunohistochemistry was done as recommended by the manufacturer with some modifications (Pedroso *et al.* 2008). Formalin fixed brain samples were thoroughly washed in running water, dehydrated in ascending grades of alcohol and acetone, cleared in benzene and embedded in paraffin at 58°C. The paraffin embedded tissues were sectioned at 5µm thickness and sister sections were taken on poly-L lysine coated slides for each sample. Then, the sections were deparaffinized and rehydrated by immersing in 250 ml EZ-AR common solution at 70°C for 10 min in EZ-retriever system V.2.1 and subsequent antigen retrieval was done in citrate buffer (0.01 M, pH 6.0–6.2) at 95°C for 10 min and at 98°C for 5 min in EZ-retriever system V.2.1. Three washings were given in PBS buffer for 3 min each. The endogenous tissue peroxidases were inactivated by immersion of the slides in a solution of 3% hydrogen peroxide in methanol for 15 min at room temperature in humid chamber followed by 3 washings with PBS buffer for 3 min each. Non-specific binding was blocked by incubating sections with ready to use power block for 10 min at room temperature in moist chamber. On one section of each slide, primary polyclonal rabbit anti-rabies antibody (1: 1000 dilution in PBS) and on second slide monoclonal antibody (1: 10, 1: 20, 1: 30, 1: 50, 1: 100, 1: 200, 1: 500, 1: 1000 dilutions in PBS and 5% BSA) was added for 1 h in moist chamber at room temperature. On the second section of each slide, PBS was added and no primary antibody was added so as to serve as a negative control. The sections were given 3 washing in PBS buffer for 3 min each, thereafter, incubated with polymer HRP (super sensitive label, one step polymer-HRPO reagent) for 30 min at room temperature in moist chamber followed by 3 washing in PBS buffer for 3 min each. The antigen-antibody-peroxidase reaction was developed with a freshly prepared 3,3 '-diaminobenzidine (DAB) solution by mixing 2 drops of DAB chromogen with 1 ml of DAB buffer supplied by the manufacturer adding 5 ml-HOH. Sections were washed in distilled water for 5 min and counterstained with Gill's haematoxylin for 30 sec and washed in running tap water for 5 min. Finally the slides were dehydrated in ascending grades of alcohol, cleared in xylene, mounted in DPX and examined under advanced microscope available in the department.

Histopathology: All nervous tissues samples, viz. cerebellum, cerebrum, hippocampus, pons, medulla oblongata from dead animals were collected in 10% neutral buffered formalin solution. These tissues were routinely processed through ascending grades of alcohol, cleared in benzene and embedded in paraffin wax. The paraffin sections were cut at 4 to 5 µ thickness and stained by haematoxylin and eosin (H & E) method (Luna 1968). Slides were examined by photomicrograph microscope.

Comparison of IHC and histopathology techniques with dFAT for sensitivity analysis: Sensitivity of IHC for brain samples and histopathology was calculated in comparison with dFAT on brain impression smears as per Perrin and Sureau (1987).

Sensitivity = true positive/ (true positive + false negative) ×100

For quantitative morphometry analysis 100 neurons were counted for presence or absence of negri bodies in complimentary serial sections stained by H&E and IHC. Total number of negri bodies in the positive neurons was counted and size of each negri body was measured by using Cell-A software of BX61 research photomicrograph microscope system both stained by H&E and IHC (Table 1).

RESULTS AND DISCUSSION

Clinical signs in rabid dogs: Our study of rabid dogs revealed that 20% (1/5) dogs showed hyper-salivation, 80% (4/5) biting behavior/aggressiveness and pica in 20% (1/5) rabid dogs (Table 2). Silva *et al.* (2004) and Eng and Fishbein

Table 1. Histopathological and immunohistochemical evaluation of brain tissues for size and number of negri bodies

Case No.	Species	No. of neurons positive for negri bodies/100 neurons		No. of negri bodies detected/100 neurons		Size of negri body (μm)			
		H &E	IHC	H & E	IHC	H & E		IHC	
						Smallest	Largest	Smallest	Largest
RL 19/09	Buffalo	78	88	177	374	0.87	6.12×4.10	0.40	8.69×3.76
RL 21/09	Buffalo	45	70	65	148	0.92	4.48×2.96	0.21	6.30×2.91
RL 24/09	Buffalo	77	84	123	242	0.43	4.43×2.61	0.35	5.01×3.82
RL 25/09	Buffalo	64	71	108	175	0.37	5.64×3.27	0.29	6.98×2.69
RL 29/09	Dog	10	17	10	17	0.54	3.84×2.52	0.32	4.01×3.25
RL 32/09	Cow calf	70	77	160	193	0.67	5.04×3.65	0.35	6.22×2.38
RL 34/09	Cow calf	58	76	100	223	0.51	5.58×3.90	0.30	5.89×5.24
RL 35/09	Cow	74	83	178	285	0.55	7.80×3.40	0.34	9.63×4.34
RL 1/10	Buffalo	12	21	16	34	0.39	1.55×1.67	0.23	5.83×4.28
RL 3/10	Cow	57	69	72	121	0.37	5.38×3.22	0.23	6.19×3.94
RL 6/10	Cow	57	73	90	107	1.39	4.32×3.56	0.73	5.56×4.02
RL 8/10	Dog	0	8	0	8	N.A.	N.A.	0.71	3.46×3.15
RL 12/10	Cow calf	84	91	239	356	1.19	3.47×3.18	0.71	6.93×3.33
RL 14/10	Cow calf	82	92	212	379	0.55	5.44×1.78	0.34	8.54×3.14
RL 15/10	Cow	72	83	122	153	1.44	8.69×3.51	0.31	9.63×5.54
RL 19/10	Dog	10	15	10	16	0.71	4.87×3.54	0.55	5.34×4.20
RL 20/10	Dog	13	29	13	30	0.86	2.41×1.81	0.68	4.54×2.43
D02-862	Dog	0	12	0	12	N.A.	N.A.	0.27	5.33×2.91
Total		863	1,059	1,695	2,873				

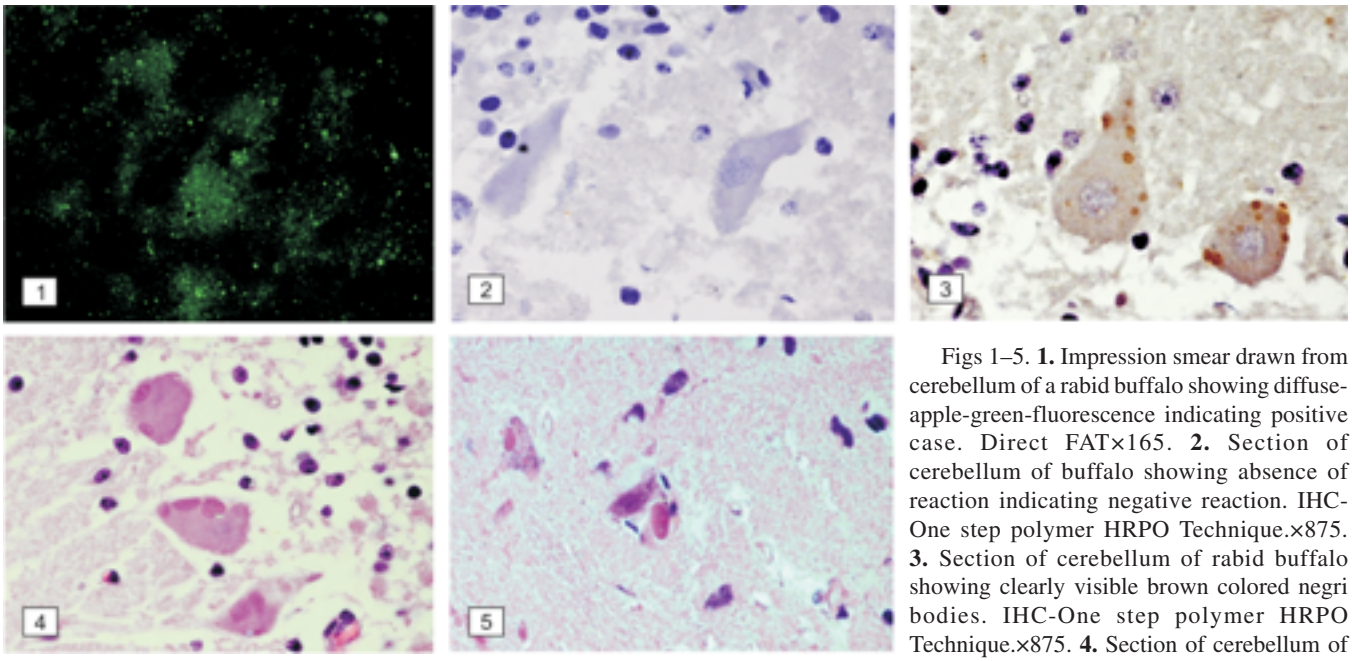
Table 2. Clinical signs in rabid dogs, buffaloes and cows

Symptoms	Species					
	Dog (n,5)		Buffaloes (n,6)		Cow (n,8)	
	No. of animals	Percentage	No. of animals	Percentage	No. of animals	Percentage
Anorexia	4	80	6	100	8	100
Hyper off-feed salivation	1	20	3	50	7	87.5
Fever	2	40	4	66.67	2	25
History of biting /aggressiveness	4	80	Nil	Nil	Nil	Nil
Not recognizing owner	2	40	2	33.33	4	50
Circling/head pressing	2	40	2	33.33	2	25
Difficulty in standing/paralysis	1	20	2	33.33	2	25
Difficult intake of food	3	60	2	33.33	4	50
Vaccination	2	40	Nil	Nil	Nil	Nil
Pica	1	20	Nil	Nil	Nil	Nil
Frequent micturition	Nil	Nil	1	16.67	3	37.5
Bellowing	Nil	Nil	5	83.33	7	75
Exophthalmia and congestion of eyes	Nil	Nil	Nil	Nil	1	12.5

N, rabies positive cases.

(1990) reported aggressiveness in 77% and 31% cases respectively. Paralysis and difficult in taking food are also considered as characteristic symptoms of rabid dogs, which were observed in 20% (1/5) and 60% (3/5) cases respectively by these researchers. The observations regarding paralysis are in accordance with that of Eng and Fishbein (1990) who reported paralysis in 29% rabid dogs. There is a common belief that rabies is a febrile, but the study revealed fever in 40% (2/5) cases; 40% (2/5) rabid dogs revealed circling and did not recognize the owner. Whereas, inappetance/anorexia was reported in 80% (4/5) cases, hence these symptoms should be taken seriously while suspecting any case of dog for rabies. Prophylactic-vaccination was done in 40% (2/5) cases only.

Clinical signs in rabid buffaloes: In rabid buffaloes, anorexia was in 100% (6/6) cases followed by bellowing



Figs 1–5. **1.** Impression smear drawn from cerebellum of a rabid buffalo showing diffuse-apple-green-fluorescence indicating positive case. Direct FAT×165. **2.** Section of cerebellum of buffalo showing absence of reaction indicating negative reaction. IHC-One step polymer HRPO Technique.×875. **3.** Section of cerebellum of rabid buffalo showing clearly visible brown colored negri bodies. IHC-One step polymer HRPO Technique.×875. **4.** Section of cerebellum of rabid buffalo showing intracytoplasmic eosinophilic negri bodies in purkinje cells. H&E.×875. **5.** Section of hippocampus of rabid cow showing intracytoplasmic eosinophilic negri bodies in neurons. H&E.×875.

83.33% (5/6), fever in 66.67% (4/6) and hyper-salivation in 50% (3/6) cases respectively (Table 2). However, paralysis, circling/head pressing and difficulty in taking of food were found in 33.33% (2/6) cases. Frequent micturition was observed in 16.67% (1/6) cases and 33.33% (2/6) rabid buffaloes did not recognize the owner. Similar symptoms were reported by Singh and Grewal (1998) and Rissi *et al.* (2008).

Clinical signs in rabid cows: In rabid cattle, anorexia was observed in 100% (8/8) cases followed by hypersalivation in 87.5% (7/8) cases and bellowing in 75% (6/8) cases (Table 2). However, difficulty in taking feed was observed in 50% (4/8) cases, frequent micturation in 37.5% (3/8), difficulty in standing in 25% (2/8), difficulty to recognize owner in 50% (4/8) and circling in 25% (2/8) rabid cattle. Exophthalmia and congestion of eyes were observed in 1 cow i.e 12.5% (1/8). Similar symptoms were reported by Srinunthapanth *et al.*(1985), Tanyi (1988), Aytekin and Mamak (2009) and Pedroso *et al*. (2009).

Fluorescent antibody test: Out of 38 cases, 19 were diagnosed positive for rabies by FAT of brain samples. The overall incidence of rabies was 50%. Characteristic apple green immunofluorescence was observed intra-cytoplasmic in neurons (Fig.1) as well as in form of diffused fluorescence in the brain tissue smears. FAT is considered an accurate method for diagnosis of rabies (Tepsumethanon *et al.* 1997) and served as a gold standard for assessment of immunohistochemistry and histopathology.

Immunohistochemistry: Brain tissues were positive in 18 cases (47.37%) out of 38 cases using polyclonal antiserum by immunohistochemistry and it revealed 94.74% sensitivity in comparison to FAT (Table 3). No positive reaction was observed with monoclonal antibody. The controls were negative and free of endogenous peroxidase (Fig.2). A large amount of distinct, granular rabies viral antigen deposits stained as sharply demarcated brown precipitates of variable sizes were found within the Purkinje cells and in the neurons of the hippocampus, in the axons, in the processes of neurons and in the stroma (Fig. 3).

Table 3. Sensitivity of immunohistochemistry on brain samples in comparison to FAT on brain tissue smears

Test	FAT on brain smears (Positive)	FAT on brain smears (Negative)	Total
IHC on brain (positive)	18	0	18
IHC on brain (negative)	1	19	20
Total	19	19	38

Sensitivity of IHC for brain samples
= true positive/ (true positive + false negative) ×100
= 18/19 ×100= 94.74%

Histopathology: Out of 38 cases, 16 (42.11%) were found positive for rabies by demonstration of negri bodies and thus, histopathology revealed 84.21% sensitivity in comparison to FAT (Table 4). Negri bodies appeared as single or multiple, eosinophilic intracytoplasmic inclusions within the Purkinje neurons (Fig.4), in the axons and in the neurons of the hippocampus (Fig. 5).

Comparison of immunohistochemistry and histopathology: Hundred neurons per case were observed for negri bodies and number of negri bodies in positive

Table 4. Sensitivity of histopathology on brain samples in comparison to FAT on brain tissue smears

Test	FAT on brain smears (Positive)	FAT on brain smears (Negative)	Total
Histopathology on brain (positive)	16	0	16
Histopathology on brain (negative)	3	19	22
Total	19	19	38

Sensitivity of histopathology for brain samples
=true positive/ (true positive + false negative) ×100
= 16/19 ×100 = 84.21%

Table 5. Comparison of histopathology and immunohistochemistry

Parameter	Histopathology (H &E)	Immunohisto-chemistry
Neurons having negri bodies (n,1800)	863	1,059
%age of neurons positive (negri bodies)	47.94	58.83
Total number of negri bodies detected	1,695	2,873
Average number of negri bodies per neuron	1.94	2.71
Smallest size of negri body (μm)	0.37	0.21
Largest size of negri body (μm)	8.69×3.51	9.63×5.54

neurons (Table 1) and a comparison of IHC and histopathology was done (Table 5). With IHC 58.83% neurons were positive for negri bodies and 47.94% with H & E. It can be concluded that IHC established many more virus infected cells than H & E stained sections which is same as reported by Feiden *et al.* (1985). Nuovo *et al.*(2005) reported average number of cells infected per 150 neurons was <1 and 94 with negri bodies (<1/150) and with immunohistochemistry (94/150).

Average number of negri bodies detected per neuron by IHC was 2.71 which were greater than H & E stained brain sections (1.94). The amount of antigen detected with IHC was much more abundant than histopathological findings which are reported by several workers (Hamir *et al.* 1992, Martinez-Burnes 1997, Jogai *et al.* 2001 and Suja *et al.* 2004). Palmer *et al.*(1985) reported that rabies antigen with IHC was apparent in 62% of the brain area in which inclusion bodies were not found in the corresponding H & E sections.

In present study a comparison of polyclonal and monoclonal antibodies employed in detection of rabies antigen in formalin fixed paraffin embedded tissue sections using IHC revealed polyclonal antibody to be highly efficacious. Immunohistochemistry in formalin fixed paraffin embedded tissue sections was found to be more sensitive than histopathology for detection of negri bodies/rabies antigen and therefore, of immense value for retrospective studies. Thus, it can be concluded that IHC was as sensitive as FAT and proved to be a valid method for rabies diagnosis and can replace FAT in examining impression smears where fluorescent microscopy is not available and when fresh samples are not available for FAT.

ACKNOWLEDGEMENTS

Authors thank Director of Research, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana for providing financial support to conduct this study. We also thank Mr Dan Singh and Mr Kewal Singh, staff of rabies diagnostic and histopathology laboratory for their help, cooperation and support extended during the period of study.

REFERENCES

Ayetkin I and Mamak N. 2009. A case of rabies in a cow. *Journal of Animal and Veterinary Advances* **8**(12): 2760–62.

Eng T R and Fishbein D B. 1990. Epidemiologic factors, clinical findings and vaccination status of rabies in cats and dogs in the United States in 1988. *Journal of American Veterinary Medical Association* **197**: 201.

Feiden W, Feiden U, Gerhard L, Reinhardt V andWandeler A. 1985. Rabies encephalitis: Immunohistochemical investigations. *Clinical Neuropathology* **4**(4): 156–64.

Hamir A N, Moser G and Rupperecht C E. 1992. Morphologic and Immunoperoxidase study of neurological lesions in naturally acquired rabies in raccoons. *Journal of Veterinary Diagnostic Investigation* **4**: 369–73.

Jogai S, Radotra B D and Banerjee A K. 2001. Immunohistochemical study of human rabies. *Neuropathology* **20**(3): 197–203.

Lembo T, Niezgoda M, Velasco-Villa A, Cleaveland S, Ernest E and Rupprecht C E. 2006. Evaluation of a direct, rapid immunohistochemical test for rabies diagnosis. *Emerging Infectious Diseases* **12**(2): 310–13.

Luna L G. 1968. *Manual of Histological Staining Methods of the Armed Forces Institute of Pathology.* 3rd edn. The Blakiston Division, McGraw-Hill Book Co. New York.

Martinez-Burnes J, Lopez A, Medellin J, Haines D, Loza E and Martinez M. 1997. An outbreak of vampire bat-transmitted rabies in cattle in northeastern Mexico. *The Canadian Veterinary Journal* **38**: 175–77.

Meslin M, Koprowski H and Kaplan M M. 1996. *Laboratory Techniques in Rabies*. 4th edn. WHO, Geneva.

Nuovo G J, DeFaria D L, Chanona-Vilchi J G and Zhang Y. 2005. Molecular detection of rabies encephalitis and correlation with cytokine expression. *Modern Pathology* **18**: 62–67.

Palmer D G, Ossent P, Suter M M and Ferrari E. 1985. Demonstration of rabies viral antigen in paraffin tissue sections: Comparison of the immunofluorescence technique with the unlabeled antibody enzyme method. *American Journal of Veterinary Research* **46**(1): 283–86.

Pedroso P M O, Colodel E M, Pescador C A, Arrudo L P and

Driemeier D. 2009. Clinical and pathological aspects in cattle affected by rabies with special reference to the rabies antigen mapping by immunohistochemistry. *Pesquisa Veterinaria Brasileira* **29**(11): 899–904.

Pedroso P M O, Pescador C A, Bandarra P M, Raymundo D L, Borba M R, Wouters F, Bezerra-Junior P S and Driemeier D. 2008. Standardization of immunohistochemistry technique for detection of rabies virus in formalin-fixed and paraffin-embedded tissue samples from central nervous system of cattle. *Pesquisa Veterinaria Brasileira* **28**(12): 627–32.

Perrin P and Sureau P. 1987. A collaborative study of an experimental kit for rapid Rabies enzyme immunodiagnosis. Bulletin WHO **65**(4): 489–93.

Pringle C R. 1991.The order mononegavirales. *Archieves Virology* **117**: 137–40.

Rissi D R, Fighera R A, Irigoyen L F, Kommers G D and Claudio S L B. 2008. Occurrence of rabies in sheep in Rio Grande do Sul, Brazil. *Pesquisa Veterinária Brasileira* **28**(10): 495–500.

Silva L H Q da, Bissoto C E, Delbem A C B, Ferrari C I de L, Perri S H V, Nunes C M de-L Ferrari C I and da-Silva L H Q. 2004. Canine rabies epidemiology in Aracatuba and neighborhood, Northwestern Sao Paulo State-Brazil. *Revista-da-Sociedade-Brasileira-de-Medicina-Tropical* **37**: 139–42.

Singh C K and Grewal G S. 1998. Comparative evaluation of rabies diagnostic sensitivity among seven laboratory techniques. *Indian Journal of Animal Sciences* **68**(12): 1210–13.

Srinunthapanth S, Triwanatham N and Khoonchan P. 1985. Rabies in cattle and buffalo; Case report. *The Thai Journal of Veterinary Medicine* **15**: 279–85.

Suja M S, Mahadevan A, Sundaram C, Mani J, Sagar B C, Hemachudha T, Wacharapluesadee S, Madhusudana S N and Shankar S K. 2004. Rabies encephalitis following fox bite, histological and immuno-histochemical evaluation of lesions caused by virus. *Clinical neuropathology* **23**(6): 271–76.

Tanyi J, Porkolab L, Fenyvesi A and Foldi J. 1988. Observations on the clinical signs, epidemiology and differential diagnosis of rabies in cattle. *Magyar Avatorvosok Lapja* **43**: 407–13.

Tepsumethanon V, Lumlertdacha B, Mitmoonpitak C, Fagen R, Wilde H, Veera-Tepsumeth A, Boonlert-Lumlertdacha and Channarong M. 1997. Fluorescent antibody test for rabies: Prospective study of 8987 brains. *Clinical Infectious Diseases* **25**(6): 1459–61.