



Histopathological alterations in nervous system of rabid animals

MADHAV MUGALE¹, B S SANDHU², C K SINGH³ and N K SOOD⁴

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

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Rabies is caused by highly neurotropic negative sense single stranded RNA (ssRNA) virus, belonging to family *Rhabdoviridae* of the Order *Mononegavirales* (Pringle 1991). Buffaloes, cattle and other livestock species get infected from rabies virus mainly through bites of rabid dogs and occasionally through rabid mongoose, cat, jackal and rodent bites. Negri bodies are considered as pathognomonic findings of rabies. Histopathological alterations in brain of rabid animals were qualitatively reported, but fewer attempts have been done to quantify these changes. The present study was conducted to quantify the histopathological alterations in brain of rabid dogs, buffaloes, cow, horse, jackal, ratel and hyena.

The brain samples were collected from 50 rabies suspected animals received for post mortem detection of rabies in the Department of Veterinary Pathology, College of Veterinary Science, Guru Angad Dev Veterinary and Animal Sciences University (GADVASU), Ludhiana, from various parts of Punjab. The tissues were also collected from the animals under observation when died at Veterinary Clinics, GADVASU. The whole brain samples were collected after observations of gross pathological changes. Three pieces of each brain tissue sample were stored in deep freezer at –20°C, in 50% glycerol saline solution, and in 10% neutral buffered formalin solution, respectively. The direct FAT was employed as diagnostic technique because of its sensitivity, accuracy and speed as recommended by World Health Organization (Meslin *et al.* 1996).

Formalin fixed brain samples were routinely processed and 4 to 5 µ paraffin sections were stained by haematoxylin and eosin method (Luna 1968). The tissues were screened for the presence of neuronal necrosis, Negri bodies,

satellitosis, gliosis, neuronophagia, edema, congestion, hemorrhage, perivascular cuffing and meningitis.

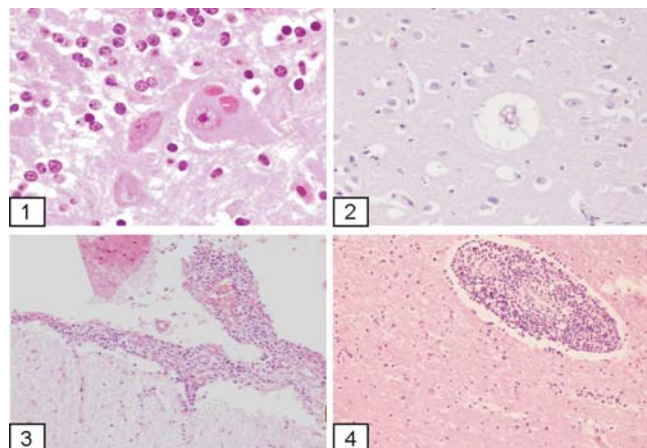
During the study period (July 2010 to June 2011) out of 50 suspected cases, 26 were diagnosed positive for rabies by histopathology of brain samples. The overall incidence of rabies was 52%. Out of 26 positive cases, 8 (30.76%) were of dogs, 8 (30.76%) of buffaloes, 5 (19.23%) of cows, 2 (7.69%) of horse, 1 (3.84%) of ratel, 1 (3.84%) of jackal, and 1 (3.84%) of hyena, respectively. Grossly 24% brains of suspected animals showed congestion, haemorrhage and edema in the present study, which was in accordance with the findings of Singh (1999) and Sumedha (2010).

Characteristic apple green fluorescence was observed intra-cytoplasmic in neurons as well as in form of diffused fluorescence in the brain tissue smears. FAT is considered an accurate method for diagnosis of rabies.

Histopathological alterations in brain of rabid dogs were observed in 8 samples of hippocampus and, satellitosis in 100% (8/8) cases, followed by Negri bodies in 87.50% (7/8) cases. Neuronophagia, gliosis, congestion and haemorrhage in 75% (6/8). However, neuronal degeneration and perivascular cuffing (Fig.4) were observed in 50% (4/8) cases. Whereas, 37.50% (3/8) cases showed oedema (Fig. 2). Babe's nodules and meningitis was seen in 25% (2/8) cases each. Spongiosis and vacuolation in neurons was observed in only one case 12.5% (1/8). Similar findings were observed by Percy *et al.* (1973) and Sumedha (2010).

Histopathological changes in cerebellum of rabid buffaloes revealed Negri bodies in 87.50% (7/8) cases (Fig.1) and which were same as reported by Jamadagni *et al.* (2008). Neuronal degeneration with necrosis and gliosis was present in 87.50% (7/8) cases. However, perivascular cuffing was observed in 62.50% (5/8) cases, satellitosis, meningitis (Fig.3) and Babe's nodule in 37.50% (3/8) samples. Congestion and oedema was shown by 50% (4/8) cases. Whereas, neuronophagia, hemorrhage and vacuolation in Negri bodies were observed in 25% (2/8), 12.50% (1/8) and 12.50% (1/8) cases, respectively. Similar histopathological alterations were qualitatively reported by Singh (1999) and

Present address: ¹Senior Executive officer (Histopathologist, madhav.mugale @gmail.com), Venus Medicine Research Centre, Chandigarh. ²Associate Professor, Veterinary Pathology (bhupisandhu@rediffmail.com), ³ Senior Veterinary Pathologist (rabiesck@gmail.com), ⁴Senior Veterinary Pathologist-cum-Head, Department of Veterinary Pathology, College of Veterinary Sciences (Nareshsood47@yahoo.com).



Figs 1–4. **1.** Section of cerebellum of rabid buffalo showing largest Negri body (Arrow). H & E staining $\times 875$. **2.** Hippocampus of rabid dog showing oedema, and neuronal degeneration. H & E $\times 875$. **3.** Section of cerebellum of rabid buffalo showing severe meningoencephalitis H&E $\times 875$. **4.** Section of hippocampus of dog showing Perivascular Cuffing (Arrow). H & E $\times 875$.

Archana (2001) and quantitatively by Jamadagni *et al.* (2008) and Sumedha (2010).

Histopathological examination of brain of cow revealed Negri bodies in all the samples (5/5), which were higher than earlier reports (Arslan *et al.* 2004, Lima *et al.* 2005). Other histopathological changes in cerebellum of rabid cattle revealed neuronal necrosis and congestion in 80% samples (4/5) and satellitosis in 60% samples (3/5), which is same as reported earlier (Jamadagni 2008 and Sumedha 2010). However, gliosis, perivascular cuffing and hemorrhages were present in 40% samples (2/5) each. Neuronophagia, spongiosis and oedema were present in 20% (1/5) cases. Similar histopathological alterations were reported by Pedroso *et al.* (2008) in cow, while histopathological alteration in cerebellum and hippocampus of rabid horses, jackal and ratel showed Negri bodies in 100% cases (2/2), other histopathological changes in cerebellum of rabid horse revealed neuronal necrosis, congestion and satellitosis in 66.66% samples (2/3) respectively. In all wild species such as jackal, hyena and ratel Negri bodies were seen in 100% cases which appeared to be highly positive for rabies showing higher number of Negri bodies per neuron.

Negri bodies were detected in more proportion in cows than in buffaloes and dogs, whereas, neuronal necrosis was present in equal proportion (100%) in all the 3 species, which is same as reported earlier by Jamadagni *et al.* (2008). Gliosis, congestion, haemorrhage, perivascular cuffing, neuronophagia were more consistent findings (100%) in the brain of rabid dogs. In rabid buffaloes, gliosis and perivascular cuffing was present in more proportion (83.33%), whereas in rabid cows, gliosis and congestion were more consistent findings (87.5%).

SUMMARY

The present study was conducted in 14 different species of 50 rabies suspected animals, in which 26 cases were positive for rabies by histopathology. This study was emphasized to quantify histopathological alterations in the nervous tissues of 8 rabid dogs, 8 rabid buffaloes, 5 rabid cows, 2 rabid horses, one ratel, jackal and hyena each received for post mortem detection of rabies in the Department of Veterinary Pathology, of the university from various parts of Punjab and the animals under observation. Gross examination of brain revealed congestion, haemorrhage and edema. Microscopically there was significant detection of Negri bodies in cerebellum in cows (100%), buffaloes (100%), ratel (100%), jackal (100%) and hyena (100%) however, in rabid horse and rabid dogs Negri bodies were found in 63.66 and 63.63% cases, respectively. Neuronal degeneration was present in all cases. Other histopathological alterations reported were satellitosis, gliosis, neuronophagia, edema, congestion, hemorrhages, perivascular cuffing and meningoencephalitis.

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