

Detection of Equine Herpesvirus Infection: Sensitivity Assay of Polymerase Chain Reaction

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Abstract

The study was designed to identify the potential diagnostic tool with minimum detection limit in a clinical sample for Equine Herpesvirus Strain -1. An approach of molecular diagnostics was used using reported primers of polymerase chain reaction using in the sample obtained from repository. The PCR primers were specific to ORF 16 gene of EHV-1. Sensitivity assay of PCR detection was performed by making dilutions of EHV-1 positive DNA sample and running each dilution in a PCR and visualizing amplicons in ethidium bromide stained agarose gel under UV radiation. Study was valuable in determining the efficiency of PCR for quick and reliable source as disease surveillance. disease surveillance is as equal important as treatment, Without surveillance and diagnostics it is not possible to prevent and control any disease.

Key words: EHV-1, Equine Herpes Virus; PCR, Polymerase Chain reaction, ORF 16.

1. Introduction

The neurologic form of EHV-1 is called Equine Herpesvirus Myeloencephalopathy (EHM). EHV-1 is an important equine pathogen, responsible for causing abortion, perinatal foal mortality, respiratory tract diseases and neurological disorders in horses throughout the world (Mumford and Eddington 1980; Crowhurst et al. 1981; Carol and Westbury 1985; Uppal et al. 1991; Matsumura et al. 1992; Rattan et al. 1998, 1999; O'Callaghan and Osterrienda 1999). The most severe problem following EHV-1 infection is late abortion in mares, which may result in abortion storm (Allen and Bryans 1986; Singh et al. 1991; Mumford et al. 1995). The virus is transmitted as a respiratory infection (Bagust et al. 1972) and has become endemic throughout the world (Allen and Bryans 1986). In India this virus has also been reported from a number of cases including abortion, still birth, neonatal foal mortality as well as cases of paresis (Tewari et al. 1987; Singh et al. 1991; Rattan et al. 1998, 1999). Since this virus is involved in number of disorders in horses its timely detection is very important. DNA based detection using PCR is one of the most precise and rapid method of detection of Equine Herpesvirus. Present study was thought to be most important in this field and undertaken to estimate the minimum detection limit of EHV-1 viral DNA in any clinical sample using Polymerase chain reaction. During course of study EHV-1 PCR utilizing reported primers was developed and various dilutions of EHV-1 DNA positive samples were run. The study was carried out at CMVL Meerut (U.P.).

2. Materials and Methods

Sample- Sample of EHV-1 positive DNA was taken from repository. DNA was extracted and PCR reaction performed to identify positive DNA for EHV-1. DNA concentration in the sample was found to be 4.90 ng/μl as reported by spectrophotometric analysis as reported by All India Institute of Medical Sciences (AIIMS) India.

Primers for PCR- Already reported primers E4A (5'-CTGTAGCATAGAATGGTACAGAGGA-3') and E4B (5'-CCCCGCAAGTAACGGCGATGATGC-3') (Telford et al. 1992) amplifying ORF-16 gene of EHV-1 were used.

Amplification- DNA was extracted using Qiagen DNA Extraction kit according to the protocol supplied with it. The PCR assay mixture (25μl) contained primers (2μM/L each), 1X- PCR buffer [750 mM/L Tris HCl (pH 8.8 at 25°C), 200 mM (NH₄)₂SO₄, 0.1% Tween20], 1.5 mM/L MgCl₂, 160μM/L dNTPs and 1.25 unit Taq DNA Polymerase. Thermal cycling parameters were 95°C for 5 minutes and 30 cycles of each 1 minute at 94°C, 1 minute at 60°C and 3 minutes at 72°C followed by final extension at 72°C for 10 minutes. Amplified product obtained after PCR reaction is 409 bp analyzed using UV illumination..

Detection of PCR amplified products- For gel based PCR detection amplicons were detected by standard ethidium bromide staining and UV illumination of 2% agarose gels using gel documentation system

Sensitivity of assay- Limit of sensitivity of PCR assays was determined by serial dilutions of the DNA. Dilutions were made in nuclease free water up to 100⁻⁸. Each tube is vigorously mixed and vortexed after each dilution. PCR was run for each dilution. Six replicates were run on PCR for last dilution.

3.Results

The positive sample of EHV-1 DNA obtained from repository was properly amplified by the PCR protocol followed. We added 5 μ L of template/25 μ L reaction. Since DNA concentration as reported by AIIMS by spectrophotometric analysis was 4.90ng/ μ L therefore DNA concentration in 5 μ L template was (5 X 4.90) 24.5ng.

4.Discussion and conclusion

Diagnosis of EHV-1 myeloencephalopathy presents difficulties for both clinicians and diagnostic laboratories. Diagnosis, treatment and prevention can only control any disease outbreak. Disease control measures, such as isolation of affected animals, segregation and monitoring of exposed horses and quarantine measures, should be established in order to prevent the spread of the virus. While there are several vaccines available against both the respiratory and abortigenic forms of EHV-1 infection, currently no vaccines are protective against the neurological strain of the virus. Currently, the standard method for diagnosing the presence of viral pathogens in clinical samples relies on culture and other techniques such as Serum neutralization testing (SN), CSF analysis, ELISA which are time consuming and cumbersome but the sensitivity of PCR is much higher than these immunological methods. PCR has become the test of choice due to its high sensitivity and specificity PCR is primer directed enzymatic amplification of specific target DNA sequences. It has emerged as an important part of modern diagnostics and also for basic research. When used with proper caution PCR can provide invaluable help in rapid identification of specific pathogens which are difficult to grow in any type of artificial media. A PCR-based method cannot be given diagnostic status, until it includes methods to determine minimum detection limit, positive control, negative control and a reagent control (blank). The minimum detection limit and thereby the diagnostic sensitivity of a PCR assay, particularly on sub clinical samples with low target pathogens, depends on an effective sample treatment procedure. Thus by estimating minimum detection limit of a diagnostic PCR one can diagnose a disease condition where infection is in subclinical phase and sample volume is less. In this study we developed a PCR using reported primers to detect EHV-1 infection Although considerable progress has been made in developing PCR assays, the lack of laboratory protocol standardization and quality assurance procedures remain ongoing challenges this study is the answer to most of the questions related to standardization, and quality assurance. In the present study DNA concentration of sample was identified using spectrophotometric analysis. Dilutions of different DNA concentrations were used as template for PCR to calculate the minimum detection limit for the viral infection. The calculated amount of minimum DNA detected by PCR to be 24.5 ng .No any other method can detect virus in such a amount of EHV-1 Infection. It means it is the way latent infection can also be detected when virus is in its primary stage of replication. The DNA of minimum concentrations was used as template for PCR reaction was able to detect the DNA every time. This demonstrates, specificity, sensitivity and reproducibility of this method.PCR therefore is found to be of immense applicability in diagnosing virus in apparently healthy animals in latent stage or very early stage long before it shows any symptoms of disease in them thus controlling infection and formulating policies on prevention and control of EHV-1 Virus infection. This is the first report of Sensivity Assay of Polymerase Chain Reaction for Detection of Equine Herpesvirus Infection.

5.Acknowledgements

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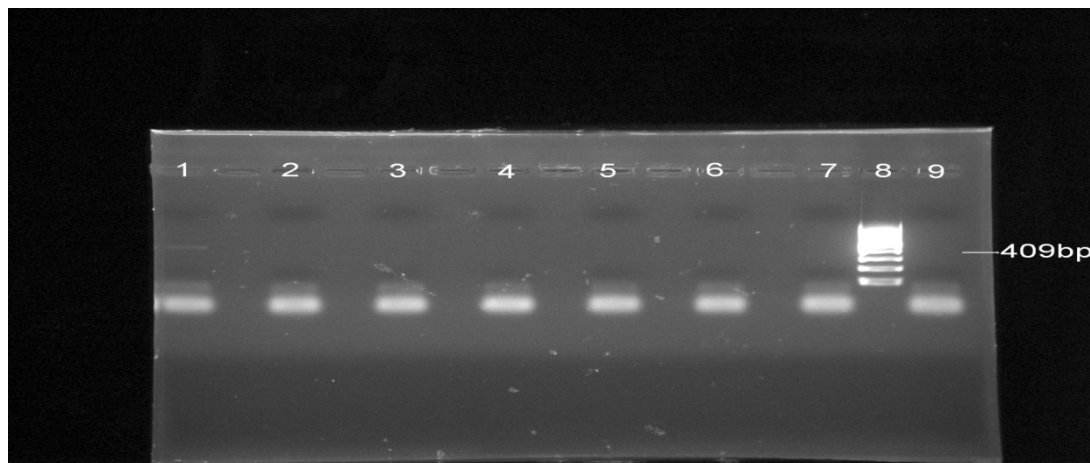


Fig. 1- Agarose gel electrophoresis with dilutions PCR products, Lane 1- 10^{-1} , Lane 2- 10^{-2} , Lane 3- 10^{-3} , Lane 4- 10^{-4} , Lane 5- 10^{-5} , Lane 6- 10^{-6} , Lane 7- 10^{-7} , Lane 8- 100 bp marker, Lane 9- negative control

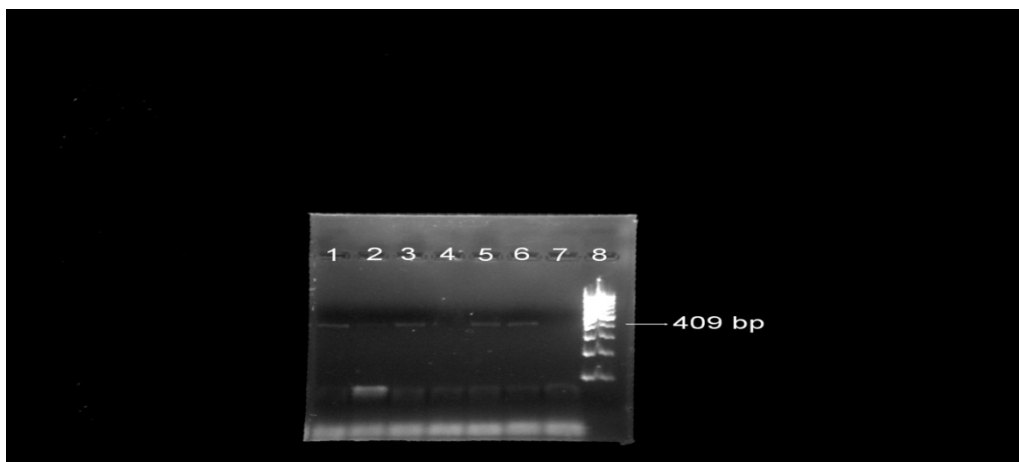


Fig. 2- Agarose gel electrophoresis of PCR products of replicates of dilution of 10^{-1} of sample DNA, Lane 1-6 - 10^{-1} dilution of sample DNA, lane 7 - Negative control, Lane 8- 100 bp marker.