

Sensitivity Assay of Polymerase Chain Reaction for Detection of Canine Adeno Virus Infection in Dogs

Prashant Sharma^{1,2*}, Kartikaye Kukreti^{*1}, Amit Rastogi ^{*1} Partap Singh Narwal^{*1}

1. Molecular diagnostic Laboratory, Central Military Veterinary Center Meerut, India
2. LSD, International Vaccine Institute, SNU Research Park, San 4-8, Nakseongdae-dong, Gwanak-gu, Seoul, Korea

* E-mail of the corresponding author: Prashant.bio@gmail.com

Abstract

This study was done with the approach of finding out minimum detection limit of canine adeno virus in clinical samples using Polymerase chain reaction. A polymerase chain reaction was performed using reported primers for detection of Canine Adeno Virus (CAV) in the blood sample obtained from different regions of India. Detection and surveillance of disease very important for diagnosis and prevention of Disease. 40 samples were screened for CAV infection in a time period of six months. DNA extracted from samples used as template in PCR reaction using primers specific to E3 gene forward primer ICHA and reverse primer ICHB of CAV1 and CAV2 virus gives a band size of 508 bp which should be the size of amplified product. Positive sample of CAV in PCR were taken for study. Assay was performed using multidilution of extracted DNA of positive sample each dilution were used as template for PCR reaction. End point of the dilution obtained where no band was visible in agarose gel when visualize in gel doc system, same DNA was used for dilutions to check repeatability of PCR. Ten replicate PCR reactions were studied. The detection limit found was at the dilution of 1:1000 is 0.20 ng per μ l. The study was valuable in determining the efficiency of PCR for detection of CAV Virus in clinical samples.

Key words: Canine Adeno Virus (CAV), reported primers, clinical samples, Infectious canine hepatitis, utilizing reported primers

1. Introduction

Adenoviruses are linear, double-stranded DNA viruses which infect a wide variety of mammals and birds. Two adenoviruses have been identified in the dog: Canine Adenovirus type 1 (CAV-1) which infects most of the major organs causing, among other diseases, hepatitis and Canine Adenovirus type 2 (CAV-2) which causes respiratory and enteric diseases. Canine Adeno Virus is known to infect dogs and cause Infectious canine hepatitis (CAV1) and infectious Laryngotracheitis or kennel Cough (CAV2). Both the viruses share some characteristics with each other and provide cross protection (Emery et al., 1978). The CAV -1 replicates in vascular endothelial tissues while CAV-2 is known to replicate in respiratory epithelium and in intestinal tract (Appel et al., 1973; Macartney et al., 1988). CAV-1 causes severe and often lethal systemic disease with hepatitis, enteritis and encephalitis, especially in pups up to 2 weeks of age and may persist in kidneys for months. CAV2 remains restricted to epithelium of upper respiratory tract. It has also been isolated from stomach and intestine but not from other tissues like liver, kidney, spleen or central nervous system. Presence of both CAV1 and CAV-2 constitutes a threat to dogs. DNA based detection using PCR is one of the most precise and rapid method of detecting CAV. Present study was undertaken to estimate the minimum detection limit of CAV PCR. During course of study CAV PCR utilizing reported primers was developed and various dilutions of CAV DNA positive sample were run. The study was carried out at CMVL Meerut (U.P.).

2. Materials and Methods

Sample-Sample of Pup No.4 CAV positive citrated blood was taken from repository. DNA was extracted and PCR was run to check Pup no.4 positive DNA for CAV. DNA concentration was found to be 438.90 ng / μ l by spectrophotometric analysis as reported by AIIMS. Primers-Already reported primers ICHA (5'-CGCGCTGAACATTACTACCTTGTC-3') and ICHB (5'-CCTAGAGCACTTCGTGTCCGCTT-3') (Hu et al., 2001) amplifying E3 gene of CAV-1 and CAV-2 were used. Amplification-DNA was extracted using Qiagen DNA Extraction kit according to the protocol supplied with it. The PCR assay mixture (25 μ l) contained primers (2 μ M each), 1X- PCR buffer [750 mM Tris HCL (pH 8.8 at 25°C), 200 mM (NH₄)₂SO₄, 0.1% Tween20], 1.5 mM MgCl₂, 160 μ M dNTPs and 1 unit Taq DNA polymerase. Thermal cycling parameters were 94°C for 3 min and 35 cycles of each 40 sec at 94°C, 40 sec at 55°C and 1 min at 72°C followed by final extension at 72°C for 10 min. Amplified product obtained in PCR assay is 508 bp. 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. Sensitivity of assay-Limit of sensitivity of PCR assays was determined by serial dilutions of the extracted DNA. Dilutions were made in nuclease free water upto 100-5. Each tube was vigorously mixed, vortexed and spinned after each dilution. PCR was run for each dilution. Ten replicates were run on PCR for final detectable dilution.

3. Results

The positive sample of Pup No.4 CAV DNA was properly amplified by the PCR protocol followed. We added 2.5 μ l of template/25 μ l reaction. Since DNA concentration as reported by AIIMS by spectrophotometric analysis was 438.90 ng μ ⁻¹ therefore DNA concentration in 2.5 μ l template was (2.5 \times 438.90) 1097.25 ng (Fig. 1). As PCR is detecting upto 100⁻² dilution of CAV DNA therefore the detection limit would be 1097.25/10000 i.e., 0.11 ng /2.5 μ ⁻¹ or = 0.20 ng / μ l (Fig. 2).

4. Discussion and conclusion

Canine adeno virus can cause many symptoms in dog including hepatitis it is very necessary to detect virus in early stage of infection. Many immunological methods can be use for detecting antigen of CAV such as HA, Elisa but the sensitivity of PCR is much higher. than these immunological methods. PCR is primer directed enzymatic amplification of specific target DNA sequences is a important part of modern diagnostic methods. When used with proper caution PCR can provide invaluable help in rapid identification of specific pathogens which are difficult to grow. PCR has emerged as the molecular method of choice in achieving objectives. 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 CAV-1 infection. PCR showed good concordance with virus isolation “gold standard” for diagnostic virology, demonstrates specificity and sensitivity of this method. In addition PCR is more economical than virus isolation because it requires neither cell culture facilities nor highly trained specialists. We got DNA concentration in sample DNA quantified from AIIMS by spectrophotometric analysis (Fig. 1). We made dilutions of concentration of the DNA and used these dilutions as template for PCR to calculate the minimum detection limit for the viral infection. We calculated the minimum amount of DNA detected by PCR to be 0.11 ng which is very less compared to other methods. When replicates of the minimum detected dilution were run PCR was able to detect the DNA every time (Fig. 2). 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 disease a very early stage at right time.

ACKNOWLEDGEMENTS

Authors are grateful to Directorate General Remount Veterinary Services for providing facilities for conduct of this work and to Dr HK Prasad, Professor Department of Biotechnology AIIMS for assistance provided.

References

- Arai, T., Aiyama, Y., Sugi, M. & Ota, J. (2001), “Holonc Assembly System with Plug and Produce”, Appel, M., S.I. Bistner, M. Menegus, D.A. Albert and L.E. Carmichael, 1973. Pathogenicity of low virulence strains of two canine adenoviruses. *Am. J. Vet. Res.*, 34: 543-550. PMID: 4348389
- Chouinard, L., D. Martineau, C. Forget and C. Girard, 1998. Use of polymerase chain reaction and immuno his to chemistry for detection of canine adenovirus type1 in formalin fixed, paraffin embedded liver of dogs with chronic hepatitis or cirrhosis. *J. Vet. Diagnostic Inves.*, 10: 320-325. <http://vdi.sagepub.com/content/10/4/320.full.pdf>
- Emery, J.B., J.A. House and W.R. Brown, 1978. Cross protective immunity to canine adenovirus type 2 by canine adenovirus type 1 vaccination. *Am. J. Vet. Res.*, 39: 1778-1783. PMID: 216291
- Hu, R.L., G. Huang, W. Qiu, Z.H. Zhong and X.Z. Xia et al., 2001. Detection and differentiation of CAV1 and CAV2 by polymerase chain reaction. *Vet. Res. Commun.*, 25: 77-84. PMID: 11214675
- Kiss, I., K. Matiz, E. Bajmoci, M. Rusvai and B. Harrach, 1996. Infectious canine hepatitis: Detection of canine adeno virus type1 by polymerase chain reaction. *Acta Vet. Hung.*, 444: 253-258. PMID: 8908749
- Linne, T., 1992. Differences in E3 regions of canine adenovirus type 1 and type 2. *Virus Res.*, 23: 119-133. PMID: 1534956

Macartney, L., H.M.A. Cavanagh and N. Spibey, 1988. Isolation of Canine Adeno virus type2 from the feces of dogs with enteric disease and its unambiguous typing by restriction endonuclease mapping. Res. Vet. Sci., 44: 9-14. PMID: 2836923

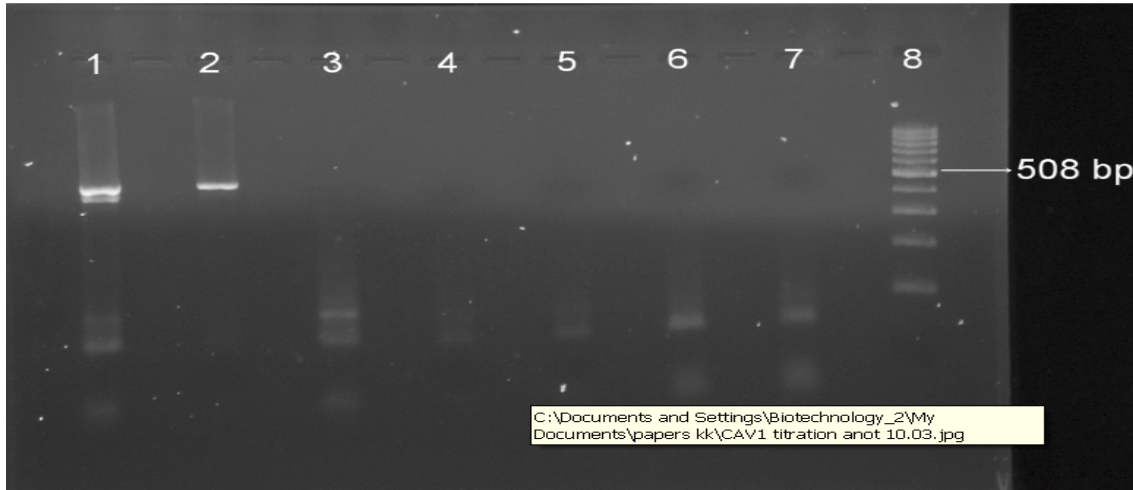


Figure 1. Agarose gel electrophoresis with dilutions PCR products, Lane 1- 10^{-1} , Lane 2- 10^{-2} , Lane 3- 10^{-3} , Lane 4- 10^{-4} , Lane 5- 10^{-5} , Lane 6- 10^{-6} , Lane 7- 10^{-7} , Lane 8- 100 bp marker

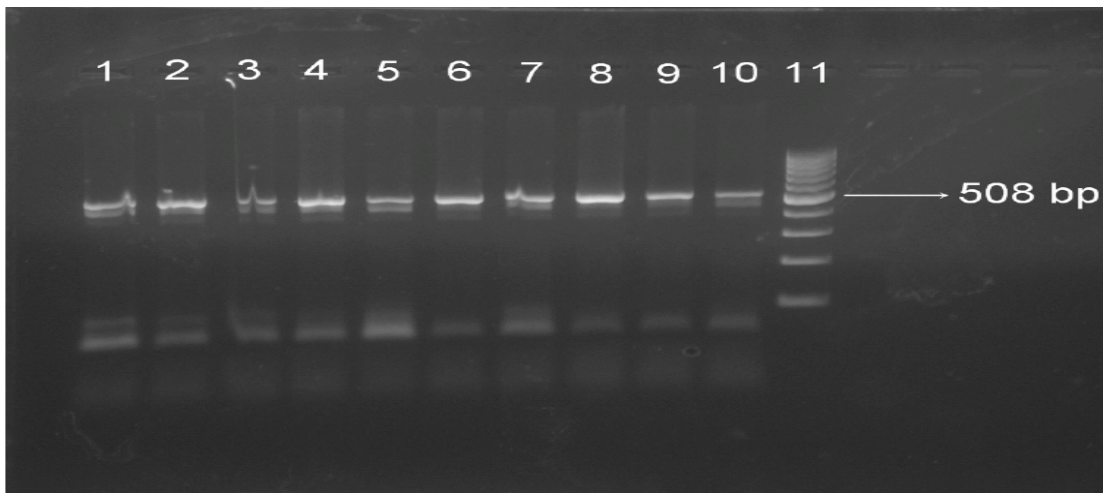


Fig. 2: Agarose gel electrophoresis of PCR products of replicates of dilution of 10^{-2} of sample DNA, Lane 1-10 - 10^{-2} dilution of sample DNA, Lane 11-100 bp marker