Recent Developments in the Diagnosis of Rabies in Humans and Animals

M. Pal, A. Hailu, R.K. Agarwal*1 and P. Dave2

Department of Microbiology, Immunology and Public Health, College of Veterinary Medicine and Agriculture, P.O. Box No.34, Debre Zeit, Ethiopia

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ABSTRACT

Zoonotic diseases constitute an important part of the global health programme of the World Health Organization. Rabies is a major viral anthropozoonosis, which infects humans, domestic and wild animals. The disease is reported from many countries of the world including Ethiopia and India. It is estimated that rabies is responsible for nearly 55,000 deaths mostly in children every year, and about 10 million people annually receive post-exposure treatments after being exposed to rabies suspected animals. Dog is the principal transmitter of rabies to humans as well as other animals, with 99 per cent of human rabies cases attributed to dog bite. The help of laboratory is imperative to confirm an unequivocal diagnosis of rabies. In recent years, new advances in the laboratory diagnosis of rabies have been reported. All the new techniques require validation before being routinely employed by public health laboratory for the diagnosis of rabies. Facility for rapid diagnosis, availability of vaccine, elimination of stray dogs, and compulsory vaccination of pet dogs, active surveillance and public awareness will certainly reduce the incidence of this dreaded disease. Further research on etiopathogenesis, aberrant course of disease and development of cheap, safe and potent inactivated cell culture vaccine is recommended.

Keywords: Anthropozoonosis, diagnosis, dog, rabies, vaccination

Introduction

Rabies is a highly fatal neurotropic viral disease of all warm blooded animals including man, caused by a virus of the genus Lyssavirus of family Rhabdoviridae (Pal, 1991; Deressa et al., 2011, Gizachew et al., 2012). The bullet shaped enveloped virus possesses a single stranded, negative sense RNA genome of 12 kbp which codes for five viral proteins namely, nucleoprotein (N), phosphoprotein (P), matrix protein (M), glycoprotein (G) and RNA-dependent RNA polymerase or large protein (L). Presently 12 species of lyssavirus are recognized which are further subdivided into two Phylogroups on the basis of the genetic distances and serological cross-reactivity (WHO, 2013). The Phylogroup I comprises of the Rabies virus, European bat lyssaviruses type 1 and type 2, Duvenhage virus, Australian bat lyssavirus, Aravan virus, Khujand virus and Irkut virus, while the Lagos bat virus, Mokola virus and Shimoni bat virus make up the Phylogroup II. The disease is mainly transmitted from rabies affected animal to man through close contact with infected saliva via bites or scratches and invariably results in death (Pal, 2007, Deressa et al., 2011).

Despite the existence of effective and economical preventive and control strategies (Bogel and Mesline, 1990, Cleaveland et al., 2003), rabies infection in humans still remains as major public health problem resulting in loss of more than 55,000 lives worldwide each year (Chakrabarti et al., 2006). The annual human deaths attributed to rabies in 2010 were estimated to be 26,400 - 61,000 (WHO, 2013), representing an estimated 1.9 million DALYs. The estimated annual cost of rabies was US$ 6 billion,
with almost US$ 2 billion (~40%) due to lost productivity after premature deaths and US$ 1.6 billion spent directly on post-exposure prophylaxis. These figures are not believed to represent the true burden of the disease due to under reporting. It is important to mention that Asia and Africa carry a larger part of the public health burden of rabies with an estimated 30,000 and 23,700 deaths, respectively, in 2003 (Knobel et al., 2005). India with its large stray dog population is reported to have the highest incidence of rabies, with 20,565 deaths, as per a multi-centric study done in 2003 (Sudarshan et al., 2007).

In order to accomplish rabies control, the early identification of the disease is imperative (Barrat et al., 2006). Therefore, highly specific and rapid diagnostic tests are essential for use in epidemiological studies and disease control. Moreover, early diagnosis aids in the timely administration of post-exposure prophylaxis, decreases the treatment cost and reduces the possibility of disease transmission to the people in contact with the patient. Rabies diagnosis cannot be solely made based on history of the case. Therefore, reliable laboratory tests are required to reveal the presence of infection. Since the virus is neurotropic by nature, the brain tissue is the most frequently used and preferred specimen for post-mortem diagnosis both in animals and humans, although the virus has been described to be present in a variety of body secretions and excretions, including in saliva of apparently healthy dogs (Fekadu, 1972, Deressa et al., 2011). Among the existing laboratory diagnostic techniques, fluorescent antibody test (FAT) is the most reputed test overseas including Ethiopia, due to its high sensitivity (Deressa et al., 2011, Gizachew et al., 2012). However, the financial cost is unaffordable to developing world where the majority of rabies cases persist. Hence, there is a need for cheap and reliable diagnostic tests. Time and again scientists have come up with rapid and reliable diagnostics for rabies. Therefore, the objective of this paper is to present an overview on the recent rabies diagnostic techniques available for humans and animals.

Rabies diagnosis in humans and animals

Sample collection and transport: The poor prognosis linked with rabies warrants for use of strict safety measures while handling the virus or other infectious material. The laboratory should have Biosafety Level II facility and the workers should wear protective gear besides having pre-exposure vaccination. However, certain procedures like bulk virus culture and those that may generate aerosol require class III facilities.

Due to the neurotropic nature of the disease, brain tissue samples are frequently used in rabies laboratory diagnosis using different techniques (OIE, 2011). A pool of brain tissues that includes the thalamus, pons, medulla, cerebellum and hippocampus are recommended for testing (Bingham and Van Der Merwe, 2002). In case of field settings the sample can be collected via the trans-orbital or trans-foramen magnum route (Barrat et al., 2006). The concept to use samples other than brain such as tears (Elmgren et al., 2002), corneal smears (Zaidman and Billingley, 1998), saliva, salivary gland (Goldwasser et al., 1959), cerebrospinal fluid, nuchal skin biopsy (Blenden et al., 1983), urine (Thiravat, 2002) and serum (Delia et al., 1983) aid in early detection of the virus.

The diagnostic materials should be frozen or preserved in 50% glycerol saline at ambient temperature. The samples stored in glycerol should be thoroughly washed before processing. Formalin fixation of brain tissue should be avoided. Wet tissue specimens should be transferred from formalin to absolute ethanol for molecular diagnosis and antigen detection.

Conventional techniques

Fluorescent antibody test (FAT): The direct fluorescent antibody detection test is the most widely used test for diagnosis of rabies. The test is considered the gold standard by the WHO and OIE. It is highly sensitive, specific, and relatively quick (Beauregard et al., 1965). The test involves the incubation of impression or smears of brain with fluorescein isothiocyanate labeled anti-rabies polyclonal globulin or monoclonal antibody and observing under fluorescent microscope. The test is
completed in just 2 h and is preferably done on fresh samples, with the sensitivity decreasing in case of autolysed samples. Additionally, for early detection of the disease, skin biopsy from neck region where hair follicles contain peripheral nerve endings can be subjected to FAT (Crepin et al., 1998). FAT has a sensitivity of 90-100%, and a 99% agreement with the mouse inoculation test. However, the sensitivity is highly dependent on various factors like examiners expertise, quality of anti-rabies conjugate and equipments like fluorescent microscope (Tepsumethanon et al., 1997, OIE, 2011, Dan, 2012). Additionally, the test requires expensive reagents and instruments.

**Histological test**

This technique is used to determine the presence of Negri bodies, the aggregates of viral protein in brain sections or impression smears by light microscopy. The intracytoplasmic acidophilic inclusion bodies appear pink or red following staining with HE and Mann’s stain, respectively. The Negri bodies are demonstrated in 10-65% of the positive cases of rabies (Sellers, 1927). They are mostly demonstrated in the hippocampus, the pyramidal cells of the cerebral cortex and Purkinje’s cells of the cerebellum (Meslin et al., 1996). However, the technique is no longer recommended due to its low sensitivity.

**Virus replication detection test**

This technique detect the infectivity of a virus containing tissue suspension in cell culture using cell culture inoculation test (CCIT) and in laboratory animals using mouse inoculation test (MIT). The murine neuroblastoma cells (NA-C 1300) are the most susceptible to rabies virus and are comparable to animal inoculation assay. CCIT is less expensive, give more rapid results, from 10-21 days with MIT to 1-2 days and avoid the use of animals (Rudd and Trimachi, 1991). The need for qualified expert and the variable preference of rabies virus (RV) variants to cell lines are drawbacks. MIT is practicable where skills and facilities are not available.

**Enzyme-linked immunosorbent assay (ELISA)**: The technique is applied to detect and quantify rabies antigen. It evaluates large number of samples simultaneously. The test is sensitive, specific and rapid for the post-mortem diagnosis of rabies in animals and humans (Perrin and Sureau, 1987). However, in cases that involves human, requires confirmatory tests (OIE, 2011).

**Anti-rabies antibody detection test**: This test aids in the diagnosis of rabies through an indirect measurement of rabies neutralizing antibody in cases where there is no history of previous vaccination. However, it possess poor sensitivity and specificity, and is cumbersome to interpret test results (OIE, 2011), also it is not suitable for early detection as antibodies only appear after 8 days of infection.

**Recent techniques**

**Antigen detection test**

**Direct rapid immunohistochemical test (dRIT)**: The dRIT detects rabies virus in brain sample using different immunoperoxidase techniques. The test uses highly purified biotinylated monoclonal antibodies against the viral nucleoprotein for staining of fresh brain impression within 1 h. It is swift, sensitive and as specific as FAT, and recognizes all genotypes of lyssaviruses (Durr et al., 2008, Tao et al., 2008). Moreover, it is applicable under field condition (Lembo et al., 2006; Niezgoda and Rupprecht, 2006).

**Rapid Rabies Enzyme Immuno Diagnosis (RREID)**: The ELISA test uses brain supernatant and salivary gland suspension for the detection of rabies nucleocapsid antigen. The test is rapid and has a 96-99% correlation with FAT (Jayakumar et al., 1994).

**Dot blot enzyme immunoassay**: It is developed to detect the rabies antigen in brain specimens of animals and humans mainly using biotinylated antinucleoprotein antibody and streptavidin peroxidase. It is described as sensitive, specific and rapid test for the post-mortem diagnosis of rabies in animals and humans (Madhusudana et al., 2004). The test is rapid, simple, does not require sophisticated equipment and trained person and is ideal for rabies antigen detection in postmortem brain samples in geographically remote areas where FAT facility is not available.
**Rapid latex agglutination test**: Latex agglutination test evaluates the presence of rabies antigen in saliva of animals. The sample used is advantageous over brain sample as it is resistant to decomposition, despite its limitations. Moreover, it is simple, safe, inexpensive, rapid technique and showed good specificity and sensitivity as compared to the fluorescent antibody test (FAT) on brain smear (Kasempimolporn et al., 2000).

**Rabies immunochromatographic diagnostic test (RIDC)**: Rapid immunodiagnostic test is a simple lateral flow test using rapid immunodiagnostic test kit where detector antibodies are attached (Kang et al., 2007). It is known for its merit over other tests for its rapidity, simplicity, and diversity of sample and needless for qualified technicians (Zhang et al., 2009). Additionally, it is sensitive and specific (Kang et al., 2007; Kasempimolporn et al., 2011) although careful interpretation and additional tests in negative results are required.

**Nucleic acid detection test**

**Reverse-transcriptase polymerase chain reaction (RT-PCR)**: Various workers have used RT-PCR targeting the conserved nucleoprotein gene for the diagnosis of rabies virus nucleic acid in ante-mortem (CSF, saliva) and post-mortem specimen (brain) and for characterization of the virus (Tordo et al., 1996; Crepin et al., 1998; David et al., 2002; Dan, 2012). It detects the rabies virus earlier as compared to FAT (Biswal et al., 2012) and appears sensitive and specific in degraded samples (Araujo et al., 2008). However, there is risk of cross contamination (Belak and Thoren, 2001). RT-PCR and enzyme-linked immunosorbent assay (ELISA) is a sandwich technique of PCR and ELISA. This test detects the six rabies virus genotypes, easy to perform, rapid (within 10 h) and highly sensitive (Whitby et al., 1997).

**Tag man based reverse-transcription PCR**: It is a type of PCR implemented to detect and quantify all genotypes of lyssaviruses isolated from a variety of specimen using fluorogenic probes. It can be used for the ante-mortem and post-mortem diagnosis of rabies. Additionally, it is sensitive, specific, requires no post-PCR manipulation thereby eliminating cross-contamination, and is amenable to high-throughput testing (Saengseesom et al., 2007). Nevertheless, the genetic diversity among lyssaviruses presents a challenge (Hughes et al., 2004).

**Nucleic acid sequence-based amplification (NASBA)**: Nucleic acid sequence-based amplification is applied to the ante-mortem saliva and cerebrospinal fluid and shown to have a ten times higher sensitivity than RT-PCR (Wacharapluesadee and Hemachudha, 2001). The assay is swift detecting rabies viral RNA as early as two days after onset of symptoms and enables to generate large number of copies and rapid throughput testing. The technique involves synthesis of multiple copy of target RNA under isothermal conditions using reverse transcriptase, RNase H and T7 RNA polymerase. The amplified RNA is detected using an automated reader in as little as four h. The relatively low temperature required for amplification of viral RNA facilitates study of in situ viral replication by maintaining the cell integrity (Sugiyama et al., 2003).

**Reverse transcription loop-mediated isothermal amplification (RT-LAMP)**: RT-LAMP can be done on ante-mortem saliva and CSF. The technique amplifies with high specificity, efficiency and without the need for thermal cycling and it is rapid, generating large quantities of target sequence within minutes. However, it is challenged by the considerable sequence variation. The product of the RT-LAMP can be further visualized by lateral flow assay (LAMP LFD). Using lateral flow devices to capture tagged LAMP products it is possible to detect positive rabies in brain samples (Hayman et al., 2011).

**Microarray technology**

This technique is being increasingly used for the diagnosis of various diseases (Hoheisel, 2006). Gurrala et al. (2009) developed a DNA microarray (LP chip) for detection and differentiation of lyssaviruses. Xi and co workers, (2012) developed Lyssachip to specifically differentiate the 7 major lyssavirus species based on generic reverse transcription–nested PCR (RT-nPCR). The chip method was 100% sensitive and highly consistent with the gold standard FAT. The chip method could detect rabies virus in highly decayed brain tissues,
whereas the FAT did not, and therefore the chip test may be more applicable to highly decayed brain tissues than the FAT.

Rabies is a dreaded viral anthroponozeugnosis which is reported from several countries of the world. Disease is important from public health as well as economic point of view. Dog is the principal transmitter of disease. Since rabies is a highly fatal infectious disease, rapid and correct diagnosis is highly imperative for medical management and in planning control programmes. Any case involving human exposure should be tested using accredited technique. Considering the heavy burden of rabies in developing countries, it is essential that they should have access to reliable diagnostic tool at hand. There is a lack of awareness about the true magnitude of the disease. Veterinary professionals must enlighten the burden arising from rabies to the policy makers in order to augment the rabies control. Close collaboration and coordination between veterinary and medical authorities at all level is recommended.

References


