

## CASE STUDY OF A RABID DOG: VACCINE FAILURE AND OWNER'S IGNORANCE

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**Summary:** Rabies is a severe but preventable anthroozoonosis of developing countries. Wandering and unvaccinated dogs are the primary source of rabies particularly, in low-income countries. The purpose of this presentation is to highlight the public health risks for pet owners. Despite pre-exposure vaccination, rabies virus was diagnosed in dog brain using histopathology and immunohistochemistry. Negligence in prophylactic measures and dog bite management constitutes the main cause of rabies cases, largely in populated cities of Pakistan. Another contributing factor is the lack of knowledge, regarding rabies prophylaxis, among both non-professionals and medical practitioners.

**Keywords:** dog bite, histopathology, immunohistochemistry, rabies virus.

### INTRODUCTION

Rabies is caused by a negative stranded RNA virus, belonging to the *Lyssavirus* genus and *Rhabdoviridae* family. Typically, it is bullet shaped neurotropic virus that produces unusual neurological and behavioral signs and symptoms, resulting in progressive and mostly lethal encephalitis in animals and humans (Jackson, 2011). Rabies is a continuous public health threat among developing countries where proper prophylactic measures are not practiced and, people are unaware about rabies control guidelines and safety measures (Parviz *et al.*, 2004). As a consequence, annually 59,000 people die of rabies worldwide, with highest death toll in Asian countries where maximum rabies cases are caused by stray dogs (Hampson *et al.*, 2015).

Rabies is endemic and non reportable disease in Pakistan and on the average, thousand numbers of animal and human dog bites are reported annually in all cities of Pakistan (Zaidi *et al.*, 2013). These numerical figures potentially cause round about 2500-5000 deaths in Pakistan as reported by Asian Global Alliance for Rabies Control. The postmortem examination of the brain tissues, using histopathology and immunohistochemistry is comparatively easy, quick and, reliable for the distribution and confirmation of pathognomonic lesions and rabies antigens respectively (Faizee *et al.*, 2011).

Several research studies have described that timely and precise application of anti-rabies vaccines is potentially excellent source for providing 100% protection against rabies (Hampson *et al.*, 2015). However, failure to do so would probably result in forth seen complications in canine and domestic animals. Effective dog bites management procedure and reliable prophylactic vaccination follow up are the best rated actions to combat this zoonosis but sadly, such kind of

positive approach is seriously deficient in different health units of the country (Parviz *et al.*, 2004; Wasay *et al.*, 2008). Moreover, published reports or data are not available about the epidemiology and pathogenesis of rabies in Pakistan. Therefore, the objectives of the case scenario were to raise the general alertness among public and health authorities regarding prophylactic measures, with a vigorous emphasis on the quick and accurate choice of treatment.

**Clinical history:** A 5-year-old male dog was brought to the civil veterinary hospital of Faisalabad, Pakistan. Apparently, the dog was lethargic, emaciated and the rectal temperature was 39.1°C. The owner complained that the food and water intake of the animal was not regular since the last 5 days and, behavioral change was peculiar. His major observation was that either the dog ingested food poison during roaming within streets or, the head trauma due to aggressive encounter with street dogs, two weeks before the onset of altered behavior. He took his pet to the nearby veterinary service unit for the administration of the post exposure vaccination thirteen days after the incidence. Using his own understandings, he also tied up a bandage of antiseptic cream on the wound in the forelimb. Upon close observations, the abdominal skin had two minute lacerated wound and a distinct bite (Category II) on the inner side of the forelimb. The dorsal surface of the head had no obvious scratches, lacerations or any blood spot. Antiseptic dressing, supportive treatment and post exposure prophylactic vaccine Rabisin (an inactivated vaccine) were administered. The owner was educated about the general precautionary measures and advised to keep the dog in segregated place. Further, the telephonic connection was put in place to update the clinical history and physical condition of the suspected dog.

After 17 days, the owner reported that the dog did respond towards the medication but, side by side he also noticed alerted behavioral signs. After the next 5 days, the owner was afraid of doubted madness by reporting nervous excitement, howling, restlessness, sticky salivation and aggressive movements in his dog. Afterward, clinical signs and symptoms progressively developed the paralytic form of rabies, leading to jerky movements of paralyzed legs in lateral recumbency. The dog was peacefully euthanized with owner's consent and, the head was separated in a confined and population free zone. Carcass and dog remains were wrapped up into a polythene bag and incinerated in an incinerator.

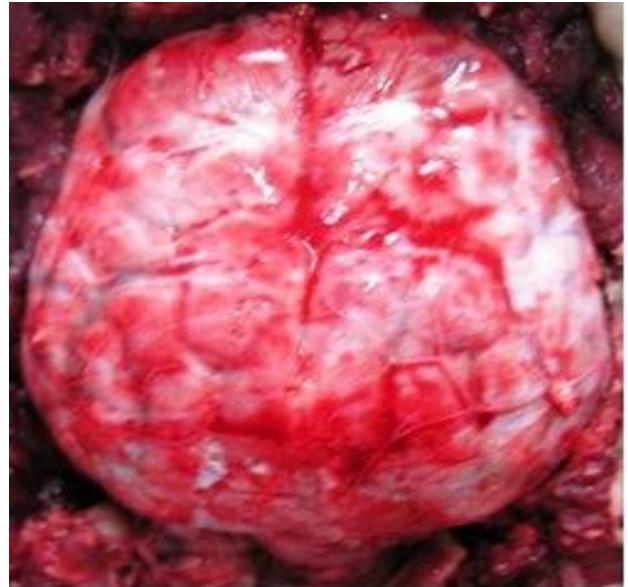
**Materials and Methods:** For the evacuation of the fragile brain tissue from dog skull, the standard laboratory procedure was adopted as recommended by the World Health Organization. In brief, four deep incisions were applied using hammer, chisel and butcher knife. Two cuts were given just above the ears on the dorso-lateral surface of the skull and, one of the remaining two was given behind the eye socket and lastly, the back of the skull cut was given to adjoin the remaining incisions systematically (WHO, 1954). Using this technique, the skull cap was carefully removed revealing the brain tissue. The brain was surgically removed and, immersed in 10% neutral buffer formalin for fixation. The cerebrum of brain was incised for the proper penetration of formalin. Standard protocols for histopathology (Bancraft and Gamble, 2008) and immunohistochemistry (Faizee *et al.*, 2011) were followed as per recommendations.

## RESULTS

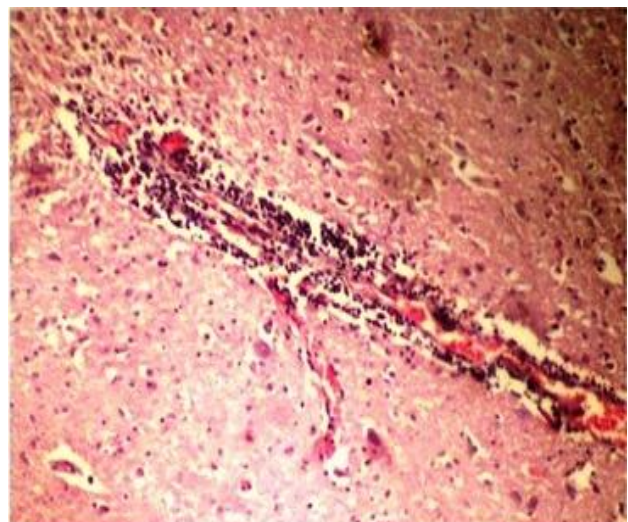
Grossly, the brain tissue was slightly edematous with severe congestion (Fig. 1). The histopathology revealed discrete lesions and pathological changes in different anatomical parts of the brain. Few sections showed pyknotic or hyperchromatic nuclei along with slender or bar-shaped neurons which, were unevenly distributed in the cerebrum. Cytoplasmic vacuolation was the salient lesions in many sections of the cerebrum. Interestingly, these vacuolated changes were located in adjacent to these injured or dowel shaped neurons. Typical babe's nodules and necrotic degeneration in neuronal cells were not abundantly seen. Furthermore, perivascular cuffs appeared as surrounded by either multi or monolayer aggregation of lymphocytes and monocytes (Fig. 2). Although, these changes are suggestive of massive tissue damage but, the tissue was devoid of intracytoplasmic inclusions or Negri bodies.

Immunohistochemical analysis revealed tiny particle of rabies virus antigens in the cerebellum and least in cerebrum. The cellular visibility and the arrangements of the neuronal cells within cerebrum were

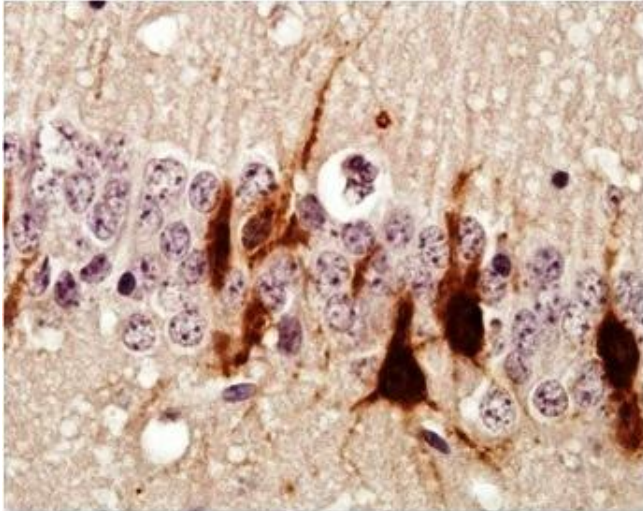
fine in overall observation. However, within the cerebellum, the Purkinje cells were stained well enough with chromogen substrate. These cells showed minute variation in terms of antigen particles and therefore, appeared little bit faint but even though, the positive reaction in the cerebellum made our diagnosis tentative by showing different sizes of the antigenic masses. They were either dispersed along the entire length of axons or covered the perikaryons of the Purkinje cells with antigenic mass (Fig. 3).



**Figure 1: photograph showing congestion and edematous brain after de-caping the dog skull.**



**Figure 2: A section of cerebrum revealing pinkish perivascular cuff surrounded by layers of lymphocytes (Hematoxylin and Eosin staining, 200X)**



**Figure 3: Photomicrograph of hippocampus showing stained Purkinje cells with deposits of rabies antigens (Mouse monoclonal antibody IgN, X-400).**

## DISCUSSION

The present case was an example of a failed handling and, similar cases have been frequently witnessed earlier in dogs (Qasim *et al.*, 2010; Habus *et al.*, 2010) and humans (Kopel *et al.*, 2012; Parviz *et al.*, 2004). In Asia and Africa, the numbers of abandoned dogs are relatively large which are difficult to vaccinate in any given area, considering the expenditure and economic cost of the whole vaccination campaign (Hampson *et al.*, 2015). Therefore, alarming consequences have to be seen in the form of human fatalities, making rabies as 10<sup>th</sup> leading infectious disease of the world (Franka *et al.*, 2012). The differential diagnosis of the case was built up with epilepsy, nervous toxicity and tetanus but, the veterinarian dealt the case as suspected rabies due to distinct bite. Among the immunopathological techniques, immunohistochemistry is equally sensitive to fluorescent antibody technique for sure diagnosis of rabies. Furthermore, in a developing country like Pakistan, the fluorescent antibody detection method is relatively expensive and precise handling is also not possible in an unfavorable weather ((Faizee *et al.*, 2011).

There may be several reasons behind the facts of the present rabies case such as, misguided clinical history of the dog, immuno-compromised host (Kopel *et al.*, 2012), mishandling or delayed administration of the vaccine (Taylor, 2013; Townsend *et al.*, 2013) and or, failure of the vaccine to induce expected immunogenic response in host immune system (Qasim *et al.*, 2010). These problems can be overcome through quick availability of reliable prophylactic measures, team up regulation of veterinary drug stores and, adequate field

serosurveillance (Townsend *et al.*, 2013). The lack of dog bite management is purely associated with the large communication gap between general public and veterinarians, which ultimately results in the lack of general awareness about rabies prophylactic measures in rural and urban communities (Franka *et al.*, 2012). These issues can be well solved through public-private partnerships (Taylor, 2013) canine herd vaccinations and raising literacy rate in general (Franka *et al.*, 2012). Keeping in context, poor attitude and response of the health practitioners is also participating indirectly in rabies cases (Wasay *et al.*, 2008).

Conversely, by establishing the close cooperation between the public and health workers would definitely diminish the incidence of sporadic cases. A similar type of coordination can be witnessed in European and developed countries where minor and major rabies cases have been wiped out, but, limited resources and low economic conditions are the barriers in the developing countries in the fight against rabies.

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