

# IMMUNOHISTOCHEMICAL DETECTION OF RABIES IN DOGS FROM SKIN

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[Received: 10.9.2014; Accepted: 30.5.2015]

It is of imperative importance to control rabies exposures by infected dogs. Thus, present study was envisaged to establish the feasibility of immunohistochemistry as least invasive diagnostic approach for antemortem diagnosis of rabies in dog from skin biopsy sample. Skin samples were collected from 13 dogs clinically suspected for rabies presented to veterinary clinics, COVAS, GADVASU, Ludhiana, Punjab. Nine out of 13 cases were confirmed to be true positive by immunofluorescence of brain tissue during postmortem examination of these cases. Out of nine true positive cases, five cases (55.55%) were diagnosed positive for antigen by IHC on skin. IHC on skin could detect rabies from skin of dogs with a sensitivity of 55.55%, accuracy of 69.23% and specificity 100%. It was concluded that immunohistochemical detection of rabies from skin offers a least invasive antemortem diagnostic alternative in absence of molecular diagnostic approaches.

**Key words:** IHC, Diagnosis, Dogs, Rabies, Skin.

## Introduction

Rabies is a zoonotic viral disease and canines pose serious threats to human beings hence it is of serious concern for medical and veterinary fraternity alike. It is caused by highly pathogenic non segmented negative sense RNA virus belonging to genus *Lyssavirus* in the *Rhabdoviridae* family (Pringle, 1991). Dog is the most significant vector for rabies virus and dog bites are responsible for the majority of human exposures and deaths from rabies (Hampson *et al.*, 2009). Antemortem rabies diagnosis is essential in canines to reduce number of exposures by dogs when animal is in incubation and to advocate timely post exposure prophylaxis to exposed persons. The outward spread of rabies virus from CNS leads to infection of various body organs (Jogai *et al.*, 2002). Thus, rabies virus (RABV) comes to lie in sensory nerves adjacent to hair follicles of infected animals (Wacharapluesadee *et al.*, 2012). WHO and OIE recommend FAT as gold standard on brain specimen, but efficiency of FAT outside nervous tissue is questionable for ante mortem rabies diagnosis. Fluorescent antibody test (FAT) had been used for identifying the presence of rabies viral

antigen in skin biopsies by various workers (Blenden *et al.*, 1983, Crepin *et al.*, 1998). IHC can be employed on formalin fixed tissue. IHC investigation of neck skin biopsy had been used for rabies diagnosis (Krause *et al.*, 2005) and for understanding pathogenesis (Tobiume *et al.*, 2009) of rabies in human being. To our knowledge IHC has not been used yet on skin biopsies for ante mortem diagnosis of rabies in canines. Hence, present study was aimed to establish the feasibility of IHC for antemortem diagnosis of rabies in canines using neck skin biopsies.

## Material and Methods

Ante mortem neck skin biopsies were collected from 13 clinically rabies suspected dogs presented to veterinary clinics, COVAS, GADVASU Ludhiana. Brain samples were also collected during necropsy to establish true positive cases using FAT. Skin biopsies were fixed in 10% buffered formalin saline for 48 hours and processed to paraffin embedded blocks. Paraffin embedded tissue sections of 4-5 $\mu$  thickness were cut and mounted on Super frost/ Plus, positively charged microscopic slides (Fisher Scientific, USA). Sections were deparaffinised and subjected to Antigen retrieval system (Biogenix