COMPARISON OF THREE DIAGNOSTIC METHODS FOR DETECTING RABIES VIRUS IN ANIMALS

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ABSTRACT

In the present study three diagnostic methods viz., Fluorescent antibody test (FAT), Direct Rapid Immunohistochemistry Test (DRIT) and Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) were evaluated to diagnose rabies. A total of 30 brain samples of rabies suspected animals were received for postmortem (including dogs (n=20), cattle (n=08) and cats (n=02) from different geographical locations in Karnataka). Out of the 30 animal brain samples tested, 22 (73.3%) were positive for rabies according to the FAT. The sensitivities and specificities of DRIT and RT-PCR were 100% equivalent with FAT. The DRIT appears to be a fast and reliable assay that can be used to analyze brain samples. The results indicated that DRIT and RT- PCR can be used as supplementary diagnostic tools for detecting rabies viruses in field and laboratory conditions respectively.

KEY WORDS: Rabies virus, FAT, DRIT, RT-PCR,

INTRODUCTION

Rabies, one of the most widespread viral zoonoses, is caused by members of different species of virus within the genus Lyssavirus. Rabies can infect all warm blooded animals and continues to be a serious problem to both humans and animals. Dogs are the major domestic reservoirs of Rabies virus and source of disease transmission to domestic cattle in India. It is difficult to accurately diagnose rabies in animals based on clinical symptoms such as frenzy, extreme tremors, salivation and paresis to distinguish this disease from encephalitic conditions caused by canine distemper virus or acute trauma (Trimarchi and Nadin-Davis, 2007). Specific histopathologic changes in the central nervous system called Negri bodies have provided a basis for diagnosing rabies for about 100 years. However, this pathological diagnostic method may be no longer suitable for providing guidance for post-exposure prophylaxis (PEP), as new diagnostic methods have been developed (OIE, 2008).

Recent technical advances can also provide more definitive evidence of rabies virus (RABV) infection and detect the presence of the entire virion, RABV proteins, and viral genes in infected tissue (Biswal et al., 2007). In the present study, the ability of three diagnostic methods (FAT, dRIT and RT-PCR) to detect RABV were evaluated.

MATERIALS AND METHODS

Thirty brain samples were collected from different species suspected for rabies [Dogs (n=20), Cattle (n=08) and Cats (n=02)] received for post-mortem at Veterinary College, Hebbal, Bangalore. These animals were from different geographical regions of Karnataka. Approximately 50-100mg of brain sample was homogenized with power homogenizer. All homogenate samples were stored at -70^{o}C until analyzed by the FAT (Goldwasser and Kissling, 1958) and DRIT (Niezgoda and Rupprecht, 2006).

Extraction of viral RNA and RT-PCR

The total RNA was extracted from the brain tissue homogenates using the TRIzol® reagent (Invitrogen, USA), according to the manufacturer's instructions. The extracted RNA was immediately used for RT-PCR with gene-specific primers as described by Johnson et al. (2004). Forward primer JW12 of the positive-sense N gene, and reverse primer JW6DPL of the negative-sense N gene sequence of the Pasteur virus (PV) strain (Tordo et al., 1986) were used for RT-PCR (Table 1). The JW12 and JW6DPL primer pair amplified a 605-bp amplicon. The RT-PCR products were visualized by electrophoresis in 1.8% agarose gel containing ethidium bromide. Samples showing a 605 bp band were considered positive (Nagarajan et al., 2006).

Table 1. Oligonucleotide primers used for RT-PCR to diagnose rabies

Primer	Nucleotide sequences (5´-3´)	Nucleotide position* (bp)	Sense	Rabies gene	Size of amplicon
JW12	ATGTAACACCTCTACAATG	55-74	+	N	605
JW6DPL	CAATTAGCACACATTTTGTG	660-641	–		

*The positions of primers were based on the Pasteur virus strain

Calculation of sensitivity and specificity

Sensitivity was calculated with the formula [TP/ (TP+FN)] × 100, where TP was the number of samples with true-positive results as determined by the reference assay and FN was the number of samples with false-negative results. Specificity was defined as [TN/ (TN+FP)] × 100, where TN was the number of samples with true-negative results and FP was the number of samples with false positive results.

RESULTS AND DISCUSSION

In the present study, a total of 30 brain samples of rabies suspected dogs (n=20), cattle (n=08) and cats (n=02) collected from different geographical locations of Karnataka for rabies diagnosis were analyzed.

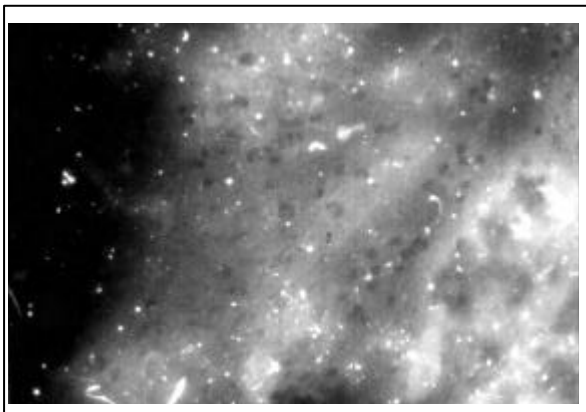

Fig. 1: FAT positive brain sample with sparkling green fluorescence and 100% of the antigen detected.

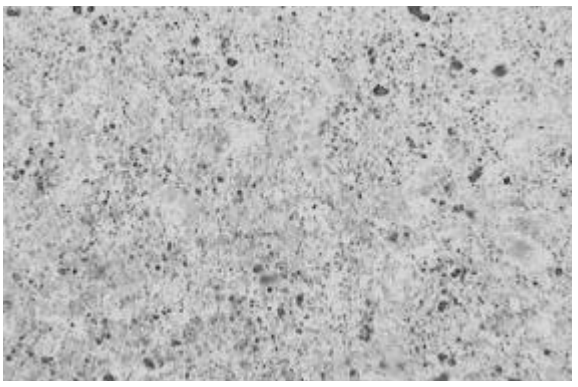

Fig. 2: DRIT positive brain touch impression (detection of rabies virus nucleoprotein) x 40.

Out of 30 brain samples dogs [(n=20), cattle (n=08) and cats (n=02)] tested, 22 were positive according to the FAT (Fig. 1) and the remaining brain samples were negative. Similarly, with DRIT, 22 brain samples tested were positive (Fig. 2) and eight samples were negative. Sensitivities and specificities of DRIT and RT-PCR were found to be 100% equivalent with the FAT.

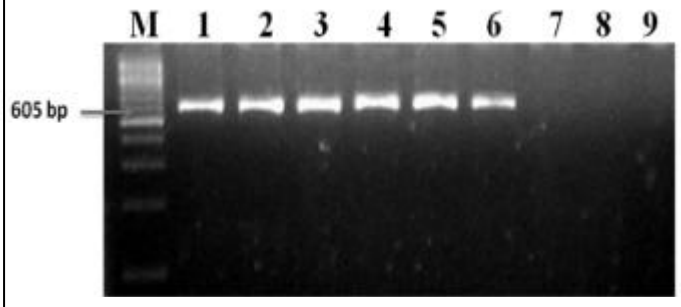

Fig. 3: Result of RT-PCR using primer sets which amplified the nucleoprotein gene of rabies virus

Lane M : 100bp DNA ladder

Lane 1 : Positive control

Lane 2-6 : PCR amplified 605bp product of RABV from suspected brain samples

Lane 7 : No template control

Lane 8 : Negative control

Lane 9 : Reagent control

Conventional RT-PCR using a primer set that amplified the N gene of RABV was able to detect RABV in 22 samples. The amplified product of 605 bp was observed among the samples (Fig. 3). However, RT-PCR could not detect the N gene in eight samples without any non-specific reactions.

The FAT has been recommended by the World Organization for Animal Health (OIE / WOAH) and WHO, and used for diagnosing rabies worldwide (OIE, 2008 and WHO, 1992). The main advantage of the FAT is that the results of this procedure can be obtained within 3-4 hrs. Even though the FAT is the most common rapid and sensitive diagnostic test for rabies, other supplementary diagnostic methods are required when a questionable FAT result is obtained in order to arrive at a definitive conclusion. Any false negative results may have a catastrophic impact and false positive results can lead to unnecessary PEP. The causes of FAT errors have been traced to inadequate sampling, unsatisfactory conjugate, and lack of experience in reading the slide (Chabbra et al., 2007).

The DRIT is a simple diagnostic method. However, as with other test, the protocol has to be followed strictly and includes more steps than the FAT. In fresh samples, the DRIT shows ideal results. Compared to the gold standard test FAT, the sensitivity and specificity were found to be 100%. To date, the DRIT has not exhibited any significant disadvantage compared to the FAT. Our study confirms the results of previous reports (Lembo et al., 2006) which found a 100% sensitivity and specificity of the DRIT compared to the FAT. A major advantage of the DRIT is that it utilizes a simple light microscope. The cost of a light microscope is ten times lower than that for a fluorescent microscope, which is needed for FAT. Thus, the DRIT has a significant potential for diagnosing rabies in low income countries, and in field locations where rabies diagnosis is unavailable.

Many kinds of diagnostic methods in molecular biology target the nucleic acids of the causative agent. Accordingly, diagnostic methods based on RT-PCR have been used to identify viruses that cause lyssaviral diseases, including RABV, since this has been confirmed to be a useful and sensitive method (Biswal et al., 2007. An important factor in determining the specificity of RT-PCR is the nucleotide sequence of the primers. The N gene, being conserved regions of the RABV genome, has been favoured for performing RT-PCR. Even if RT-PCR is capable of detecting the N gene in brain tissue of doubtful rabies cases, it is important to use fresh brain samples because viral RNA can be easily degraded through the action of ubiquitous RNases (David et al., 2002).

Rabies virus was detected by RT-PCR in the 22 FAT-positive samples using primer sets that amplified the N gene. The DRIT in the present study used for rapid detection of RABV in clinical samples. The sensitivity and specificity of the DRIT was also shown to be equivalent to that of the FAT. The FAT and RT-PCR diagnostic methods require a fluorescent microscope and thermal cycler, respectively. However, the DRIT can be performed in less than an hour (52 min) without any special equipment or facilities. In the present study, sensitivities and specificities of DRIT and RT-PCR were shown to be 100% equivalent with FAT. These results were similar to those of previous findings (Salome et al., 2008). Based on the results from this study, FAT, DRIT and RT-PCR were the most sensitive and specific diagnostic methods for detecting RABV. In conclusion, these demonstrated that it is possible to use these diagnostic methods to make rapid decisions for detecting and controlling rabies.

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