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DETECTION OF BOVINE CORONAVIRUS FROM A PRIVATE FARM IN THRISSUR, KERALA USING REVERSE TRANSCRIPTASE POLYMERASE CHAIN REACTION

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Abstract: Bovine coronavirus is one of the most important viral agents causing neonatal diarrhoea in calves. The present study aims to detect the presence of bovine coronavirus in calves using Reverse Transcriptase-Polymerase Chain Reaction. The faecal samples were collected from five diarrhoeic calves from a private farm in Paralam, Thrissur district of Kerala. The primers specific for N gene were designed using Primer 3 software and subjected to Reverse Transcriptase-Polymerase Chain Reaction and found that one out of five samples was positive revealing an amplicon of 187 bp. This study is the first of its kind in Kerala. Hence, further detailed research is warranted to analyse the actual magnitude of bovine coronaviral infections among calves in Kerala.

Keywords: Bovine coronavirus, Reverse Transcriptase-Polymerase Chain Reaction, Nucleocapsid (N) gene.

Introduction

Bovine *coronavirus* (BCoV) belongs to the order *Nidovirales*, family *Coronaviridae* and subfamily *Coronavirinae.* The virus is grouped under the genus *Betacoronavirus* and species *Betacoronavirus 1* [4]. The main routes of transmission of coronavirus are through oral, faeco-oral and nasal cavity [1, 2, 8]. The calves infected with BCoV shows signs such as anorexia, pyrexia, dehydration and listlessness [10]. The virus is one of the important causative agents of gastroenteritis and respiratory disease in bovines with worldwide distribution creating a huge economic loss in cattle industry. The disease has to be differentially diagnosed from other enteropathogenic viral and bacterial infections to prevent the spread of the disease.

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Coronavirus infection has been reported from many countries world-wide and also from different states of India. However, there is only a single report of occurrence of BCoV in Kerala [5]. A rapid diagnosis is absolutely necessary to control and prevent the spread of the disease. The present study was undertaken to detect the presence of BCoV in faecal samples of calves with diarrhoea by Reverse Transcriptase Polymerase Chain Reaction (RT-PCR).

Materials and methods

The faecal samples were collected from diarrhoeic calves in the age group 0-14 days-old from a private farm in Paralam in Thrissur district. The clinical signs observed were anorexia, pyrexia and diarrhoea. Five samples were collected using sterile swabs and immersed in RNA later and stored at -80°C until use.

The primers used in the study for detection of BCoV were designed from the conserved region of N gene (GenBank accession number NC003045.1) using Primer 3 software (V.0.4.0). The forward and reverse primer sequences are as follows

Forward primer (F): 5' ATTTGCAGAGGGACAAGGTG 3'

Reverse primer (R): 5' GTCGGTGCCATACTGGTCTT 3'

The primers were custom synthesised commercially by Sigma-Aldrich Inc., US. Both the primers obtained were reconstituted and working solution was made upto 10p*M*/µL for further use. The RNA was extracted from the faecal samples using TRIzol LS reagent (Sigma-Aldrich Inc., US) as per the manufacturer's protocol, with minor modifications and then the concentration and purity of extracted RNA were checked in nanodrop 2000C (Thermo Scientific, USA). The RNA was converted to cDNA using Verso cDNA synthesis kit (Thermo Scientific, USA) according to the manufacturer's protocol. The concentrations of reagents were optimised for the amplification in a total volume of 25 µL. The reagents consisted of 12.5 µL of 2X PCR Master mix (Takara), 2 µL (10p*M*) of each of the forward and reverse primers, 4 µL of template (cDNA) and 4.5 µL of nuclease free water (HiMedia, India). The PCR was performed in a thermal cycler (Bio Rad, USA) with an initial denaturation of 94°C for 4 min. 30 sec., followed by 40 cycles of denaturation at 94°C for 1 min., annealing at 58.6°C for 1 min. 30 sec., extension at 72°C for 2 min., then one cycle of final extension at 72°C for 7 min. The PCR products along with a positive control and a negative control without template were run in two per cent agarose (GeNei, India) prepared in 1X TAE buffer using horizontal submarine gel electrophoresis (Hoefer, USA) at 50 V for 30 min. Then, the gel was documented in gel documentation system and the image was recorded (Bio-Rad, USA).

Results and Discussion

The conventional methods such as virus isolation and transmission electron microscopy employed for the detection of BCoV are considered as expensive, laborious and time consuming. Hence, nucleic acid based techniques like RT-PCR can be used for the detection of BCoV from faecal samples as the assay is more sensitive and specific than the conventional methods [3,6].

The concentration and purity of all the extracted RNA was checked using Nanodrop 2000c (Thermoscientific, USA) and the values were >80 ng/µL and 1.82-1.92, respectively indicative of good quality RNA. The cDNA prepared from the samples were subjected to RT-PCR for the amplification of N gene of BCoV. Out of the five samples tested, one showed an amplicon of approximately 187 bp. The gel image was depicted in fig. 1. The BCoV was detected in the calf within the age group 0-14 days-old [7,9]. The result indicated the occurrence of BCoV in Thrissur.

The number of samples collected in this study was less. Hence, it is necessary to conduct more studies on BCoV to control and prevent the disease thereby reducing the economic loss to the farmers.

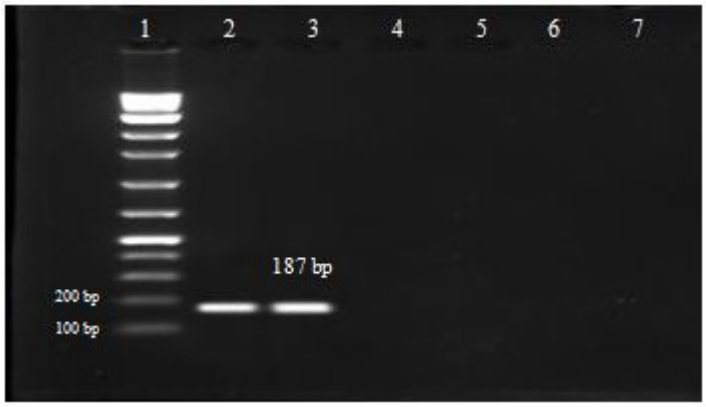

Fig 1. Agarose gel electrophoresis of RT-PCR amplified products of bovine coronavirus (representation)

Lane 1: 100 bp ladder
Lane 2: Positive control
Lane 3: Positive sample
Lane 4: Negative control

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