

## Research Article

# Epidemiology and Genetic Diversity of Rotavirus Strains Associated with Acute Gastroenteritis in Bovine, Porcine, Poultry and Human Population of Madhya Pradesh, Central India, 2004–2008

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### ABSTRACT

Virus induced enteritis is one of the grave problems accounting for maximum deaths in neonatal animals and human throughout the world. In developing countries like India, the socio-economic conditions favors the occurrence of higher disease frequency of gastro-enteric infections, majorly dominated by rotavirus (RV). The dearth of appropriate surveillance programs and laboratory facilities have resulted in scarcity of accessible data on RV associated enteric disease affliction and epidemiological evidence in the country. We describe here the epidemiological and genotypic distribution of RV in various animal species and human during 2004 to 2008 from Madhya Pradesh, the central region of India. The overall prevalence rate of RV was 17.19% (126/733), maximum in humans (30.23%), followed by buffaloes (22.01%), cattle (13.33%), swine (13.04%) and poultry (6.47%). The nested-polymerase chain reaction (PCR) based genotyping results revealed circulation of genotypes of G10 and G3 specificity and P[1] and P[3] types in the region. In humans, only the G1 genotype was detected and for the first time group B rotavirus was detected in an adult. Based on the typical migration pattern of RNA genome segments in the gel electrophoresis (5:2:2:2), group D rotavirus was also identified in poultry diarrheic samples. The results emphasize the need for extended molecular surveys for understanding the interspecies transmission, evolution and especially in designing future vaccine.

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### INTRODUCTION

Acute diarrhea has been recognized as one of the foremost causes of death in neonates of animal and human globally (Estes and Kapikian, 2007; Dhama et al., 2009). As per the National Animal Health Monitoring System (NAHMS), 2011 report, diarrhea is responsible for more than a half of the mortality (52%) in animals. Rotavirus, the member of family *Reoviridae*, has a triple layered protein capsid of 75 nm, surrounding the viral genome consisting of 11 segments of double-stranded RNA, which encodes six structural (VP1, VP2, VP3, VP4, VP6 and VP7) and six non-structural proteins (NS1, NS2, NS3, NS4, NS5 and NS6) (Greenberg and Estes, 2009). Rotaviruses have been classified into seven serological species or groups (A–G) based on the antigenic properties of inner capsid protein (VP6) according to the International Committee on Taxonomy of Viruses (ICTV) (Ball et al., 2005). The RVs belonging to species A, B and C (RVA, RVB and RVC, respectively) are known to infect human and various animals, whereas species D, E, F and G (RVD, RVE, RVF and RVG, respectively) thus far have only been recovered from animals, mostly birds (Ball et al., 2005; Matthijnsens et al., 2010). Epidemiologically, RVA is the most important cause of human and animal diarrhea. Specifically, RVA strains have been categorized based on (i) the antigenic

properties of VP6, VP7 and VP4 (subgroups, G-serotypes and P-serotypes, respectively); (ii) the migration pattern of the RNA genome segments when subjected to polyacrylamide gel electrophoresis (long, short, super-short or atypical electropherotypes) and (iii) nucleotide sequence analysis (genotypes) (Estes and Kapikian, 2007). To date, 27 G types and 35 P types of RVA have been identified in humans and animals (Matthijnsens et al., 2011).

The update on prevalence of various genotypes of RVs in different host species belonging to distant geographical locations of India is required for better understanding of interspecies transmission; evolution and in designing vaccine, but several geographical locations in India remain unexplored, with regard to detection and prevalence of RV genotypes. To further explore the epidemiology and genotypic diversity of RV in Madhya Pradesh state, part of central India, we report the findings of RV prevalence and genotypes distribution in different livestock animals and human.

### MATERIALS AND METHODS

#### *Clinical samples and processing*

A total of 733 stool samples were collected from buffaloes (309), cattle (105), goats (25), pigs (69) and poultry (139) in addition

to diarrheic human patients (n=86) during April, 2004 to August, 2008 from Jabalpur and surrounding areas of Madhya Pradesh, central India. Majority of these samples were collected from young ones of animals (less than 6 months of age) or children below 5 years of age who were hospitalized with suspected rotavirus gastroenteritis or visited hospital for treatment of acute dehydration due to enteric infection. All the samples were processed by making 10% (w/v) suspension in phosphate buffered saline (pH 7.2) and centrifuged at 10,000g for 30 min to remove coarse materials. Supernatants were filtered through 0.45 µm syringe filters before storage at -20°C for further use.

#### **Electrophoretic characterization of dsRNA**

The collected stool samples were processed for the detection and confirmation of rotavirus by RNA-PAGE and the genome segments were visualized by silver staining as described in our previous studies (Malik et al., 2011, 2012). The extracted RNA was assessed qualitatively and quantitatively using a Nanodrop Spectrophotometer (ND-1000, ThermoScientific, USA).

#### **Reverse transcription-polymerase chain reaction (RT-PCR)**

The first strand cDNA synthesis following PCR for the amplification of full length VP7 (1062 bp) and partial length VP4 gene (864 bp) was carried out as described in our previous studies (Malik et al., 2012; 2013) using the published primers for VP7 (Taniguchi et al., 1992) and VP4 (Isegawa et al., 1993). The multiplex nested PCR was done for genotyping as per the method and primers used in earlier studies (Gouvea et al., 1990, 1994; Isegawa et al., 1993; Iturriza-Gomara et al., 2004). Amplified products were resolved by agarose gel electrophoresis (1% w/v) at 100 V for 1 hour in IX TAE buffer with 0.5 µg/ml ethidium bromide and viewed under UV trans-illuminator and documented. Amplified products were directly sequenced for VP7 and VP4 genes using the Big dye terminator sequencing kit v3.0 by automated Genetic analyzer ABI 3130xl (Applied Biosystems).

## **RESULTS AND DISCUSSION**

Enteric infections with severe diarrhea are accountable for more than 5% of all deaths among children below 5 years of age and in animals mainly below 6 months of age. Viral pathogens are the most common cause of gastroenteritis in both developing and developed countries (Boschi-Pinto et al., 2008; Dhama et al., 2009; Black et al., 2010) especially rotaviruses (WHO, 2007, 2008; Ramani and Kang, 2007; Tanaka et al., 2007; Parashar et al., 2009; Tate et al., 2009), noroviruses (Radford et al., 2004; Scipioni et al., 2008), astroviruses (De Benedictis et al., 2011) and adenoviruses (Baber and Candy, 1981; McFerran and Smyth, 2000). Rotaviruses belonging to species A, B and C (RVA, RVB and RVC, respectively) are known to infect humans and various animals, whereas species D, E, F and G (RVD, RVE, RVF and RVG, respectively) thus far have only been recovered from animals, mostly birds (Ball et al., 2005; Matthijnsens et al., 2010; Otto et al., 2012). Epidemiologically, RVA is the most important cause for animal as well as human diarrhea.

#### **Prevalence of rotavirus in cattle and buffalo calves**

Though extensive epidemiological studies have described the occurrence of RVs from animals in India (Minakshi et al., 2005, 2009; Ghosh et al., 2008; Manuja et al., 2008; Nataraju et al., 2009; Basera et al., 2010; Kusumakar et al., 2010; Dash et al., 2011; Rai et al., 2011; Singh and Jhala, 2011; Malik et al., 2012), still the information on prevalence and genotypes distribution of RV from central part of India is scarce. The samples collected during the period of April-June, 2004 revealed the presence of group A rotavirus in 34.78% (24/69) of diarrheic buffalo calves, while 2 out of 5 diarrheic samples from cattle calves were also found positive for RV based on genomic pattern migration in RNA-PAGE. Age wise, higher susceptibility to RV infection

was seen in calves of 3-6 weeks old age group (55.55%) followed by up to 3 weeks of age (36.36%). Sex wise, male calves (42.85%) were found more susceptible compared to female calves (28.2%). Subsequently, in 2006 sampling was done to know the prevalence of RVs in animals, in which maximum prevalence was recorded in buffalo calves (23.61%, 17/72) than cattle calves (21.43%, 6/28). In both the study periods, younger ones of less than 3 months of age were more susceptible to virus infection. In contrast to previous year report, in which male calves showed higher susceptibility to infection, during 2006, female calves (25.32%) were slightly more susceptible than males (22.39%). In 2007, 13.3% (6/46) buffalo calves were found positive in comparison to 20% (2/10) cattle calves. In 2008, the prevalence of RV in buffalo and cattle calves was 17.2% (21/122) and 6.45% (4/62), respectively. Overall, RV prevalence was 22.01% in buffaloes and 13.33% in cattle calves over the period, 2004-2008 (Fig.1).

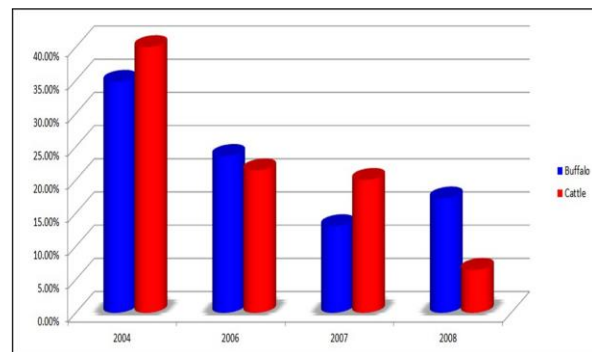


Figure1: Prevalence of rotavirus in cattle and buffalo calves during 2004-2008

#### **Prevalence of rotavirus in piglets**

In 2006, sampling was done from diarrheic piglets to know the prevalence of RV in swine population of the state. Group A rotavirus was detected in 25.7% piglets (9/35), where all RV positive samples showed typical migration pattern of 4:2:3:2 in RNA-PAGE. Surprisingly, in the subsequent year (2008) all the diarrheic piglets (n=34) were found negative for RV infection, which need further evaluation.

#### **Prevalence of rotavirus in human**

In 2006, sampling was done to know the prevalence of RVs in animals as well as humans and its cross species transmission possibility, in which 26.09% (12/46) prevalence of RV was recorded in diarrheic children. Younger ones of less than 1 year of age were found more susceptible to RV infection. In 2008, 35% (14/40) of samples from children were detected positive. All the RV positive samples exhibited 4:2:3:2 type migration pattern (group A rotavirus), while one sample of adult human showed 4:3:3:1 indicative of group B rotavirus. Also long electropherotypes were observed in all except two human samples in which short electropherotypes were observed. Overall, RV prevalence in human was 30.23% over the period, 2006-2008 (Fig.2).

#### **Prevalence of rotavirus in poultry**

The study on avian RV prevalence was conducted during 2006 and virus was detected in 11.68% (9/77) of faecal samples based on migration pattern in RNA-PAGE. More than 75% of birds below 3 months of age showed RV infection, and broilers were comparatively more susceptible to infection than layers. Interestingly, RNA-PAGE profiles of genomic RNA segments of avian RVs revealed the presence of two different groups i.e. group A (5:1:3:2) in seven samples and group D rotaviruses (5:2:2:2) in two samples. This was the first report of group D

rotavirus from poultry in India based on genomic profile in PAGE after silver staining (Savita et al., 2008).

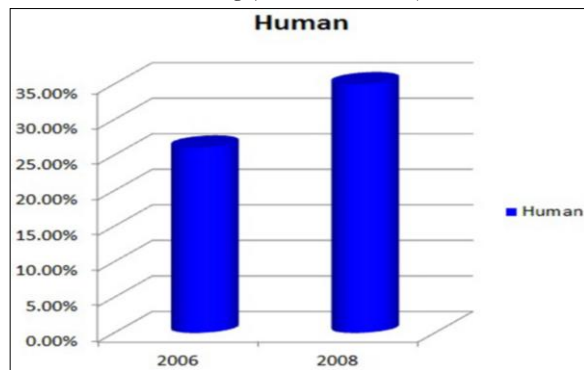


Fig. 2. Prevalence of rotavirus in children below 5 years of age during 2006-2008.

Overall, the prevalence of RVs in central India (Fig. 3a,b) was found maximum in human (30.23%, 26/86), followed by buffaloes (22.01%, 68/309), cattle (13.33%, 14/105), pigs (13.04%, 9/69) and poultry (6.47%, 9/139).

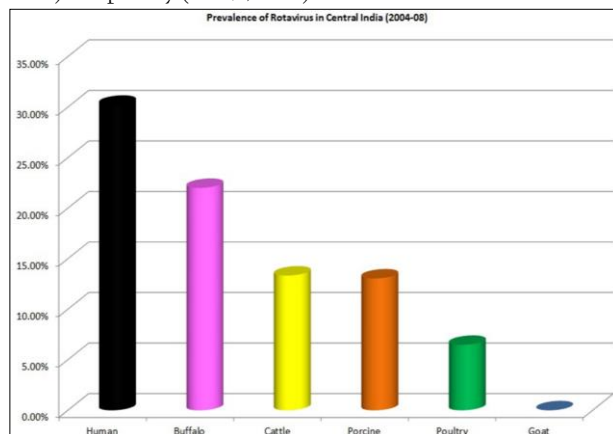


Figure 3(a): Prevalence of rotavirus in human, buffalo, cattle, pigs, poultry and goats (2004-2008).

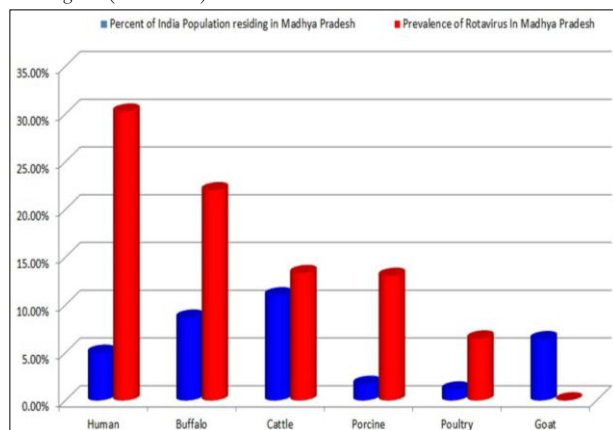


Figure 3(b): Percent of India's population residing in Madhya Pradesh with prevalence of rotavirus in different species of animals and human

In past, it has been observed that the under-five child mortality rate is highest in Madhya Pradesh (89 deaths/1000 live births), approximately three times higher than the Indian under-five child mortality rate (30.15 deaths/1000 live births) (Sample Registration System, Office of Registrar General, India, 7<sup>th</sup> July, 2011) which could be associated with the higher prevalence of rotavirus like fatal infections in children of the state. Though diarrheic samples collected from goats were found negative for RV, but needs further investigation on large number of sample size for the exact scenario of its existence from the region.

#### Genotypic diversity

The rapid evolution of RVs by means of various mechanisms especially through the exchange of genomic segments among the different host specific RVs lead to diverse genotypes and emergence of novel RVs strains having the capability to jump cross-species barrier (Jagannath et al., 2000; Laird et al., 2003; Esona et al., 2004, 2009, 2010; Mascarenhas et al., 2007; Matthijnsens et al., 2008, 2009; De Donno et al., 2009; Bonkoungou et al., 2011; Mukherjee et al., 2012). In India, during late 1990's, the first report on animal group A rotavirus genotypes distribution was published by Gulati et al. (1999) based on G & P typing of rotavirus-positive samples collected during 1994 and 1997, in which G10 was found predominating (83%) over others G6 (6%). The majority (94%) of bovine RVs had P[11] while one isolate exhibited P[1] genotype specificity. The most common combination of G and P types detected was G10P[11] (81%), followed by G6P[1] (3%) and G6P[11] (3%). Since the first report on genotypic distribution of bovine RVs in 1997, it has been identified in diarrheic calves from northern region: Kashmir (G6, G8 and G10), G10P[11] G8+G10P[11]; G10P[11], Haryana and adjoining region (Minakshi et al., 2005; Malik et al., 2013) with predominance of G10 genotype (Basera et al., 2010; Rai et al., 2011), G6P[11] and G10P[3], Haryana and Punjab (Manuja et al., 2008); Eastern region: G3P[3] strains from Kolkata (Ghosh et al., 2007) [G10]; Western region: G8P[14] strains from Pune (Chitambar et al., 2011). Novel and/or unusual bovine group A RV strains have also been reported viz. G10P[3] from northern (Manuja et al., 2008) and G8P[14] from western India (Chitambar et al., 2011). Rare G15P[11] and G15P[21] strains have been identified and characterized from eastern India (Ghosh et al., 2008). G10P[11] has been found in asymptomatic and symptomatic infections in Indian children, and thought to be acquired through zoonotic transmission (Rao et al., 2000; Varshney et al., 2002; Iturriza-Gomara et al., 2004).

During the study period, genomic diversity was studied based on both genomic RNA migration pattern in RNA-PAGE and multiplex nested PCR. The RV isolates detected during 2006 revealed eight different electropherotypic patterns in animals i.e. four in buffaloes, three in cattle, one in swine and five electropherotypic patterns in human. Interestingly, two unique electropherotypes (one common in cattle and swine; another common in buffalo, cattle and humans) were found suggestive of possible cross-species transmission of RVs. Subsequently, in 2007, all (n=8) the RNA-PAGE positive RV samples were genotyped as of G10 specificity, while none of the sample was typed in nested RT-PCR assay for P-genotype. Studies conducted with bovine RV recovered worldwide showed that serotype G6 and G10 predominate in cattle, although samples with specificity to serotypes G1, G3, can also be found (Suresh et al., 2012). The most frequent RV P genotypes detected in bovine faeces are P[1]; P[5] and P[11] (Gulati et al., 1999; Rao et al., 2000; Minakshi et al., 2005; Ghosh et al., 2007; Manuja et al., 2008; Chitambar et al., 2011; Malik et al., 2012; Suresh et al., 2012). Differences were more frequent in the migration of 10<sup>th</sup> and 11<sup>th</sup> segments of RV isolates from cattle, buffalo and human. Both short and long



electrophenotypes were recorded. In human, the most common genotypes identified are G1 genotype. More recently, Malik et al. (2012) described the genotypic distribution of RVs in Indian bovine population with unexpectedly higher proportions of G3 genotype. PCR-genotyping confirmed that 39.4% (13/33) of the prevalent RVs were the G3 type and P typing revealed that 93.9% (31/33) of the samples were P[11]. The changing pattern of genotypic circulation in bovines over the time further needs more extensive molecular epidemiological studies in other species also so as to reach a stage where precise situation of most predominant genotypes can be known. In this study, we found the common bovine rotavirus of G10 genotype in central India and in humans it was of G1 specificity.

#### CONCLUSIONS

The present findings conclude that the occurrence of rotavirus in bovine, porcine, poultry and human population in central India is quite consistent. It also adds to the epidemiological data on rotavirus genotypes circulating in dairy herds of Madhya Pradesh, central India. However, still more elaborate studies should be conducted to estimate the gravity of the situation as well as the epidemiological understanding of contributing viral genotypes in future for the development and implementation of efficient immunization approaches, thereby controlling infection and reducing economic losses.

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