Short Communications

Differentiation of rabies fixed and street viruses using RT-PCR coupled with restriction endonuclease analysis

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A method based on RT-PCR and restriction endonuclease digestion of amplified PCR product was used to differentiate rabies laboratory “fixed” and “street” viruses. The primer sets from glycoprotein and nucleoprotein gene of rabies virus were used to amplify 406 and 533 bp amplicons, respectively. After amplification in RT-PCR, the PCR product was digested with Haelli restriction endonuclease and analysed for the presence or absence of restriction site on amplicons. The presence of Haelli restriction site on amplicons indicated rabies fixed virus while absence indicated street virus.

Keywords: rabies, virus, differentiation, street, fixed, RT-PCR, restriction endonuclease analysis

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Rabies is a disease of central nervous system that constantly produces victims in human beings as well as in domestic and wild animals. Rabies virus belongs to Lyssavirus genus in the Rhabdoviridae family. The genus Lyssavirus consists of 6 genotypes, of which genotype 1 includes the classical rabies viruses, including majority of both laboratory “fixed” and “street” viruses1. The fixed virus is a laboratory passaged attenuated virus with predictable properties, in terms of incubation period, pathological and clinical effects. In contrast, the virus isolated from naturally infected animals is termed as street virus. The street virus may have incubation periods varying from 2 weeks to more than 1 year and variable clinical effects2. The vaccines available today against rabies are killed vaccines prepared from laboratory fixed virus.

The diagnostic tests for detection of rabies in clinical samples are fluorescent antibody test (FAT), mouse inoculation test (MIT) and reverse-transcription polymerase chain reaction (RT-PCR)3-5. These methods can only detect presence of virus in clinical samples but cannot differentiate between street and laboratory fixed virus. In this study, the authors report differentiation of street and fixed rabies viruses using RT-PCR coupled with restriction endonuclease analysis of the amplified product.

The laboratory fixed rabies viruses used in this study were the mouse brain adapted challenge virus standard (CVS) strain and BHK-21 adapted PV-11 strain. The street rabies viruses were brain tissue samples received from Pasteur Institute of India, Coonoor, India. All the tissue samples used in this study were found positive in MIT and RT-PCR5.

Total RNA was isolated from all the laboratory fixed and street rabies viruses using Trizol LS reagent (Life Technologies, New York, USA) following manufacturer’s instructions. The isolated total RNA samples were dissolved in DEPC-treated RNase free distilled water. The primer sets used in this study were from two different genes (glycoprotein, G and nucleoprotein, N) of rabies virus. The primer set from G gene (forward, 5’- TAA TCC CAG AGA TGC AAT CA -3’; reverse, 5’- CCT CAG AGT CTG GTC TCA CC -3’) was used to amplify 406 bp amplicon. Similarly, the primer set from N gene (forward, 5’- ACT GAT GTA GAA GGG AAT TG -3’; reverse, 5’- CCT CAG AGT CTG TCA CC -3’) was used to amplify 533 bp amplicon5.

For RT-PCR reaction, 8-10 µg of total RNA from each sample was used. The single tube RT-PCR reaction was performed using G or N gene primer sets following the method described earlier5. Briefly, RNA samples mixed with G or N gene primer set and other ingredients were incubated at 42°C for 30 min for RT reaction and then PCR was done in the same tube following 35 amplification cycles with denaturation at 94°C for 1 min, annealing for 1 min and extension at 72°C for 5 min. The annealing temperatures were 55
and 50°C for primer set for G and N gene, respectively.

An aliquot of 5 μl from each sample was analysed for amplification on 1% agarose gel. For restriction digestion, 5 μl of PCR product was digested with 10 units of HaeIII restriction endonuclease (Promega, USA) in 25 μl volume at 37°C for 2 hrs. The digested PCR fragments were separated on 1% agarose gel along with 100 bp DNA ladder (Promega, USA) and analysed.

The 533 bp amplicon from N gene and 406 bp amplicon from G gene from reported nucleotide sequences were analysed for HaeIII restriction site using MapDraw software (DNAStar, USA). For HaeIII restriction site analysis of 406 bp amplicon from G gene, GenBank accession numbers used were: AF042824 (CVS-B2c), AF042823 (CVS-N2c), AJ506997 (CVS), M31046 (SAD B-19), M13215 (PV), M38452 (ERA), M32751 (Flury), M81059 (Street 1), M81060 (Street 2), M81058 (Street 3) and U52947 (Street 4). For HaeIII restriction site analysis of 533 bp amplicon from N gene, GenBank accession numbers used were: D42112 (CVS), M13215 (PV), M31046 (SAD B19) and AB010491 (Nishigahara).

The diagnostic tests available for detection of rabies in field samples, viz. FAT, MIT, virus isolation, histopathological examination of Negri bodies, immunoperoxidase staining and PCR based diagnosis, though sensitive and reliable, are unable to differentiate between rabies fixed and street viruses.

Therefore, for differentiation of rabies fixed and street viruses, the amplicons from G and N gene were amplified and analysed for the presence of HaeIII restriction site. Both the groups (fixed and street viruses) yielded 406 bp amplified amplicon. When the PCR products from rabies fixed viruses i.e. CVS and PV strains were subjected to restriction digestion with HaeIII restriction endonuclease, there was presence of restriction site in the amplicon due to which the PCR product of 406 bp was digested into fragments (Figs 1 & 2). When the amplified products from street viruses (field samples) were subjected to HaeIII digestion, the absence of restriction site was found in all the samples used in this study (Figs 1 & 2). This indicated that HaeIII restriction site on 406 bp amplicon was present only in fixed viruses and absent in street viruses. The street viruses (field samples) used in this study were already confirmed as rabies isolates using MIT and RT-PCR.

When the amplicon from nucleotide sequences submitted to GenBank for fixed viruses (CVS, PV, ERA, HEP-Flury) and few of the reported street viruses (Street 1-4) were analysed using DNA Star software, the restriction site for HaeIII restriction endonuclease was found at nucleotide position 225 in fixed viruses while absent in street viruses (Fig. 2). One additional restriction site at position 235 was also found in CVS. But the resultant 10 bp fragment could not be located in 1% agarose gel.

Similarly, the amplified amplicon (533 bp) from N gene demonstrated the presence of restriction site for HaeIII at nucleotide position 280 in fixed viruses (CVS and PV) used in this study. However, no site was found in PCR amplified products from street viruses (Figs 1 & 2). The same amplicon from reported fixed viruses (from GenBank sequences) when analysed using DNA Star software, the presence of HaeIII restriction site for fixed viruses was well corroborated (Fig. 2).

The results from this study indicated that the presence of HaeIII restriction sites on amplicon from G or N gene at nucleotide position 225 and 280, respectively, for rabies fixed virus while absence for
street virus. This finding will be of help in grouping a rabies field isolate (sample) either in fixed or street rabies viruses.

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References