The Journal of Animal & Plant Sciences, 26(3): 2016, Page: 636-642
ISSN: 1018-7081

IMMUNOPATHOLOGICAL STUDIES OF CANINE RABIES IN FAISALABAD, PAKISTAN

W. Ahmad[†1, 2], F. Mahmood[†2], Y. Li[†1], M. Duan[†1], Z. Guan[1], M. Zhang[1], M. A. Ali[3] and Z. Liu*[1]

[†1]Key Laboratory of Zoonosis, Ministry of Education, Institute of Zoonosis, Jilin University, 5333 Xian Road, Changchun 130062, People's Republic of China
[2]Department of Pathology, Faculty of Veterinary Science, University of Agriculture, Faisalabad, Pakistan,
[3]Department of Clinical Sciences, Faculty of Veterinary Science, Bahauddin Zakariya University, Multan, Pakistan
[†]These authors contributed equally
*Corresponding author's email; zsliu1959@sohu.com

ABSTRACT

Rabies is a fatal disease particularly in developing countries like Pakistan, where annual death toll ranges from 2,000 to 5,000 due to insufficient prophylactic measures. The present study was conducted to screen out randomly selected stray dogs (n= 80) for rabies virus at eight sites in the district of Faisalabad, Pakistan. Brain samples were surgically removed and fixed in neutral buffer formalin. Paraffin-embedded brain tissues were processed for the diagnosis of the rabies virus using immuno histochemistry and histopathology. Post dog-bite vaccine data from the year 2006 to 2010 were obtained from two public hospitals in district Faisalabad. The highest number of rabies cases was found in 2010 (n=1,980) while, the lowest number of anti-rabies shots (n=312) was injected in 2009. Most importantly, only 3.75% of brain samples were diagnosed positive for rabies virus. Considerable immuno-reactivity and characteristic pathological lesions (neuronal degeneration, babe's nodules and Negri bodies) were observed in granular and Purkinje cells of the cerebrum and cerebellum respectively. Proper monitoring and regulatory framework are essentially required to fully eradicate rabies cases from rural and urban areas of Pakistan.

Keywords: Brain, rabies virus, dog bite, immune histochemistry, histopathology, stray dog.

INTRODUCTION

Rabies is a deadly zoonotic infection caused by neurotropic virus of genus *Lyssavirus*, belonging to the family *Rhabdoviridae*. Annually, extensive number of fatalities occurs in a wide part of Asia and Africa where canine rabies is enzootic (Knobel *et al*., 2005). World Health Organization sponsored a multi-centric study which estimated that more than 30,000 deaths occur annually in Asia with an excessive numbers in urban areas of Indian subcontinent (Sudarshan *et al*., 2007). In urban areas, domestic as well as stray dogs play a significant role in dissemination of the disease to humans and domestic animals (Zaidi *et al*., 2013).

Hydrophobia, spasm of inspiratory muscles of larynx and pharynx, episodes of hallucination and excitement, aggression, excessive salivation, weakness, flaccid paralysis and ultimate death are salient symptoms of rabies exhibited by rabid animals and humans with variable intensity (Jackson, 2010). Characteristic intracytoplasmic oval shape Negri bodies with granular appearance are pathognomonic lesions that are more commonly observed in rabid brains, skin and salivary glands (Jamadagni *et al*., 2006). Significant pathological lesions have been reported in rabies infected animals including neuronal degeneration, necrosis, perivascular cuffs surrounded by inflammatory cells and, presence of focal cell aggregates (babe's nodules) in different parts of brain (Jackson. 2011). Furthermore, several reports about epidemiological and disease surveillance data about rabies have been reported in USA (Blanton *et al*., 2009), North and South Africa (Faizee *et al*., 2011), India (Shah *et al*., 2012) and South East Asia (Knobel *et al*., 2005).

Various techniques have been used to diagnose rabies in laboratories as well as in field conditions including virus isolation, direct rapid immune histochemical test (Lembo *et al*., 2006), fluorescent antibody test (FAT), serodiagnos is using ELISA and molecular diagnosis through RT-PCR (Tenzin *et al*., 2010). Among these, FAT is considered to be the gold standard test for the diagnosis of rabies virus yet, histopathology and immune histochemistry are the most reliable and routinely used tests in developing countries.

Recently, a large number (> 97000) of dog bite cases have been reported by the National Health Management Information System (NHMIS) in Pakistan. Sufficient lack of conversance regarding post exposure prophylaxis (PEP), serosurveillance, rabies diagnosis and management has been seen in public hospitals. Moreover, rabies pathogenesis and lesions have never been reported earlier neither in humans nor in domestic animals of Pakistan. Hence, the present study was designed to assess the data about pre exposure, PEP and load of circulating rabies virus in district Faisalabad.

MATERIALS AND METHODS

The study area covered eight sites/towns (*Chakjhumra, Jaranwala, Tandlianwala, Jinnah, Iqbal, Samundari, Madina and Layalpur*) of district Faisalabad, Pakistan. Dog bites cases and vaccinations against rabies are usually reported in the District Head Quarter Hospital (DHQ) and Allied Hospital (AH). These are the two main hospitals, located in the center of the city. Annual dog bite vaccine data from the year 2006 to 2010 were obtained from these hospitals.

Screening of Dog Brains: Eighty (n=80) dogs were randomly selected during dog capture campaign of the district government from eight sites of the city. The dogs were peacefully euthanized and skulls were surgically operated in a confinement to withdraw brain samples aseptically using complete bio-safety measures. For this purpose, specified pattern of incisions was adopted at the dorsal surface of skull with the help of autopsy hammer and chisel as prescribed earlier (Antona, 1954). Extreme care and precautionary measures were followed to avoid any injury to the operator and the underlying fragile nerve tissue. Brain samples were fixed in 10% neutral buffered formalin and protocols for paraffin sectioning and Hematoxylin & Eosin (H&E) staining were followed as described previously (Bancroft and Gamble. 2008).

All steps pertaining to animal handling were carried out according to Centers for Disease Control and Prevention (CDC, China), Institutional Animal Care and Use Committee guidelines.

Immuno histochemistry: About 5µm thick brain sections were deparaffinized using citrisol solution (Fisher Scientific. PA) and proceeded according to Stein *et al.,* (2010). Primary antibodies (Polyclonal rabbit anti-rabies) were diluted 1:500 and covered for 1 hr at room temperature, while, secondary antibodies (anti rabbit antibodies, Vector Lab.) were used to incubate the tissue sections for 30min followed by incubation with ABC ELITE (Vector Laboratories, CA) and differentiation through DAB chromogen. At last, sections were counter stained with hematoxylin, cleared in xylene and mounted with DPX.

Descriptive analysis of the data was conducted to differentiate significant means using SPSS software.

RESULTS

Histopathology: Most of the brain samples appeared healthy in gross and microscopic appearance. However, experimental evaluation showed that 3.75% brain samples were positive one from each of three sites (*Chakjhumra*, *Jaranwala* and *Tandlianwala* town). The presence of intracytoplasmic Negri bodies was seen only in single case from one site (*Tandlianwala* town).

Grossly, these positive samples were edematous and appeared congested with naked eye. In addition, brain samples from *Samundari* town and *Jinnah* town were slightly congested with glistening meninges but found negative. Similarly, four brain samples belonging to *Madina* town also exhibited certain areas of discoloration but again, these findings also lead to negative results. Nevertheless, brains collected from remaining sites of district Faisalabad were healthy in color, consistency, shape and other parameters visible with naked eye.

Microscopic examination of infected and non-infected brain samples demonstrated variable number of pathological lesions in various sections of brain but, positive brain samples revealed severe congestive changes in cerebrum, with perivascular cuffing of mononuclear cells (Fig. 1). Babe's nodules were seen in granular layers along with degenerative and necrotic cells of rabid brain cerebrum (Fig. 2). Likewise, few brain sections showed satellitosis and definite rod shape neurons and, the later kind of neurons markedly appeared as slender or pole shape under light microscope. These rod shape neurons were scattered among granular layers of cerebrum with distinct morphological appearance. Cavernous lesions and vacuolation were also present with characteristic honey comb shapes within the cerebrum of positive brain samples. The distinctive oval shaped intracytoplasmic Negri body was present in Purkinje cells of single positive sample.

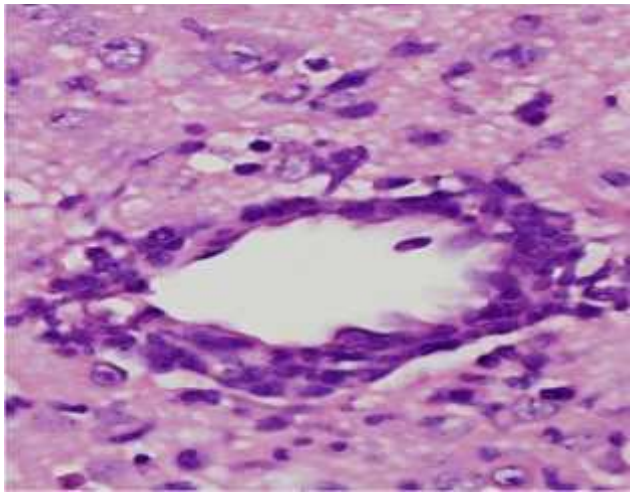

Figure 1. Photomicrograph showing a typical perivascular cuff of mononuclear cells in brain tissue of *Chakjhumra* town (H&E stain, X-200).

Statistical Analysis: Statistical analysis showed that values for means of cases were higher for summer in both hospitals which indicated that the incidence of the dog bites was significantly elevated in summer as compared to other seasons (Table 2, 3).

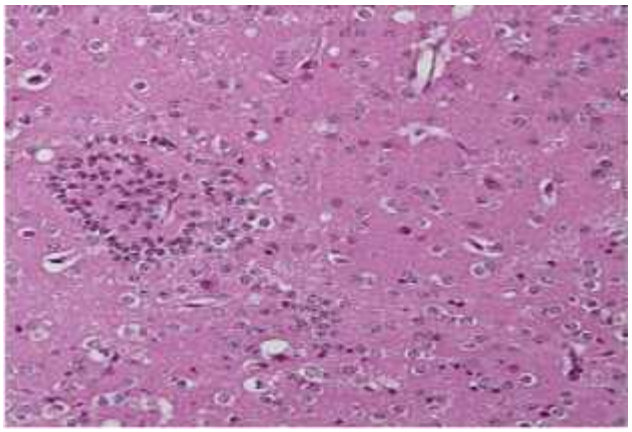

Figure 2. Babe's nodule; a typical focal aggregation of mononuclear cells in the cerebrum of *Jaranwala* town brain tissue (H&E stain, X-400).

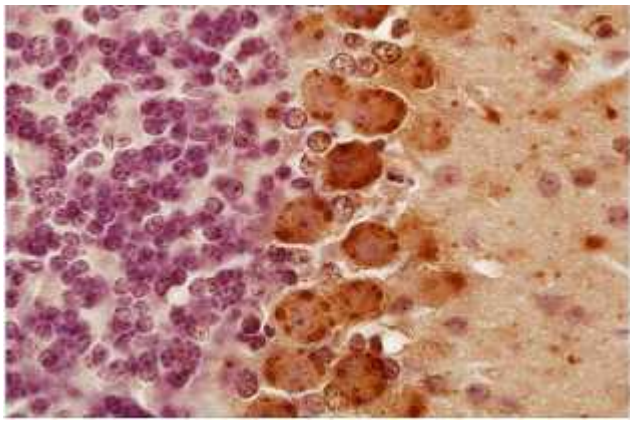

Figure 3. Photomicrograph of rabies positive dog cerebellum from *Tandlianwala* town revealing antigenic localization in perikaryons (DAB substrate, X-200).

Immunohistochemistry: In accordance with these results, discrete intracytoplasmic inclusions in Purkinje cells of cerebellum were also suggestive of extensive rabies virus infection (Fig. 3) in rabid brain samples of dogs. The antigen particles of different size were dispersed within the cell bodies and cytoplasm of neurons and clearly visualized under light microscope. Characteristic antigen and antibody reaction with the distribution of antigenic stained particles in rabies infected samples was extensively seen in hippocampus followed by cerebellum and cerebrum.

Post-Dog Bite Vaccine Record: The data obtained from DHQ clearly showed that out of total 1980 cases, 15.76% and 21.67% were the lowest and highest percentages during the year 2009 and 2010, respectively. Highest numbers of cases (n=285) were recorded in June while, the lowest were found in February (n=78) between 2006 and 2010. Similarly, out of total 2110 cases from AH Faisalabad, only 17.11% and 22.46% were lowest and highest percentages of anti-rabies shots injected in the year 2009 and 2010 respectively. However, monthly distribution showed highest cases in July (n=250) and lowest cases in September (n=97) during that five year time period (Table 1).

Table 1. Total number of human post exposure vaccine shot cases from AH and DHQ, Faisalabad.

Month	Hospitals	Tl	2006	`2007	2008	2009	2010
January	AH	224	18.30%	17.86%	35.71%	20.09%	8.04%
	DHQ	110	31.82%	13.64%	9.09%	10.91%	34.55%
February	AH	172	29.65%	29.07%	24.42%	11.05%	5.81%
	DHQ	78	25.64%	30.77%	15.38%	5.13%	23.08%
March	AH	311	23.47%	22.51%	24.12%	13.83%	16.08%
	DHQ	222	27.03%	14.41%	18.92%	15.77%	23.87%
April	AH	172	15.70%	14.53%	17.44%	29.07%	23.26%
	DHQ	156	19.23%	22.44%	27.56%	18.59%	12.18%
May	AH	102	21.57%	24.51%	9.80%	2.94%	41.18%
	DHQ	136	15.44%	24.26%	29.41%	0%	30.88%
June	AH	168	23.81%	22.62%	2.98%	26.79%	23.81%
	DHQ	285	20.70%	22.46%	19.30%	12.28%	25.26%
July	AH	250	23.60%	20.00%	12.00%	22.00%	22.40%
	DHQ	212	16.51%	24.53%	32.08%	15.09%	11.79%
August	AH	177	16.95%	14.12%	23.73%	14.12%	31.07%

	DHQ	234	21.37%	25.21%	23.50%	19.23%	10.68%
September	AH	97	15.46%	8.25%	1.03%	18.56%	56.70%
	DHQ	150	20.00%	16.67%	23.33%	14.67%	25.33%
October	AH	111	18.02%	16.22%	22.52%	9.01%	34.23%
	DHQ	196	11.22%	14.29%	12.76%	36.73%	25.00%
November	AH	143	20.98%	24.48%	23.08%)	13.99%	17.48%
	DHQ	99	23.23%	25.25%	22.22%	4.04%	25.25%
December	AH	183	16.39%	27.32%	16.39%	15.30%	24.59%
	DHQ	102	19.61%	19.61%	14.71%	21.57%	24.51%
Total	AH	2110	20.76%	20.57%	19.10%	17.11%	22.46%
	DHQ	1980	20.45%	20.81%	21.31%	15.76%	21.67%

Table 2. Comparison among mean values of rabies cases for hospitals, years and seasons.

		N	**Means**	**SE**		**s.e**
Hospital	AH	60	34.17	2.23	A	3.10
	DHQ	60	32.31	2.23	A	
Year	2006	24	33.64	3.58	A	5.06
	2007	24	33.29	3.58	A	
	2008	24	32.99	3.58	A	
	2009	24	28.64	3.58	A	
	2010	24	37.65	3.58	A	
Season	Summer	40	39.55	2.69	A	4.11 to 4.91
	Spring	30	37.03	3.10	AB	
	Autumn	20	27.70	3.80	B	
	Winter	30	28.70	3.10	B	

Table 3. Analysis of Variance (ANOVA) for rabies cases.

Source	**DF**	**SS**	**MS**	**F**
Hospital	1	104.5	104.5	0.36^{NS}
Year	4	922.5	230.6	0.80^{NS}
Season	3	3138.1	1046.0	3.61*
Year*Season	12	2708.3	225.7	0.78^{NS}
Error	99	28689.4	289.8	

DISCUSSION

Rabies status, in terms of control and eradication, is still poor at domestic and national level of Pakistan which always evokes health authorities to take strict measures. Present study results confirmed that stray dogs are potential source of rabies in animals and humans that correspond to sporadic incidences of animal and human deaths in different regions of Pakistan (Perviz *et al*., 2004). These cases are witnessed everywhere but, barely reported at clinical and research levels (Wasay *et al*., 2008; Zaidi *et al*., 2013). Correspondingly, many research studies about rabies outbreaks have been witnessed earlier in different regions of the world (Manickam *et al*., 2009; Tenzin *et al.*, 2010; Faizee *et al*., 2011; Camila *et al*., 2014).

Similar to the present study, multiple gross and pathological lesions have been reported earlier in different brain sections of cattle and buffalo (Jamadagni *et al*., 2007), mice (Bernardi *et al*., 2006), wild and domestic animals (Faizee *et al*., 2011), humans (Hsu *et al*., 2005) as well as experimentally induced sheep (Brookes *et al*., 2007). The characteristics babe's nodules were mostly found in the cerebrum of positive cases whereas, previously these have also been observed in brainstem (Jamadagni *et al*., 2007). Only single positive brain sample demonstrated the presence of pinkish Negri bodies. This finding also govern that pathognomonic Negri bodies could only be seen in 20 to 60% of rabies infected cases (Woldehiwet, 2005) and further, histopathological analysis could not be set as tentative diagnostic test for rabies as compared with immune histochemistry (Stein *et al*., 2010). Moreover, similar features of Negri bodies have also been reported in the salivary glands, tongue or other organs (Hamir *et al*., 1992). Strong immuno-reactivity in hippocampus followed by cerebellum and cerebrum suggested that the intense staining was directly correlated with the amount

of rabies virus antigens present in the brain tissues (Jogai *et al*., 2000). In the same way, several studies conducted in dogs coincide with the results of present study and, detected a wide range of rabies virus antigens in the same order (Stein *et al*., 2010; Faizee *et al*., 2011; Johnson *et al*., 2011). In few paraffin sections, very weak staining was observed that could be due to prolong fixation in formalin (Faizee *et al*., 2011).

Retrograde transport of rabies virus within neuronal cells and considerable hijacking of TLR3 receptors are the reasons to produce granular shape Negri bodies (Menager *et al*., 2009). Intracellular rabies virus replication generates different necrotic and degenerative pathological changes as described in these results (Fu and Jackson, 2005). Resultantly, these necrotic neurons produce nitric oxide (N_2O) and extensive amount of chemokine and, together both increase blood brain barrier (BBB) permeability in brain that may dilate capillary network (Donald and Oliver, 2001). Apoptosis and other pathogenic infections could also be responsible for necrosis and dead cells in rabid brain tissue (Fazakerley *et al*., 2001).

Recently, a large number of dog bite cases have been reported in Pakistan with highest number reported from large cities; Karachi (59.7%), Peshawar (13.1%) and Hyderabad (11.4%). Such a huge number of dog bites are the principle source in producing spontaneous, scattered and unrepeatable human and animal deaths in different regions of Pakistan (Perviz *et al*., 2004; Numan *et al*., 2011; Afzaal *et al*., 2013). In these areas, sheep brain vaccine (SBV) is still being used against rabies PEP but unfortunately, it is ineffective and induces lower immune response against rabies. Likewise, huge number of animal bite cases (n=150,068) was seen earlier in Dhaka (Bangladesh) with the highest percentages in January, April and December as seen in present study (Hossain *et al*., 2011). In the same way, 96% of human cases were victimized by stray dogs of rural and urban areas of India in a total number of 1112 animal bites (Shah *et al*., 2012). During 2011 in China, dogs and cats accounted for 95% and 4% of human bite cases respectively. Consequently, 77% people were unaware about dog bite management whereas, 91% were failed to get timely vaccination. Most number of bites to humans (51%) was reported from five southern provinces (Guangxi, Hunan, Gunagdong, Guizhou and Yunnan) of China that possess relatively hot climate similar to present study area (Yin *et al*., 2013). Higher number of rabies infected cases (38 to 53 per million per year) was associated with the dense population and hot weather of Karachi (Zaidi *et al*., 2013). Pakistan, Burma, Bangladesh, Peru and Argentina have still not shifted itself to follow the WHO recommended cell culture vaccine and thus, fight against rabies remains static since the emergence of rabies in Pakistan (Burki. 2008). The above mentioned research studies are strongly in accordance with present findings and validate that stray dogs are the principal reason of rabies infection (96% cases) in Asian countries especially Pakistan, India and China (Sudarshan *et al*., 2007; Zaidi *et al*., 2013).

The different rabies incidences in central Asia are much probably due to more stray dogs roving in housing areas, hot and dry weather, unvaccinated wild and domestic animals and illiteracy rate among people of Pakistan. The reasons for continued human deaths due to rabies are negligence by the people in following and practicing complete vaccination schedule. Additionally, practice among general medical practitioners and attitude towards rabies is considerably poor. The current literacy rate of Pakistan, Bangladesh and India is round about 55%, 57% and 71% respectively but, rabies in India and elsewhere is concentrated among the poor people of rural areas (Burki. 2008; Zaidi *et al*., 2013).

It is strongly recommended that health authorities and disease surveillance teams should monitor the occasional outbreaks within wild and domestic animals at veterinary hospitals to avoid future cases. The hospitals must maintain detail clinical record of dog bite patients that could help health officials to envisage future control strategies, which in turn help us to eradicate unexpected rabies deaths. Moreover, people and health workers must be educated in general about rabies virus control and prevention.

Acknowledgements: We are extremely thankful to Dr. Bilal Asghar and the district government of the city for their cooperation during the period of sampling. The work was supported by the Grants No. 31472208 and 31272579 from the National Natural Science Foundation of China.

REFERENCES

Afzaal, S., M. Numan, Z.A. Qureshi, M. Shaukat, I.A. Khan, Z. Hussain, Z. Abidin, A.W. Manzoor and M. Habib (2013). Ante-Mortem Diagnosis of Rabies in Cows and Buffaloes. J. Adv. Vet. Res. 3: 27-30.

Antona, D'd and contributors. 1954. Laboratory Techniques in Rabies. Monographic Series (World Health Organization, No. 23). 1-143

Bancroft, J.D. and M. Gamble (2008). Theory and Practice of Histological Techniques. 6th Ed., USA: Elsevier: Churchill Livingstone.

Bernardi, F., A.A. Barros and F.H. Gomes (2006). Biological and immunological studies of five Brazilian rabies virus isolates. Braz. J. Vet. Res. Anim. Sci. 42(4): 307-312.

Blanton, J.D., D. Palmer and C.E. Rupprecht (2009). Rabies surveillance in the United States during 2009. J. Am. Vet. Med. Assoc. 237(6): 646-657.

Brookes, S.M., R. Klopfleisch, T. Muller, D.M. Healy, J.P. Teifke, E. Lange, J. Kliemt, N. Johnson, L. Johnson, V. Kaden, A. Vos and A.R. Fooks (2007). Susceptibility of sheep to European bat lyssavirus type-1 and -2 infection: A clinical pathogenesis study. Vet. Microbiol. 125: 210–223.

Burki. (2008). The global fight against rabies. The Lancet. 372 (9644): 1135-1136.

Camila, C.A., A.N. Priscilla, I.N. Clayton, P.M. Leonardo, F.R.L. Priscilla, W. Faldemir, M.S. Varaschin and S.B.J. Pedro (2014). Histopathology and immune histochemistry of tissue outside central nervous system in bovine rabies. J. Neurovirol. 20: 388-397.

Donald, W.L and J.A. Oliver (2001). Insights into shock: The pathogensis of vasodilatory shock. New. Eng. J. Med. 345(8): 588-595.

Faizee, N., N.Q. Hailat, M.M.K. Ababneh, W.M. Hananeh and A. Muhaidat (2011). Pathological, Immunological and Molecular Diagnosis of Rabies in Clinically Suspected Animals of Different Species Using Four Detection Techniques in Jordan. Blackwell Verlag GmbH. Transb. Emerg. Dis. 1865: 1-11.

Fazakerley, J.K., T.E. Allsopp and F.F. Zhen (2001). Programmed cell death in virus infections of the nervous system. Curr. Top. Microbiol. Immunol. 253: 95-119.

Fu, Z.F. and A.C. Jackson (2005). Neuronal dysfunction and death in rabies virus infection. J. Neuro. Virol. 11: 101-106.

Hamir, A.N., G. Moser and C.E. Rupprecht (1992). Morphologic and immunoperoxidase study of neurologic lesions in naturally acquired rabies of raccoons. J. Vet. Diag. Invest. 4: 369-373.

Hossain, M., B. Tania,K. Ahmed, Z. Ahmed, M. Salimuzzaman, M.S. Haque, A. Ali, S. Hossain, K. Yamada, K. Moji and A. Nishizono (2011). Five-year (January 2004–December 2008) surveillance on animal bite and rabies vaccine utilization in the Infectious Disease Hospital, Dhaka, Bangladesh. Vaccine. 29: 1036-1040.

Hsu, Y.H., L.S. Wang, L.K. Chen, J.J. Lee and H.H. Yang (2005). Rabies Virus Infection: Report of an Autopsy Case with Comprehensive Pathologic, Immuno fluorescent, Immuno histochemical and Molecular Studies. Tzu. Chi. Med. 17(4): 219-225.

Jackson, A.C., (2010). Rabies pathogenesis update.Rev. Pan-Amaz. Saude. 1(1): 167-172.

Jackson, A.C., (2011). Research and Reports in Tropical Medicine. Res. Rep. Trop. Med. 2: 31-43.

Jamadagni, S.B. and C.K. Singh (2006).Studies on prevalence and pathology of rabies in Punjab. Vet. Path. 10: 534-549.

Jamadagni, S.B., C.K. Singh and B.S. Sandh (2007). Histopathological alterations in brains of rabies infected buffaloes and cattle. Int. J. Anim. Sci. 6: 872-874.

Jogai, S., B. D. Radotra and A.K. Banerjee (2000). Immuno histochemical studies of human rabies. Neuropath. 20: 197-203.

Johnson, N., A. Nunez, D.A. Marston, G. Harkess, K. Voller, T. Goddard, D. Hicks, L.M. McElhinney and A.R. Fooks (2011). Investigation of an Imported Case of Rabies in a Juvenile Dog with Atypical Presentation. Animal.13: 402-413.

Knobel, D.L., S. Cleaveland, P.G. Coleman, E.M. Fevre, M.I. Meltzer, M.E.G. Miranda, A. Shaw, J. Zinsstagand and F.X. Meslin (2005). Reevaluating the burden of rabies in Africa and Asia. Bull. WHO. 83: 360–368.

Lembo, T., M. Niezgoda, A.V. Villa, S. Cleaveland, E. Ernest and C.E. Rupprecht (2006). Evaluation of a direct, rapid immune histochemical test for rabies diagnosis. Emer. Infect. Dis.12: 310-313.

Manickam, B., I.S. Sajitha, R. lakshmi and N.D. Nasir (2009). Prevalence, Gross and Histopathological Study of Brain Disorders in Cattle-Kerala State, India. Int. J. Trop. Med. 4: 9-20.

Menager, P., P. Roux, F. Megret, J. Bourgeois, A.L. Sourd, A. Danckaert, M. Lafage, C. Prehaud and M. Lafon (2009). Toll-Like Receptor 3 (TLR3) Plays a Major Role in the Formation of Rabies Virus Negri Bodies. Plos Path. 5: 1-15.

Numan, M., Z.A. Qureshi, M. Shauket, H.A. Hashmi, M. Iqbal, Z.J. Gill, M. Habib and M. Siddique (2011). Rabies out-break in mules at Mansehra, Pakistan. Res. Vet. Sci. 90: 160-162.

Parviz, S., R. Chotani, J. McCormick, S.F. Hoch and S. Luby (2004). Rabies deaths in Pakistan: results of ineffective post-exposure treatment. Int. J. Inf. Dis. 8: 346-352

Shah, V., D.V. Bala, J. Thakker, A. Dalal, U. Shah, S. Chauhan and K. Govani (2012). Epidemiological determinants of animal bite cases attending the anti- rabies clinic at VS General Hospital, Ahmedabad. Heal. Line. 3: 66-68.

Stein, L.T., R.R. Rech, L. Harrison and C.C. Brown (2010). Immunohistochemical study of rabies virus within central nervous system of domestic and wild life species. J. Vet. Patho. 47: 630-636.

Sudarshan, M.K., S.N. Madhusudana, B.J. Mahendra, N.S.N. Rao, D.H.A. Narayana, S. Abdul Rahman, F.X. Meslin, D. Lobo, K. Ravikumar and Gangaboraiah (2007). Assessing the burden of human rabies in India: results of a national multi-center epidemiological survey. Int. J. Inf. Dis. 11: 29-35.

Tenzin, D.N., K.J. Dorjeeand and M.P. Ward (2010). Reemergence of rabies in dogs and other domestic animals in eastern Bhutan. Epid. Infect.139: 220-225.

Wasay, M., A.K. Ismail and S. Naseem (2008).Tetanus and rabies eradication in Pakistan; a mission not impossible. J. Pakistan Med. Ass. 58(4): 158-159.

Woldehiwet, Z. (2005). Clinical laboratory advances in the detection of rabies virus. Clin. Chim. Act. 351: 49–63.

Yin, W., J. Dong, C. Tu, J. Edwards, F. Guo, H. Zhou, H. Yu and S. Vong (2013). Challenges and needs for China to eliminate rabies. Infect. Dis. Pov. 2(23): 1-10.

Zaidi, S.M.A., A.B. Labrique, S. Khowaja, I. Lotia-Farrukh and J. Irani (2013). Geographic Variation in Access to Dog-Bite Care in Pakistan and Risk of Dog-Bite Exposure in Karachi: Prospective Surveillance Using a Low-Cost Mobile Phone System. PLos. Negl. Trop. Dis. 7 (12): 1-13.