Rabies: diagnostic challenges and immunohistochemistry as an alternative candidate

Rabies is a neuroparalytic viral disease of animal species. The disease is popularly known as hydrophobia in humans. It is probably the oldest known zoonotic disease recognized for more than 5000 years. The disease is transmitted to humans and animals mainly by bite of infected dog (rabid dog) and human deaths due to rabies in Bangladesh is estimated 2500 a year, although the exact figure might be far higher, and no data is available on death of animal species from rabies. There is no known test which can detect rabies infection during the incubation period (the time course required for development of disease symptoms from the point of bite), which is generally 1 to 8 weeks 1. Rabies diagnosis could be done, with 100% certainty, only through postmortem examination of central nervous system or trigeminal ganglia. Until today, there is no effective therapy and exposed individual must die if left untreated. Despite high fatality, rabies could be prevented with 100% certainty by post-exposure vaccination. This means that a single step, the accurate and rapid diagnosis of rabies infection, can impact significantly on the reduction of rabies related death every year. It is important to note that rabies diagnosis in animals needs to be rapid and accurate to facilitate post-exposure treatment decisions for exposed humans. This article focuses on different aspects of rabies diagnosis in animal species.

Histologically, rabies is characterized by a viral encephalitis with intracytoplasmic accumulation of viral particles, called Negri bodies/inclusion bodies, in neuron but these changes can be mild or even absent. Although the presence of Negri bodies are considered confirmatory for rabies, they are absent in 20% to 60% of the cases 2. Additionally, the encephalitis of rabies can be challenging to distinguish from other viral encephalitides, and hence the diagnosis of rabies require ancillary tests such as direct fluorescent antibody test (FAT) and intracerebral inoculation in suckling mice, the standard tests for rabies today. Despite its efficiency in quickly detecting rabies, FAT has many drawbacks that can limit its usefulness, including equipment cost, potential exposure of laboratory staff to live viruses, fading nature of fluorochrome dyes, and necessity of getting fresh specimens to potentially distant laboratories in adequate condition for testing. Immunohistochemistry (IHC) using polyclonal antibody (commercial preparation available) can be used for detection of rabies. The sensitivity of IHC is equal to that of FAT and even more sensitive in early diagnosis of suspected cases when traditional histologic and FAT techniques can not detect viral antigens or lesions 3. The advantages of using polyclonal antibody over monoclonal antibody is that it detects a wide range of rabies viral strains compared to reactivity of monoclonal antibody on conformational epitopes.

Rabies does not always infect all regions of the brain equally and viral dissemination varies among species and the erratic distribution of viral antigen can compromise the reliability of test results if all parts of the brain are not sampled or if a particular sample has low levels of viral antigen. Therefore, optimal sampling is essential for optimizing rabies diagnosis. Immunohistochemical procedures demonstrate that, the signal for rabies antigens is observed almost exclusively in gray matter and characterized by sparse to diffuse granularity throughout the perikaryon of neurons and their axonal and dendritic processes (Figure 1-2). In cats and dogs most intense signal is observed in the hippocampus followed by the cerebrum, brainstem, and cerebellum in descending order of immunoreactivity. In cattle, the most intense signal is seen in the brainstem nuclei followed by the cerebellum, especially in the Purkinje cells. In horses, the cervical spinal cord exhibit the strongest signal followed by the brainstem with sparing or even absence in sections of the hippocampus, cerebellum and cerebrum. Compared to other parts, signal is stronger in brainstem in pig, cerebral cortex followed by the brainstem in fox, and brainstem and cerebrum in deer. Because clinical manifestations of rabies in many species are unpredictable, the optimal rabies diagnostic test must detect viral antigen in any mammalian species.
As mentioned earlier, although FAT is the standard technique for quick rabies diagnosis, it requires the use of expensive ultraviolet light microscopes, which is difficult to maintain, and fresh samples, which contain live virus and entail public health risk. In our country, only a few laboratories, mainly located in Dhaka, have FAT facility, and hence transporting fresh samples from remote area is a problem. Additionally, lack of proper refrigeration and high ambient temperatures ensue autolysis of brain sample and interfere with the FAT hindering diagnostic accuracy. By contrast, in the formalin fixed specimens used in IHC, the rabies virus is rapidly inactivated by formaldehyde, making the transport and laboratory processing of specimens much safer. Recently, a new technique, known as direct immunohistochemical test (dRIT), has been evaluated under field and laboratory conditions to detect rabies virus antigen in glycerol preserved, field collected brain samples. This technique uses touch imprint brain samples that are further fixed in formalin with application of a monoclonal antibody. Diagnosis using the dRIT technique is rapid, with high sensitivity and specificity. However, if the rabies test is negative with dRIT, the touch imprint specimen is not suitable for additional diagnostic testing. In contrast, a major advantage of IHC is that the pathologic changes in the brain are clearly observable. Formalin fixation of brain preserves the tissue architecture and allows histologic evaluation to formulate a differential diagnosis.

Figure. (1) Cerebellum; bovine. Several brown, circular, strongly positive inclusion bodies for rabies viral antigen are within the cytoplasm of Purkinje cells. The dendrites of many Purkinje cells are also strongly positive. The granular cell layer and the basket cells of the molecular layer have widespread fine staining. IHC. Hematoxylin counterstain. (2) Hippocampus; dog. Distinct labeling for rabies virus antigen within the perikaryon of several neurons. IHC. Hematoxylin counterstain.


Certain parts of the brain have historically been considered reliable for detecting Negri bodies and are routinely sent for FAT to confirm the diagnosis of rabies. These parts include the hippocampus in carnivores and the cerebellum in herbivores. The initial diagnostic focus on hippocampus can attribute to its high frequency of large antigen aggregates or inclusion bodies, which are easily identified by traditional histological stains. The accuracy of laboratory diagnosis becomes inconsistent when based solely on the presence of Negri bodies, because Negri bodies are found only in 40-80% of rabies cases. Along with such variable and inconsistent Negri body formation, some hematoxylin and eosin stained inclusions in the cytoplasm of neurons resemble Negri bodies but might not be rabies specific. Such pseudo Negri bodies are protein related inclusions and can lead to an initial false positive diagnosis of rabies. These cytoplasmic inclusion bodies have been described in the neurons of nonrabid cats, cattle, mongoose, woodchucks, and skunks. For these reasons, rabies diagnosis should not be based solely on the presence of Negri bodies in the hippocampus, especially in cases without inflammation, and IHC or FAT should be applied to rabies suspect cases to confirm the presence of viral antigen. As the distribution of rabies virus antigen in the brain varies among species, so sampling site in some species might be important in diagnosing rabies. However, although collection of the whole brain is preferable and thalamus is recommended for area of choice for testing when composite sampling is not possible, specific parts of the brain should be chosen for preliminary IHC. Knowledge of the optimal area of the brain for detection of rabies viral antigen will enhance the quality and efficiency of the diagnosis.

One important issue concerning transmission of rabies is that whether bite of cat can cause rabies or not. Studies have shown that unvaccinated cat may contract rabies from wildlife and be the source of infection for humans.
In summary, IHC for rabies detection using targeted sections of brain could enhance accurate diagnosis in various species. The public health implications of this disease warrant continued efforts to develop more accurate sampling and testing modalities. The IHC provides an alternative to FAT and can be used safely.

References

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