NATURAL CASE OF RABIES IN STRAY DOGS: PATHOMORPHOLOGY AND ANTIGEN DISTRIBUTION STUDIES OF BRAIN AND SALIVARY GLANDS

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Abstract

Stray dogs with positive rabies that were used in this study collected from Disease Investigation Center Bukittinggi area. The microscopic diagnose were confirmed based on direct Fluorescence Anti-body Technique (dFAT). The immunohistochemistry evaluation and distribution of virus antigen were visualized by using polyclonal antibody againt rabies. Brain tissues (hippocampus, cerebellum, cerebral cortex) and salivary glands (submandibullary and parotid glands) were collected, and fixed in 10% Buffered Neutral Formalin (BNF) then routinely processed for histopathological examination. Multifocal infiltration of lymphocytes and perivascular cuffing were mildly observed in evaluated brain samples. Careful examination on hippocampus, cerebellum, and cerebral cortex showed appearance of Negry Bodies in Pyramidal and Purkinje cells. The found lesions in submandibulary glands were in severely diagnosed level, with moderate to severe infiltration of lymphocytes in acini septal and necrosis of epithel mucogenic acini cells. However, there were no significant lesions found in parotid glands. Immunohistochemical examination demonstrated that rabies virus/antigen granules were predominantly found in ammons horn of hippocampus, purkinje cells of cerebellum and pyramidal cells in cerebral cortex. Antigens were localized in epithel cells of mucogenic acini of submandibulary glands. In addition, antigen rabies was also observed mildly in granular and striated duct lumina of parotid glands.

Keywords: rabies, street dogs, antigen distribution, brain, salivary glands.

Introduction

Rabies is a zoonotic disease causes fatal encephalomyelitis in humans and animals. It remains an important public health problem in Indonesia. The disease is caused by a highly neurotropic RNA virus belonging to rhabdoviridae family *lyssavirus* genus. In Indonesia street dogs is the principal reservoir of rabies infection and plays important role in transmission to humans. Dogs accounted for almost 95% of the rabies cases, followed by cats (3,5%) and captive monkeys (1%) and 0,5% were found in cattles, goats, buffalos, porcines, sheeps and deer (Soegiarto 2010). Data from Disease Investigation Centre Bukittinggi in 2010, there was 56,8% rabies positif case from 250 samples.

Rabies virus is usually transmitted by bite at peripheral site, from where, after entering axon terminals, it is transported in a retrograde direction to the central nervous system (CNS). With centifugal direction via peripheral nerves, it spreads to salivary gland and transmittes to animals or humans. Clinical manifestation characterized by progressive infection of the central nervous system that causes paralysis,

encephalitis, ataxia, hydrophobia and photosensitivity (Lafon 2004). Occasionally the infected animal may even look apparently normal but continue to secrete virus in saliva, thereby remaining asymptomatic (Fekadu 1988).

Based on the the neurotropic and the ability of the rabies virus to infected non neuronal organ, knowledge the distribution of viral antigen in CNS and salivary gland is essential in understanding its pathogenesis. In this study patomorphological and viral antigen distribution in the brain (hippocampus, cerebellum, cerebral cortex and salivary gland (submandibulary and parotid gland) rabid street dogs, from Disease Investigation Centre Bukittinggi area were identified using histopathological and immunohistochemical technique. The purpose of this study to elucidate the patomorphological and distribution antigen virus in natural case of rabid street dogs. The resul of this study could inform the best location to collect sample with minimal risk virus exposed in the field.

Material and Methods

Eight street dogs were suspected rabies from Disease Investigation Centre Bukittinggi area were euthanized by Livestock Services technician. It was sent to the laboratory for necroption. There was not quarantine maintained before dogs death. Hippocampus was collected for preliminary diagnosed by using direct Fluorescent antibody Technique (dFAT) test. If the result was positif, brain and salivary gland tissues (submandibullary and parotid gland) were fixed in buffered neutral formalin (BNF) 10% overnight. Whole formalin fixed brain was sliced in 9 (Suja *et al*. 2009) and fixed again overnight. Hippocampus, frontal lobe of cerebral cortex, cerebellum, submandibulary and parotid gland were subjected to examination. Tissues were routinely processed and stained with hematoxylin - eosin (HE) for histopathology inspection.

Additional section were prepared for immunohisto-chemistry using the REAL EnVision Detection System Labeled Polymer–HRP Method (Dako Denmark). Antigen retrieval by boiling 0,01 citrate buffer pH 6,0, and blocking of endogenous peroxidase activity with 3% H_2O_2 in methanol. Section treated with normal goat serum to block non specific reaction. For detection of rabies virus antigen using rabbit anti-rabies virus phosphoprotein antibody was kindly provided by Dr. Y. Sunden, Lab. Comparative Pathology, Graduate School of Veterinary Medicine, Hokkaido University, at a 1:1000 dilution. Antigen-antibody binding was detected with peroxidase-conjugated polymer (Dako K5007). The immunoreaction was visualized using chromogen 3-3'-Diaminobenzidine (DAB). Section counterstained with mayer hematoxylin, examined under a light microscope. The patomorphological features with hematoxylin-eosin (HE) stained were recorded. The number of antigen positif neuron

in different region was visualized with graded; none (-), 1-30% neuron (+), 30-60% neuron (++), 60-100% neuron (+++) (Suja *et al*., 2009).

Results and Discussion

Clinical Signs and Macroscopic Findings

There was not complete clinical history reported regarding the time of onset before dogs were euthanized. The information only unprovoked aggressiveness and human or animal bite by suspected rabid dogs. From necropsy inspection, the meningeal vessels of the brain are congested and sometimes found hemorrhage. There were not found macroscopic finding in submandibulary and parotid gland. The macroscopic finding in the brain of rabid dogs are unremarkable (Iwasaki and Tobita 2002). No macroscopic lesions found in the brain mice at necropsy throughout the experimental study with fixed rabies virus (CVS-11 strain) were inoculated intramuscularly (Kojima et al. 2008)

Patomorphological Findings.

In the histopathological examination of brain, seven of the eight dogs showed inflammatory reaction, mild to moderate multi focal non-suppuratif meningoencephalomyelitis characterized by multifocal monocuclear cells , predominantly lymphocytes and small amounts of macrophages in the grey mater and white mater frontal lobe cerebral cortex. One dogs showed severe non-suppuratif meningoencephalomyelitis. All of dogs showed many of the blood vessels hyperaemic and surround with lymphocytes and macrophages cells in cerebral cortex, hippocampus and cerebellum. Some neurons in the location found to be degenerative to necrosis. Negry bodies, that characterizes of rabies virus infection found predominantly in the pyramidal cell of ammons horn hippocampus, purkinje cells in the granular layer of cerebellum and in the pyramidal cell of frontal lobe cerebral cortex. Glial cells proliferation distribute focally and diffusely in the cerebral cortex, hippocampus, cerebellum. In that area were also observed neuronophagi. Moderate sialodenitis were found in the septa mucogenic cells submandibulary gland with predominantly mononuclear cells. Degeneration and necrosis were also found in the epithelial mucogenic cells. There were no significant lesions found in parotid gland.

Distribution of The Viral Antigen

With immunohistochemical staining antigen rabies viruses were found in a large number of cytoplasma neurons in pyramidal cells of ammons horn hippocampus, purkinje cells cerebellum and pyramidal cells frontal lobes cerebral cortex (Vural *et al*. 2001). In this study the similiar result was found. Pyramidal neurons of ammons horn of the hippocampus and purkinje cells of cerebellum have GABA as the main inhibitory neurotransmitter in addition to the cholinergic input, involvemet of

this part had been implicated the altered behavior and aggressiveness in the rabid animals (Suja *et al*. 2009). Lesions in the frontal lobes cerebral cortex result in change in behavior and personality (Akers and Denbow 2008)

In submandibular salivary glands, antigen rabies virus localized in epithel mucogenic cells. Maturation of rabies virus upon cell membranes predominated in salivary glands, virions were observed budding from the marginal membranes of mucogenic acinar cells exclusively in areas apical to nuclei (Dierks *et al*. 1969). While, there were not found lesions from histopathological inspection in parotid gland, in immunohistochemical staining antigen rabies virus detected in lumina of striated duct. A naturally infected skunks indicated that concentration of virus was highest in the submandibular glands, moderate in parotid glands and low in the sublingual glands (Howard 1981).

Conclusion

Based on antigen distribution hippocampus is the best location to collect sample for routine diagnosed natural case of rabies virus. Frontal lobe cerebral cortex and submandibulary gland could be alternative location for rabies sampling, attention on to make easier in collecting sample in the field than hippocampus. Further study are needed to elucidate sensitivity and spesifisity frontal lobes cerebral cortex and submandibulary glands than hippocampus as a sample rabies virus for laboratory test.

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Tables.
Distribution of Rabies Viral Antigen

	1	2	3	4	5	6	7	8
Hippocampus	+++	+++	+++	+++	++	+++	+++	++
Cerebelum	+++	+++	N/A	+++	++	N/A	N/A	++
Cerebral cortex	+++	+++	+++	+++	++	+++	+++	++
Submandibullary	+++	+++	+++	N/A	++	N/A	N/A	++
Parotid	+	+	+	N/A	+	N/A	N/A	+

N/A = Not available samples

Legends

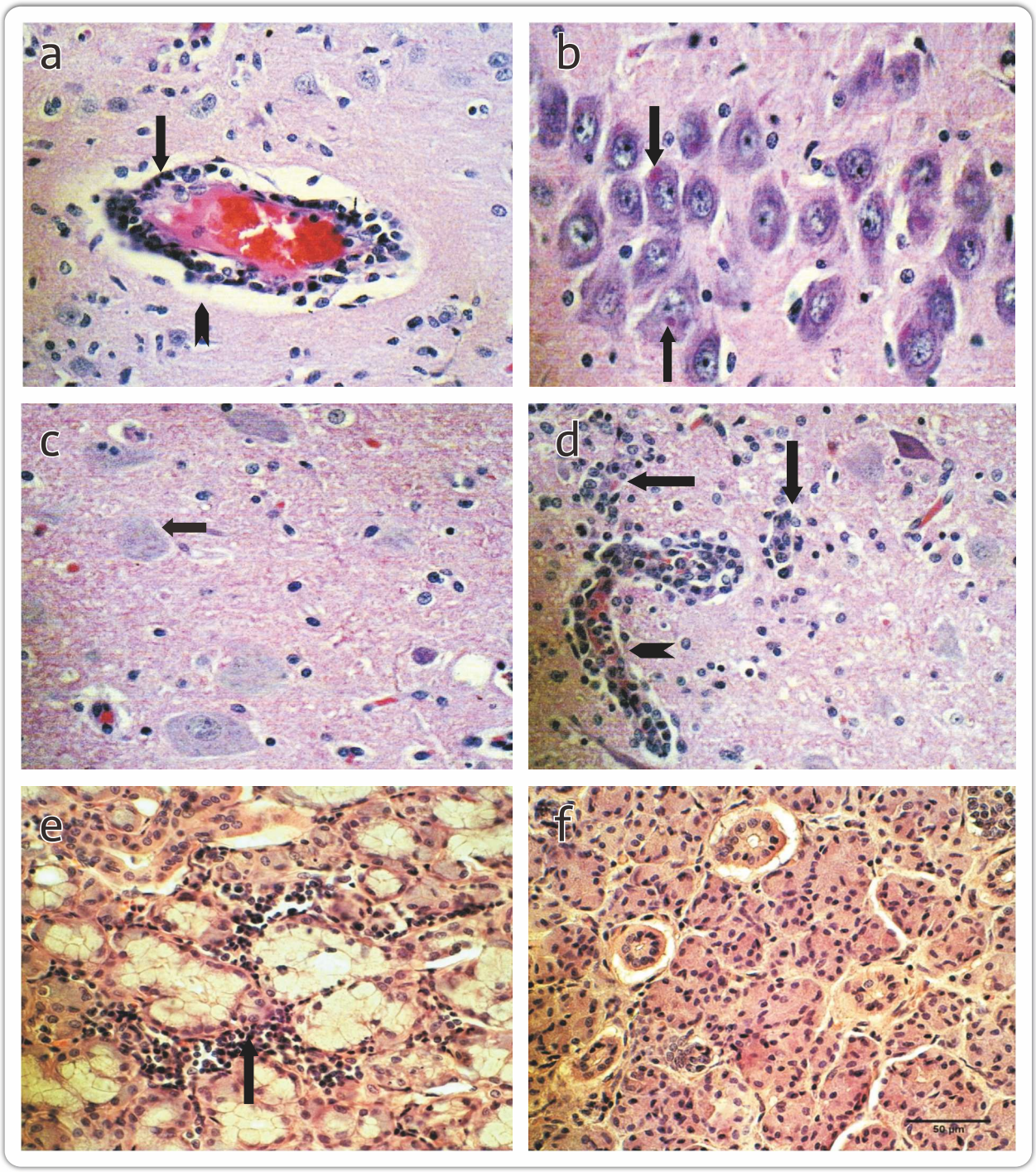

Fig. 1 A-F. Histopthological changes (HE). (A). Frontal lobe cerebral cortex Perivascular cuffing (arrowhead) associated with infiltration mononuclear cells (arrow). (B). Cerebelum. Negry bodies in cytoplasmic purkinje cells (arrow). (C). Necrosis neuron (arrow) in frontal lobe cerebral cortex (D). Nodular aggregates of microglia (arrow) surrounding neuron, Perivascular Cuffing (open arrowheads). (E). Infiltration mononuclear cells in septa mucogenic cells (arrow) submandibulary glands. (F). No significant lesions found in parotid glands. (Bar = 50 µm).

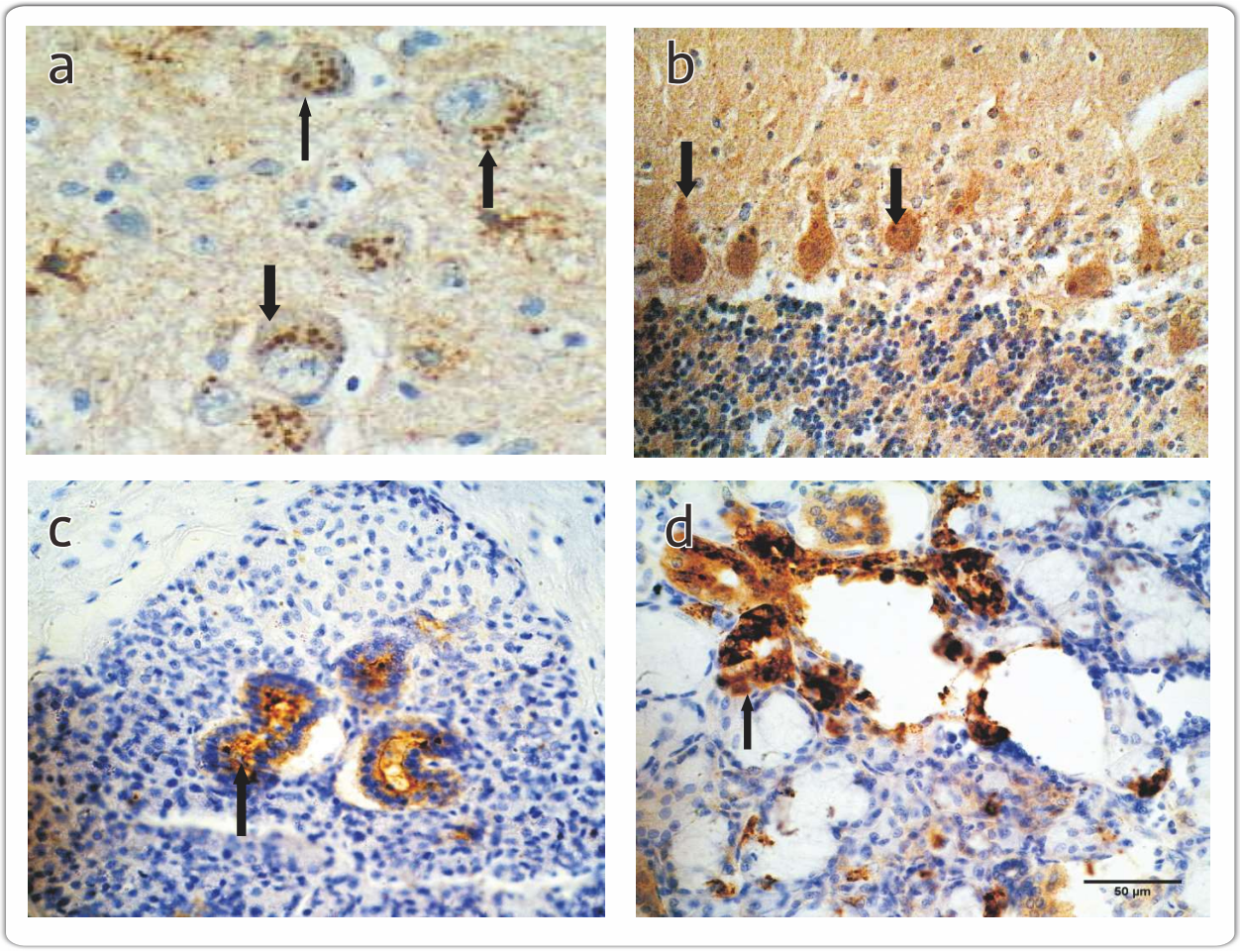

Fig 2. A-F. Immunohistochemistry staining. (A) Viral antigen was detected in the cytoplasma (arrow) pyramidal cells hippocampus. (B). Viral Antigen was detected in cytoplasma (arrow) Purkinje cells cerebellum (C). Viral antigen was dertected in lumina striated duct (arrow) parotid glands (D). Viral antigen was detected in epithel mucogenic cells (arrow) submandibullary glands. (Bar = 50 µm).