Research Paper

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Rabies – The Incurable Wound of The Century

Veterinary Science

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Divya C

MVSc Pathology, Veterinary Officer, TRCMPU Ltd, P&I office, Mannar, Alappuzha, Kerala - 689622

ABSTRACT

Rabies is one of the most important zoonotic diseases causing significant health hazards in almost all warm blooded animals. The disease is caused by an RNA virus which causes disease in the brain and result in death. Rabies is present in the animal populations of almost every country in the world. Thousands of human deaths occur annually from rabies worldwide. Rabies is unique compared to other infections in that the development of disease following exposure to the virus is preventable even in patients that have not been previously vaccinated, through timely administration of post-exposure prophylaxis. Though symptoms can be considered as a factor suggestive of rabies, the disease has to be confirmed by reliable laboratorytests. Direct Fluorescent Antibody Test on impression smears from brainproves to be the gold standard technique for rabies diagnosis because of its sensitivity, accuracy and speed; and dFAT is established as a reliable diagnostic test.

INTRODUCTION

Rabies, derived from the Latin word meaning "madness", is one of the most important zoonotic diseases causing significant health hazards. It is an irredeemable deadly viral infection affecting almost all warm blooded animals.Rabies is caused by a bullet shaped RNA virus belonging to family Rhabdoviridae and genus Lyssa virus.The disease is most often transmitted through close contact with saliva of a rabid animal. There exist noeffective curative measures once the clinical symptoms are manifested.

HISTORY OF RABIES

Rabies was recognized throughout the world as one of the earliest disease of mankind and the human implications of the disease were found in early Egyptian hieroglyphics and in the writings of Asclepiadae, Democritus, Aristotle and others. The origin of rabies virus appears to be in Africa; the disease being first described in the Sumerian law code from the city of Eschunna in Mesopotamia dating about 1885 BC. Rabies was first described in dogs by the ancient philosopher Democritus (500 BC) and in humans by Hippocrates during 400BC.

CURRENT SCENARIO AND INCIDENCE OF RABIES

Rabies is present in the animal populations of almost every country in the world, except Australia and New Zealand. In some countries, such as those in western Europe and Oceania, rabies is considered to be prevalent among bat populations only. The major foci of rabies in the world towards the end oflast millionaire was observed to be the Indian subcontinent, south east Asia and most parts of Africa (Plotkin, 2000). India has the highest rate of human rabies in the world, primarily because of the increasing stray dog population in the country. Twenty thousand people are estimated to die every year from rabies in India. In India, the virus is primarily transmitted through canines. In India, rabies was endemic except in the Lakshwadeep, Andaman & Nicobar islands, which were found free of the disease (Saseendranath, 1996).

Rabies is a highly fatal disease with varying incubation period ranging from few days to years. The public health impact of rabies has been underestimated (Coleman *et al.,* 2004). In Asia, the disease is found to be endemic in dogs and wild life and numerous cases occur each year in humans. According to the estimates of World Health Organisation (WHO), 55000 human deaths occur annually from rabies worldwide, with about 31,000 in Asia and 24000 in Africa.This is a modest estimate and the actual incidence of human rabies may be 100 times greater than what is officially recorded. (Knobel *et al.,* 2005). Hence a more accurate estimation of rabies incidence is probably a necessity.

DIAGNOSIS OF RABIES

Animals with changes in behaviour and various neurological signs should always be suspected of being rabid.Though clinical symptoms can be considered as a factor suggestive of rabies, the disease has to be confirmed by reliable laboratory tests. A confirmatory laboratory test should be accurate, fast and economical. The confirmatory diagnosis of rabies is usually done by the examination of brain on necropsy. The various diagnostic methods currently employed for this purpose include histologic examination of brain tissue, DME to detect Negri bodies, dFAT, RRIT, MIT, ELISA, PCR and IHC on histologic sections of brain. All activities related to the handling of animals and samples for rabies diagnosis were to be performed following the appropriate standard guidelines and practices to avoid direct contact with potentially infectious fluids or tissues.

CLINICAL PATHOLOGY

Typically there is no significant clinical pathology findings associated with rabies. However, the cerebral spinal fluid may contain increased protein levels, monocytes, and neutrophils.

NECROPSY LESIONS

Gross lesions: According to Burkhart *et al.,* 1950, there are nospecific gross lesions in brain evident at necropsy. Therefore, it is important to remove the brain and perform diagnostic testing to make a diagnosis.

Histopathology:

Histological examination of rabid brain is characterized by non-suppurative encephalitis, brain edema, meningeal congestion and focal areas of hemorrhage. Perivascular mononuclear cell infiltration, glial cell proliferation, neuronal degeneration, neuronophagia and demyelination has been frequently reported as histopathological changes in the cerebral and cerebellar cortices, pons, spinal cord and Gasserian ganglion by various scientists (Burkhart *et al*., 1950; Field, 1951; Johnson, 1965; Jackson and Park, 1998). The presence of acidophilic intracytoplasmic inclusions called Negri bodies, found prominently in the Purkinje cells of the cerebellum and the pyramidal cells of the hippocampus, whichis virtually pathognomonic for the disease is the most remarkable histopathologic alteration associated with the disease

LABORATORY TESTS FOR DIAGNOSIS OF RABIES

Direct Microscopic Examination (DME) / Seller's staining

Confirmatory diagnosis of rabies based on the presence of Negri body has a long history to go through. Since it is easy, rapid and economical to perform, the Direct Microscopic Examination - DME (Seller's staining to detect Negri bodies) remains the standard screening test for rabies in most of the laboratories in India. Impression smear collected from hip-

pocampus in small animals and from cerebellum in ruminants forms the specimen for rapid Seller's staining. Examination of stained, air-dried smear reveals the presence of acidophilic intracytoplasmic inclusion bodies called Negri bodies in positive cases. There is great variation in the size and shape of Negri bodies. They are found most frequently in the pyramidal cells of Ammon's horn of hippocampus, and the Purkinje cells of the cerebellum.A major limitation of the DME is its low sensitivity, which is reported to range from only 75% to 85% (Miranda and Robles, 1991). According to Jogai *et al.* (2000), Negri bodies in stained preparations can be reliably detected only in 50%–80% of infected animals. Because of this, the absence of Negri bodies should not be considered as confirmatory negative in diagnosis of rabies.

Rapid Rabies Immunochromatographic Test (RRIT)

RRIT is done using commercially available kits. The test is capable of detecting rabies within 5 to 10 minutes. Absence of characteristic line in rabies antigen test card doesn't ensure negativity of the sample for rabies.

Direct Fluorescent Antibody Test

Direct Fluorescent Antibody Test (dFAT), recommended by World Health Organisation (WHO), is employed as the gold standard technique for rabies diagnosis because of its sensitivity, accuracy and speed; and FAT is proven to be a completely reliable and highly sensitive test for diagnosis of rabies. dFAT is performed on cold acetone fixedimpression smear of brainto which lyophilized adsorbed antirabiesnucleocapsid fluorescein isothiocyanate conjugate is added as antibody to react with rabies virus antigen. Presence of 'apple green fluorescence' indicate positive test result. Combining the speed of histological techniques and the accuracy of experimental inoculation, the fluorescent antibody technique showed itself as a promise of being of great help in deciding early, whether or not exposed persons should be administered a post-exposure anti-rabies treatment(Goldwasser and Kissling,1958).

Enzyme-Linked ImmunoSorbent Assay (ELISA)

ELISA tests are commonly used to confirm a positive diagnosis of rabies, since they are highly sensitive.Commercial kits available to perform the test uses immunoglobulin G antirabies virus glycoprotein from human and animal serum and plasma to detect the rabies virus. A history is crucial to diagnosis, because a vaccinated animal will also have a titer.ELISA offers the benefit of sensitivity when applied to poorly preserved specimens and manual or automated reading, which made it well suited for use in field conditions, but sensitivity on well-preserved specimens might be low in comparison with other methods (Franka *et al.,* 2004).

Direct Rapid Immunohistochemical Test (DRIT)

A direct rapid immunohistochemical test, employing a short formalin fixation of fresh or glycerol-preserved brain impressions and requiring no specialized equipment, had been demonstrated to be of utility for testing conducted under field conditions and for countries with limited diagnostic resources (Lembo *et al.,* **2006). IHC is done on 5µ thick sections of brain using polyclonal anti-rabies antibody.** It is not as sensitive as other tests available.

Mouse Inoculation Test (MIT)

The mouse inoculation test (MIT) was developed as a tool for confirmatory diagnosis of rabies by Webster and Dawson in 1935 and proved as a sensitive and reliable procedure by BourhyandSureauin 1991. MIT involves intra-cerebral inoculation of the suspected material into weanling laboratory mice and observing for signs of rabies for 28-30 days. Mice typically die at day 9. Brains are harvested from the mice when they die, and rabies is confirmed using dFAT. MIT is not used much anymore, due to SPF mice being expensive and the time taken for diagnosis. Also differentiating this disease from other neurological disorders may require extensive investigation.

Polymerase Chain Reaction (PCR)

PCR is not used much in laboratory diagnostics. It is used exclusively for epidemiology studies.

CONCLUSION

Rabies is unique compared to other infections in that the development of clinical disease following exposure to the virus is preventable even in patients that have not been previously vaccinated, through timely administration of post-exposure prophylaxis. This rationalizes the need for prompt diagnosis of rabies in animals especially those which are in close contact with humans.

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