Rotavirus diarrhea in piglets: A review on epidemiology, genetic diversity and zoonotic risks

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ABSTRACT

Pig farming is considered as backbone of rural poor farmers, belonging to lowest socio-economic strata, where mainly unorganized means of pig farming, improper housing, feeding and management are the constraints, and such practices are well known to expose the pig population to a number of infectious and non-infectious disease causing agents. One among those infectious diseases agents is rotavirus (RV), which is the foremost cause of gastrointestinal infections in mammalian and avian species all over the world, also a predominant cause of enteric infections in pigs, and has potential public health concerns. This review provides information on the frequency of RV infection, genotype diversity and zoonotic potential of its emerging reassortants in Indian porcine population. Prevalence studies done so far revealed gruesomely higher porcine RV prevalence in north-eastern region (46.4%) of India. Analysis of available sequence data of VP7 gene (G genotype) and VP4 gene (P genotype) of porcine group A rotaviruses (Po-RV As) showed the presence of G4, G6, G9, G12 and P[6], P[7], P[13] and P[19] genotypes. Lately, few of the porcine RV strains also exhibited unique genomic constellations with evidence of interspecies transmission events, mainly involving RV strains of human origin. Evolution of rotaviruses is fast due to point mutation and reassortants generation in case of multiple infections. Thus it is imperative to generate the baseline information for formulating better disease preventive and control strategy. At present, nationwide surveillance and monitoring is prerequisite to assess the distribution of common and unusual genotypes of RVs circulating among pig population in India.

Key words: Diarrhea, Genotypic diversity, India, Porcine, Prevalence, Rotavirus, Zoonoses

Viral gastroenteritis is arguably the most significant public health problem today throughout the world, with higher momentous situation in developing countries. Rotavirus (RV) has been identified as an assured cause of severe gastroenteritis in mammalian and poultry species throughout the world. The disease is usually seen in young animals and susceptibility to the disease decreases as the age progress presumably because of change in the animal physiology and/or acquired immunity due to previous exposure (Estes and Kapikian 2007). The RV associated viral gastroenteritis in piglets is of great concern not only because of their economic impact in the livestock sector in terms of mortality and morbidity, but also as potential source of heterologous RV infection in humans and many other animal species (Varghese et al. 2006, Chitambar et al. 2009, Dhama et al. 2009, 2010, Tate et al. 2009). Unlike group A, B, C and E rotaviruses reported in porcine population throughout the world so far (Chasey et al. 1986, Smitalova et al. 2006, Kuga et al. 2009), group A rotaviruses (RVA) remain more prominent (Estes and Kapikian, 2007, Miyazaki et al. 2013). Further, RVAs are the most important due to their high prevalence and pathogenicity in human as well as in pigs (Papp et al. 2013). RVs are member of the genus Rotavirus within the family Reoviridae. Its genome entails 11 genomic segments of double stranded RNA (dsRNA) with size from 0.6 to 3.3 kb encoding 6 structural (VP1-VP4, VP6 and VP7) and 6 non-structural proteins (NSP1-NSP6) (Estes and Kapikian 2007). VP1 protein is a RNA-dependent-RNA polymerase and for its correct functioning, VP2 protein is required. VP3 and VP1 forms complex and plays a central role in
transcription. The outer spike protein is VP4 in the outer capsid, which helps the virus for attachment to the host cells thereby contributing in RV virulence. A non-structural protein NSP3 plays a major role in the process of translation. NSP4 protein acts as an enterotoxin of the virus as well as virulence factor that increases the concentration of calcium ion (Ca2+) intracellularly thus distressing the host cellular homeostasis (Estes 2003). Exploration of the physico-chemical characteristics of RVs revealed higher stability as well as resistance to ether, chloroform, quaternary ammonium compounds and chlorination procedures, but sensitivity to phenol as well as formaldehyde is high (Murphy et al. 1999, Song et al. 2006).

The convenience of modern biotechnology assays added a newer dual classification system (G and P genotypes) for RVA targeting the outer-layer capsid proteins of virus i.e. VP7 and VP4, which independently elicit neutralizing antibodies (Varghese et al. 2004). Thus, RVA strains are classified into VP4 or P types (for protease-sensitive) and VP7 or G types (for glycoprotein) (Estes and Kapikian 2007). Till now, 27 G and 37 P types have been documented in human and animal RVA infections (Mukherjee et al. 2013, http://rotac.regatools.be/classificationinfo.html). During a surveillance in New Delhi molecular characterization of several non-typeable strains of rotaviruses have been done and it has been found that most of the strains belong to G1 genotype or P[8] genotype based on sequencing of nucleotide fragments from the VP7 as well as VP4 genes. Acquisition of the VP7 along with VP4 and NSP4 genes can result in generation of new strain of the virus (Sharma et al. 2009). Though, several RV genotypes or new genetic variants of a specific genotype have been witnessed in connotation with outbreaks of RV diarrhea in suckling piglets throughout the world (Barreiros et al. 2003, Lorenzetti et al. 2011, Miyazaki et al. 2011), this review presents the information on prevalence of RVs, genotypic distribution in piglets from India and public health concerns of the emerging reassortants pig RV strains.

**Epidemiology of porcine RVs**

The diversity of RV strains is predominantly increased by accretion of point mutations leading to genetic/antigenic drift and reassortment of cognate genes leading to genetic/antigenic shift. The documentation of emerging RV strains in animals is of utmost prominence for instigating the control measures for the virus infections in both animals and humans. Therefore, more apprehension has been on the information congregation on RV flowing in the environment. In the adult pigs, the rate of sero-conversion due to RV infection is almost 100%. Thus, in 2–4 weeks age group of piglets, the rotaviral outbreak is common (Murphy et al. 1999). The prompt RV evolution as of a variety of mechanisms offers the foremost challenges in epidemiological surveys of RV infection. Substantial focus has been paid on RV disease in India. Since the very first RV detection in porcine of India (Barman et al. 1998, Jhala and Raghavan, 1998, Jain et al. 2001a), the porcine RVs have been documented from eastern, north eastern region, northern, central and western parts of the country (Barman et al. 1998, 2003, Nath et al. 2004, 2007, Bora et al. 2007, 2009, 2011, Kusumakar et al. 2008, Kumar et al. 2011, Dubal et al. 2013) as summarized in Table 1.

In India, most of the records on RV epidemiology have originated from north-eastern part of the country where pig farming constitutes major share (40%) of pig population of the country (Fig. 1a), and is directly related to the livelihood of poor farmers in general and tribal farmers in particular. The very first porcine RVAs prevalence study came out from North-eastern part of the country in late 1990’s, where 26.9% serum samples were found positive for RV antibodies using sandwich enzyme-linked immunosorbant assay (ELISA) (Barman et al. 1998, Kang et al. 2005). The highest incidence was recorded in 4-week-old piglets (53.1%) followed by 2-week (46.1%) and 3-week old piglets (48.8%). In a subsequent study, Barman et al. (2003) detected RV antibodies in 51.1% of the serum samples by ELISA. The prevalence of RV antibodies was more in...
During winter (41.1%), semi-intensive system (24.2%), diarrhoeic pigs (38.2%) and 0–2 month’s old piglet (31.25%). Higher percent of RV (128/560) of the faecal samples with highest incidence in 7.33±0.67 days. The excretion of RV in faeces of these duration of excretion in pre- and post-farrowing was 5.33±0.33 days, respectively, while in gilts the average excretion at pre- and post-farrowing in sows was 5.66±0.33 days.

The first report of isolation of porcine RV recovered from diarrhoeic piglets in MA-104 cell line came in the year 2007 from Asom (Bora et al. 2007). The prevalence of RV in pigs (53.9%) compared to open grazing pigs (45.5%). Later, in a study from the same region, 73.8% (307/416) of pigs were recorded positive for RV infection employing indirect ELISA (Kelkar et al. 2004, Nath et al. 2004). They established the higher prevalence of RV infection (85.48%) in pigs of more than 8 months age than any other age group, and pigs reared under organized semi-intensive farming were more susceptible to RV infection in comparison to unorganized semi-intensive and open range rearing. In a succeeding cross-sectional and cohort comparison to unorganized semi-intensive and open range intensive farming were more susceptible to RV infection in any other age group, and pigs reared under organized semi-intensive farming was accomplished.

On exploitation of the ribonucleic acid-polyacrylamide gel electrophoresis (RNA-PAGE) technique easy separation of all the 11 segments of the RV has become possible and researchers were able to generate an RNA electropherotype, which is a characteristic visual pattern (Chauhan and Singh 1993, Song et al. 2006). The prevalence of RV in pigs from Madhya Pradesh was 25.7% (9/35) with detection of long electropherotypes (based on migration of 10th and 11th segment of RV 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 (Kusumakar et al. 2008, Malik et al. 2013).

The RV infection was more common in piglets of up to 8 week age or weaned piglets (50%) than piglets of above 3 months of age (7.7%). In another study from northern region of the country (Bareilly, Uttar Pradesh), no RV was detected in pig population using RNA-PAGE and reverse transcription polymerase chain reaction (RT-PCR) (Kumar et al. 2011). The RV infection was more common in piglets of up to 2 months age from the date of farrowing. The piglets excreted RV from eighth day onwards and continued up to sixth week of their life. Some of the studies carried out in past demonstrated that the enteritis in piglets is complicated by secondary pathogens (Das et al. 2008, Neog et al. 2009). The clinicopathological and hematological studies conducted in RV infected piglets alone or in combination with bacterial infections have revealed rise in body temperature (103–105°F) followed by profuse diarrhea and dehydration, along with lymphopenia and neutrophilia. Significant increase in haemoglobin (Hb), packed-cell volume (PCV), erythrocyte sedimentation (ESR) and serum potassium level and decrease in the levels of serum sodium, chloride and bicarbonate in RV infected animals were also documented (Desselberger et al. 2001, Das et al. 2008, Jain et al. 2001b, Neog et al. 2009).

Recently, the screening for the presence of RV in faeces of piglets from different parts of the India was accomplished by Dubal et al. (2013), in which they tested 275 faecal samples (180 diarrheal and 95 non-diarrheal) from piglets from the western, southern, northern and north-eastern hill

### Table 1. Descriptive porcine rotavirus surveillance studies done so far in India from 1998–2013.

<table>
<thead>
<tr>
<th>Authors</th>
<th>Regions surveyed</th>
<th>% Prevalence</th>
<th>Detection methods</th>
<th>Antigen (RV)/antibody (Abs) detected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Barman et al. (1998)</td>
<td>North-Eastern Region</td>
<td>26.9% (34/126)</td>
<td>ELISA</td>
<td>RV</td>
</tr>
<tr>
<td>Barman et al. (2003)</td>
<td>Asom</td>
<td>51.1% (270/528)</td>
<td>ELISA</td>
<td>RV</td>
</tr>
<tr>
<td>Nath et al. (2004)</td>
<td>Asom</td>
<td>73.8% (307/416)</td>
<td>ELISA</td>
<td>RV</td>
</tr>
<tr>
<td>Nath et al. (2007)</td>
<td>Asom</td>
<td>22.86% (128/560)</td>
<td>PAGE</td>
<td>RV</td>
</tr>
<tr>
<td>Bora et al. (2007)</td>
<td>Asom</td>
<td>29.35% (423/1441)</td>
<td>ELISA</td>
<td>RV</td>
</tr>
<tr>
<td>Bora et al. (2009)</td>
<td>Asom</td>
<td>65.4% (942/1441)</td>
<td>PAGE</td>
<td>RV</td>
</tr>
<tr>
<td>Kusumakar et al. (2010)</td>
<td>Madhya Pradesh</td>
<td>25.7% (9/35)</td>
<td>PAGE, PCR</td>
<td>RV</td>
</tr>
<tr>
<td>Kumar et al. (2011)</td>
<td>Uttar Pradesh</td>
<td>0% (0/47)</td>
<td>PAGE, PCR</td>
<td>RV</td>
</tr>
<tr>
<td>Dubal et al. (2013)</td>
<td>Western Region</td>
<td>7.4% (10/135)</td>
<td>PAGE, PCR</td>
<td>RV</td>
</tr>
<tr>
<td></td>
<td>Northern Region</td>
<td>0% (0/20)</td>
<td>PAGE, PCR</td>
<td>RV</td>
</tr>
<tr>
<td></td>
<td>Southern Region</td>
<td>0% (0/60)</td>
<td>PAGE, PCR</td>
<td>RV</td>
</tr>
<tr>
<td>North-Eastern hills</td>
<td></td>
<td>30% (18/60)</td>
<td>PAGE, PCR</td>
<td>RV</td>
</tr>
</tbody>
</table>
vaccines. It is easy to derive neutralizing monoclonal antibodies thereby allowing antigenic classification of the viruses most frequently detected and so also they may be detected in non-diarrheic piglets. Both enzootic as well as epizootic forms of diarrhea are caused by RVs that result in losses commercially in piggeries. The mechanisms of synergism is triggered by other pathogens that worsen the disease condition in piglets. In India more than 50% of the piglets positive for RVA are also found positive for either caliciviruses or rotaviruses belonging to group C (Saif and Fernandez 1996).

Global epidemiologic surveys have identified G3, G4, G5 and G11 as the most common G genotype and P[6] and P[7]) as the most common P genotype associated with diarrhea in pigs. Also rare genotypes (G1, G2-like, G6, G8, G9, G10, and G12) were reported in pigs which are commonly associated with humans and cattle (Bellinzoni et al. 2005, Marthaler et al. 2007, http://rota.regatools.be/classificationinfo.html). On analysis of the VP1, VP2, VP3, VP4, VP7 and NSP4 genes identification of genetic relatedness has been done in comparison to recent human rotavirus (HRV) strains. These research findings cumulatively points to the facts that there may be evolution of new reassortant viruses (Steele et al. 2004, Varghese et al. 2004, Mascarenhas et al. 2007). By employing RNA electrophoresis several porcine samples were found positive for rotaviral infection between the years 1998–2000 at Kolkata. Genotyping of the G (VP7 genotype) as P (VP4 genotype) has been done by reverse transcription as well as multiplex PCR (Gentsch et al. 1992).

High degree of genetic reassortment was found in samples collected from Manipur (Das et al. 2002). It was found that group A rotaviruses (RVAs) of porcine are found in association with weaning as well as post-weaning enteritis in case of piglets both worldwide as well as in India. In piglets aged between 1 and 8 weeks of age RVAs are the viruses most frequently detected and so also they may be detected in non-diarrheic piglets. Both enzootic as well as epizootic forms of diarrhea are caused by RVAs that result in losses commercially in piggeries. The mechanisms of synergism is triggered by other pathogens that worsen the disease condition in piglets. In India more than 50% of the piglets positive for RVA are also found positive for either caliciviruses or rotaviruses belonging to group C (Saif and Fernandez 1996).

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The available information on epidemiological surveys from India clearly indicated that RVAs are the most significant viral contributors to the burden of diarrheal diseases in piglets and huge genetic diversity within porcine RV strains exists. In India, 4 types of G (G4, G6, G9, G12) and 4 types of P genotypes (P[6], P[7], P[13], P[19]) of RVAs have been detected from pigs so far. Hitherto, there is no report till now on occurrence of group B and C RVs in pigs from India. Both the diagnosis as well as molecular typing of RVs are performed at present by using RT-PCR (serotype specific); nested / multiplex RT-PCR as well as restriction fragment length polymorphism (RFLP) (Santos et al. 1999, Song et al. 2006). Sequencing analysis of the VP6 as well as VP7 genes as well as microarray hybridization (oligonucleotide) are in use for identification of mixed viral infections (Fischer and Gentsch 2004, Ghosh and Kobayashi, 2011). Diarrhea due to RV must be
differentiated from *E. coli* associated infectious diarrhea in pigs, *Clostridium perfringens*, coronavirus, calicivirus or astrovirus infections. For this use of multiplex RT-PCR was found suitable (Santos et al. 1999, Smitalova et al. 2009, Wang et al. 2010). Detection of new/rare genotypes or mixed infection evokes the need for continued surveillance to assess and consider the strain variability in the design of the suitable vaccine candidate.

**Zoonotic potential of novel RV strains**

Usually the rotaviruses are species-specific but there is a possibility of cross-species transmission. Various case studies worldwide and in India indicated that infection in humans can be caused by the animal rotaviruses. Close identity is often revealed between the human and animal rotaviruses when the genetic sequences are compared. It is suggestive by seeing the incidence of uncommon strains at lower rate that such transmission or at least establishment of animal rotaviruses or a reassortants human/animal virus in the human population does not occur with a very high frequency. Many researchers therefore suggested that the input of strains of rotaviruses or their genetic sequences from animal to human population may be a continuous process but of course at a very low level (Cook et al. 2004, Tate et al. 2009).

The evolution of porcine RVs, especially through the exchange of genomic segments among the different host specific RVs, leads to emergence of novel porcine strains having the capability to infect human beings (Fig. 2). Explicit reports are available confirming the reassortment events going on between human and porcine RV strains and emergence of RVs strains with rare genotypes. In one of such study, Varghese et al. (2004) characterized a rare genotype with G9P[19] specificity having long

![Fig. 2. Porcine rotavirus interspecies transmission cycle. Exchange of genomic segments among different hosts. Specific rotaviruses within the host leads to emergence of new strains/genotypes. RVA genotypes (G and P) circulating in Indian porcine population are also presented. HRA: Human group A rotavirus; PRA: Porcine group A rotavirus.](image)

electropherotype with subgroup I specificity, isolated from an epidemic (1987–88) of infantile gastroenteritis in Manipur, India. The nucleotide sequences of VP4, VP6, VP7, NSP1, NSP2, NSP3, NSP4 and NSP5 genes of this rare genotype were deciphered and on phylogenetic analysis, all the genes except VP7 were found closely related to porcine RVs, while VP7 gene of this RV strain clustered with human RV strains. Isolation of porcine rotaviral strains like G3 and G5 was done from the human population from various parts of the world (Ra´cz et al. 2000, Desselberger et al. 2001, Malik et al. 2005). In another study, Ghosh et al. (2006) reported first time detection of a porcine RVA strain (RU172) with G12P[7] genotype specificity from suburban area of Kolkata city, India and this strain on comparative sequence analysis clustered with human G12 strains with maximum identities of 93.6% to 94.5% at deduced amino acid level. In spite of its G12 genotypic nature, RU172 strain formed a separate lineage with human G12 strains on phylogenetic analysis. The other gene segments (VP4, VP6 and NSP5) of this strain showed porcine origin on analysis. Detection and characterization of strains like RU172 provided dynamic insight into the role of reassortment events in genomic diversity of RVA of man and pigs.

Subsequently, Ghosh et al. (2007) characterized 2 porcine RVA strains (HP113 and HP140) from eastern India and these strains were unique in the sense that VP7 gene was closely related to those of human G6P[14] strains, VP4 with a borderline P[13] genotype, and VP6 related to bovine and human sub-group I (SGI) strains rather than porcine SGI and/or SGII RVAs. Both the strains had NSP4 and NSP5 genes of porcine origin. This was the first report of detection of RVA strains with G6P[13] genotype specificities with evidence for independent segregation of the VP6- and NSP4-encoding genes in porcine RVAs. Chitambar et al. (2009) characterized a novel human RV strain (NIV929893) with a rare specificity of G1P[19]. The phylogenetic analysis of amino acid sequences of the VP7 and VP4 gene products showed clustering of the VP7 gene with G1 strains of human origin while the VP4 gene with P[19] strains of porcine origin. The findings of this study provide evidence of reassortment between VP7/VP6 genes of humans and VP4/NSP4 genes of porcine species. Recently, Dubal et al. (2013) reported prevalence of porcine RV of G4, G9 and P[6] specificity with identification of an uncommon strain of G4P[6] specificity. The Indian porcine RV genome sequences retrieved from The National Center for Biotechnology Information (NCBI) database are compiled and presented in Table 2. The sequence data compilation from public domain illustrates that till collation of this review, 19 sequences are available in the sequence databank from India and mostly have been used a partly overlying portion of the RV genes. The HP 113, HP 140 and RU172 PoRV strains isolated from pig farms of Kolkata, India have been elucidated in detail. The VP7 gene of one more PoRV strain (Ro1) was also typed as G4 (EU445113, 1062 bp).
Future perspectives

RVs are well-recognized cause of substantial losses and health problems both in developed and developing countries. Results validate existence of huge genetic diversity among Indian porcine RV strains and present that pig farms may be the plausible source and likely reservoirs for human infections. In India, the rotavirus epidemiological profile is complex in nature thereby highlighting the requirement for a unified surveillance protocol of the strains that are circulating by the laboratories in India. Porcine RV prevalence in India was unexpectedly higher when surveyed, with maximum in the north-eastern region. Overall, epidemiological studies revealed the occurrence of porcine RVA strains with G4, G6, G9, G12 specificity and P types were P[6], P[7], P[13], P[19]. Emergence of some novel/unusual strains by mechanism of reassortment among the rare combinations of prevailing porcine RV strains showed the need for exhaustive surveillance of RV infection in Indian settings, before entering attempts for a new vaccine. Compiled epidemiological profile of porcine RVs in India will be of considerable importance to both policy makers and vaccine inventors for designing an effective and potent vaccine for safeguarding porcine industry from this important pathogen having public health concerns. Along with monitoring, frequent updating of the methods of virus genotyping are essential on a national scale.

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