Prevalence of Group A Bovine Rotavirus in Neonatal Calves in Punjab, India.

G.V.D. Sravani, Gurpreet Kaur*, Mudit Chandra and P.N. Dwivedi
Department of Veterinary Microbiology, College of Veterinary Science, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab-141001, India.

Abstract
Group A rotaviruses play an important role in causing gastroenteritis and mortality in buffalo calves. To investigate the epidemiological characteristics and prevalence of bovine rotavirus (Group A rotavirus) in Punjab, 120 fecal samples were collected from buffalo calves (n=93) and cow calves (n=27) up to 3 months age group calves exhibiting diarrhoea during the period from July 2012 to March 2013 and screened for the presence of rotavirus using RNA-PAGE. Twenty-three samples were positive by RNA-PAGE having electrophoretic pattern that corresponded to mammalian group A rotaviruses. Male diarrheic calves were found to be more susceptible to rotavirus infection (22.2%) than female diarrheic calves (12.8%). Calves below 20 days of age and buffalo calves were found to be more susceptible to rotavirus infection. High incidence was seen in winter months at low relative humidity. Thus from the above study the overall prevalence of Group A rotavirus in diarrheic calves was found to be 19.16%.

Key words: Bovine rotavirus, RNA-PAGE, Prevalence, Buffalo calves, Cow calves.

1. Introduction
Livestock farming plays an important role among the development programs in Punjab, India. Buffalo contributes significantly to the economy of dairy farmers in Punjab. The country has over 98 million buffaloes which constitute 56.4% of the world buffalo population and contribute 66.3% of total buffalo milk in the world (http://dahd.nic.in/stat_files/BAGHS2006). Rotaviruses (RVs) are recognized as the single most significant cause of severe gastroenteritis, malnutrition and diarrhoea, affecting a wide range of mammalian and avian species (Estes and Kapikian, 2007). Among rotaviruses, Group A rotaviruses are a significant cause of neonatal diarrhoea in several animal species including calves (El-Attar et al., 2002; Dhanze et al., 2014). The virus causes diarrhoea in 5-15 days old calves although it can affect calves up to several months of age (Gay et al., 2012). It is a non-enveloped virus which belongs to the family Reoviridae, and contains a genome of 11 segments of double stranded RNA, enclosed within a triple layered capsid protein (Estes and Kapikian, 2007). Rotaviruses are classified into seven groups (A to G) based on antigenic differences on the inner capsid VP6 protein (Estes, 2001). In India, the incidence of rotaviral infection ranges from 11% to 24% in buffalo calves and 10% to 52% in cow calves (Singh and Pandey, 1988). As the virus has zoonotic nature the study on the prevalence of rotavirus is important (Martella et al., 2010). Thus this study was undertaken to study the prevalence of rotavirus infection in diarrheic buffalo and cow calves in Punjab region.

2. Materials and Methods

2.1 Collection of Specimen
A total of 120 fecal samples were collected, out of which 47 samples were from an organized dairy farm and 73 samples from unorganised dairy farms. The farms used in the study were termed ‘organised’ if daily records of production and health were maintained, or ‘unorganised’ if no such records were held by the farm. The samples were collected from diarrhoeic cow calves (n=27) and buffalo calves (n=93) under 3 months of age during the period from July 2012 to March 2013 from Guru Angad Dev Veterinary and Animal Sciences University dairy farm and areas in and around Ludhiana.

2.2 Preparation of Fecal Suspension
A 10% suspension of each fecal sample was made in phosphate buffered saline, (pH 7.2).
2.3 Extraction of Viral RNA from Fecal Suspension

Viral RNA was extracted from fecal suspensions by using a QIAamp RNA Mini Kit (QIAGEN) following the manufacturer’s protocol.

2.4 RNA-Polyacrylamide Gel Electrophoresis

The extracted RNA was subjected to RNA-PAGE according to the method of WHO, (2009) with minor modifications. The staining of the gel was done as described by Bassam et al. (1991). The stained gel was photographed by Alphaimager (USA). The positive control used in the RNA-PAGE was the RNA extracted from the Rotavirus procured from the Division of Virology, IVRI, Mukteshwar. Nuclease free water was used as negative control.

3. Results and Discussion

Out of 120 fecal samples screened using RNA-PAGE, 23 (19.16%) samples were found to be positive for bovine rotavirus. These positive samples had a migration pattern of 4:2:3:2 which is typical of group A mammalian rotavirus, with segments 2, 3 and 4 migrating close together, segments 7, 8 and 9 migrating as a triplet. All exhibited long electropherotyping pattern (segments 10 and 11 were wider apart) (Fig 1). This corroborate with Dash et al. (2011); Okada and Matsumoto (2002) who reported group A rotavirus as major cause of diarrhoea in calves all over the world. Saravanan et al. (2006) and Kumar et al. (2011) also reported that they found long electropherotyping pattern in their studies.

In the present study the overall prevalence was found to be 19.16% in diarrhetic calves. Similarly, in other studies conducted on rotavirus, prevalence of 16.83%, 22% and 10.15% have been reported from different parts of the India (Dash et al., 2011; Ghosh et al., 2007; Basera et al., 2010).

The results showed that 18 of 81 (22.2%) male calves were found positive whereas rotavirus was detected in 5 of 39 (12.8%) samples of female calves (Table 1). The higher rate of susceptibility was seen in male calves compared to female calves (Fig 2). The possible reason for this might be that more number of samples were collected from male calves. Another possible justification for this could be due to the managemental practices, as in most of the dairy farms female calves are taken care better than male calves. Our findings are more or less similar to the observations reported by other workers (Sharma, 2004; Dash et al., 2011) where higher susceptibility of male bovine calves were found in comparison to female calves.

Age wise susceptibility was also evaluated in the present study (Table 1) and the results indicated that newborn calves of 1-20 days age group were more susceptible to rotavirus infection (Fig 3). Dash et al. (2011) indicated that newborn calves of first 8 weeks of age were more susceptible to rotavirus infection. Bellinzoni et al. (1987) recorded peak prevalence (53%) of rotavirus infection in calves between 10-19 days age group. In another study conducted by Dhanraj et al. (1996) also reported the prevalence of infection with Rotavirus is greater during the first week of life and when liquid faeces are present. There is also a possibility that immune system of very young calves is not fully mature to handle rotavirus pathogens and the lack of sufficient amount of maternal antibodies can be another possible reason.

When species wise distribution of bovine Rotavirus was evaluated (Table 1), 19.35% (18/93) of prevalence was found in buffalo calves and 18.51% -
Fig 1: RNA-PAGE showing electrophoretic migration of bovine Rotavirus

Legends: Lane N- Negative control; Lane S- Positive control; Lane S3, S4, S51, S52, S53, S54, S9 and S13- Positive diarrhoea samples

Fig 2: Sex wise distribution of bovine Rotavirus in neonatal calves
Fig 3: Age Wise distribution of bovine Rotavirus in neonatal calves

Fig 4: Species wise distribution of bovine Rotavirus in neonatal calves

(5/27) was found in cow calves (Fig 4). Similarly Kusumakar et al. (2010) found 23.61% of incidence rate in buffalo calves (17/72) and 21.43% in cow calves (6/28) for the rotavirus. Jindal et al. (2000) also reported that in the unorganised dairy herds, 25% and 29.4% cow and buffalo calves, respectively were positive.

Month wise distribution of bovine Rotavirus was also evaluated (Table 1) and it revealed more number of positive cases during winter months in -
diarrhoic calves with peak prevalence during December 2012 to January 2013 (38.8%), followed by October to November 2012 (15%) and February to March 2013 (11.9%) (Fig 5). The present study recorded an increasing trend in number of rotavirus cases with decrease in environmental temperature. Broor et al. (2003) reported the seasonal variation of rotavirus diarrhoea in different geographical regions with high incidence in winter months at low relative humidity in north India. Other workers also reported rotavirus was predominant in winter, particularly in December to February (Borade et al., 2010; Sherchand and Haruki, 2004).

4. Conclusion

Thus it could be concluded from the above study that the overall prevalence of Group A rotavirus in diarrheic calves was found to be 19.16%.

References


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