Detection of Corona virus antigen by ELISA from diarrhoeic cow calves in Mathura, India

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Abstract

Neonatal diarrhoea is one of the most important conditions of calves, associated with morbidity and mortalities. Diarrhoeal diseases have an adverse effect on calf health status, survival and productive performances. Corona virus is one of the etiological agents responsible for calf diarrhea worldwide. However there is paucity of literature stating the disease status in India. The present study was carried out to determine the prevalence of corona virus infection among cow calves in Mathura and adjacent regions. During the present study 63 diarrhoeic stool samples collected from cow calves were screened for corona virus. Of the 63 diarrhoeic samples 3 samples (4.76%) were found to be positive for corona virus by ELISA.
Keywords: Bovine corona virus; Rotavirus; Diarrhoea; ELISA.

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Introduction

Major etiological agents responsible for calf diarrhoea are bacteria (*E. coli, Salmonella*), Viruses (Rotavirus, corona virus) and protozoa (Cryptosporidia) [4]. Corona viruses and rotaviruses are the most common viruses involved in neonatal calf diarrhoea. Coronaviruses belong to family *Coronoviridae*. Coronavirus particles are irregularly-shaped, 80-220 nm in diameter, with an outer envelope bearing distinctive, 'club-shaped' peplomers (20 nm long). This 'crown-like' appearance (Latin, *corona*) gives the family its name.

The Viruses have non-segmented, single-stranded RNA with helical symmetry [5]. Corona viral diarrhea in young calves is characterized by profuse watery or hemorrhagic diarrhoea lasting for 2 to 6 days along with listlessness, anorexia, pyrexia, and dehydration. Morbidity is high (30-100%) but mortality is influenced by the age of the calf. Calves with bloody diarrhoea can die of hypovolemia within a few hours of the onset of clinical signs [1].

Viral transmission can be through aerosols of respiratory secretions, via the faecal-oral route, or by mechanical transmission [1].

Materials and Methods

Collection of specimens: A total of 63 diarrhoeic fecal samples were collected from calves from both organized and non-organized dairy farms located in and around Mathura during winter months of the study period, 2007-2009. The stool samples were collected in sterilized plastic containers, transported under ice and stored at -20°C till further processing.

Screening by ELISA: ELISA was performed to detect corona virus antigen in the fecal samples as described by the kit manufacturer (Corona virus ELISA kit, Bio-X Diagnostics, Belgium). The 96

well plates provided in the kit contained two different capture antibodies. Rows A, C, E and G were coated with corona virus specific capture antibodies and rows B, D, F, H coated with non-specific antibodies, which acted as controls. These control rows allow the differentiation between specific immunological reaction and non-specific bindings so as to eliminate false positives. Faeces were diluted in the dilution buffer provided in the kit. A volume of 100µl of diluted sample was added to corresponding wells of specific and non-specific antibody coated rows respectively. The plate was incubated for one hour at 25°C and washed 3 times with washing solution (diluted in the ratio 1:20 with distilled water) provided in the kit. The conjugate, a corona virus specific monoclonal antibody labeled peroxidase was used as such and poured in 100µl quantities per well. The plate was incubated for one hour at 25°C in a dark room and washed thrice with the washing buffer.

Then 100µl of chromogen (tetramehtyl-benzidine) were added and the plates allowed to stand at room temperature without excess light for 10 minutes. Finally the reaction was stopped by adding stop solution (1M phosphoric acid) provided in the kit. The optical density was measured at 450nm after stopping the reaction with 50µl of stop solution. The test was validated using the positive control and data sheet provided by the kit. The net optical density of each sample was calculated by subtracting the reading for each sample well from corresponding negative control.

Net optical density (O.D.) = O.D. of specific binding - O.D. of non-specific binding. Any sample that yieldied an O.D difference of 0.15 or greater was considered positive.

Results and Discussion

Till today a variety of methods are used to detect bovine corona virus (BCV) infection in stool samples. Currently used methods include electron microscopy, haemagglutination test, enzyme immune assay, virus isolation in cell culture and RT PCR. Each method has its own advantage and disadvantage. Electron microscopy (EM) is very expensive and identifies only complete BCV particles accurately. Partial or complete loss of spikes on the viral envelope which occurs during sample processing can mislead the diagnosis [11]. In spite of this EM is still used as a basic test procedure [7]. The results of haemmaglutination test are affected by non-specific agglutinins present in the faeces [2]. Virus isolation based tests are laborious and time consuming. RT PCR based tests are highly sensitive and widely accepted. For this, good RNA handling facilities, proper standardisation and appropriate positive controls are required. The results of enzyme immunoassays largely depend on the quality of reagents. Polyclonal antibody based tests produce high levels of non-specific back ground and cross reactions with other antigens present. However, monoclonal antibody based tests overcome these difficulties and are widely used [7]. In the present study we have analyzed fecal samples obtained from single diarrhoeic episodes.

Out of the 63 diarrhoeic stool samples processed, 3 (4.76%) were found positive by ELISA. Other studies also revealed that prevalence of corona virus in neonatal calf diarrhoea is slightly lower than that of rotavirus and varieed between 3.64 to 54%. [8, 10 and 12]. However, there is paucity of literature stating the corona virus prevalence status in India. As per Niture et al. [6] prevalence of rotavirus induced diarrhea in calves varied between 7.49 to 43% in India. Previous work by our group in the same Mathura and adjacent regions during same study period showed a rotavirus prevalence of 16.83% [3]. Hence prevalence of corona virus induced diarrhea is less compared to that of rotavirus in the studied regions during this study period (2007-2009). In addition to neonatal diarrhea, bovine corona viruses cause winter dysentery in adult cattle and respiratory tract infections in calves and feed lot cattle [1, 2 and 9].

In conclusion, corona viruses are associated with multiple bovine disease conditions and should as a result not be neglected, but consistently monitored and controlled. Monoclonal antibody based ELISAs are simple and easy to perform,

and are ideal for utilization when generating important epidemiological data.

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Conflicts of interest

Authors declare that they have no conflicts of interest.

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