**INTRODUCTION**

Rotavirus (RV) is a major cause of acute gastroenteritis in human children (Bishop et al., 1973; Flewett et al. 1973), as well as in other mammals (Adams & Kraft, 1963; Malherbe & Harwin, 1963; Mebus et al., 1969) and birds (Otto et al., 2012; Trojnar et al., 2009). It still causes around 453,000 deaths annually among young children (Tate et al., 2012). More than half of the reported childhood mortality cases were reported from less-developed nations (Parashar et al., 2009; Tate et al., 2012). Despite lower mortality rates, RV is also an issue in developed nations. Before the wide use of vaccines, RV only caused an estimated 25-37 deaths annually in the United States. However, there were approximately 59,600 hospitalizations and the cost to the economy was around $893 million (Fischer et al., 2007; Widdowson et al., 2007; Esposito et al., 2011). RV-induced morbidity and mortality in farm animals also result in further economic losses (House, 1978; Martella et al., 2010).

*Viral Genome*

RV is a Group III double-stranded RNA virus in the *Reoviridae* family. Its genome is around 18,000 bp and consists of 11 segments numbered by decreasing size, ranging from 3,300-3,500 bp (segment 1) to 630-730 bp (segment 11) (Nagashima et al., 2008). These segments encode for six structural viral proteins (VPs: VP1-VP4, VP6 and VP7) and six non-structural proteins (NSPs: NSP1-NSP6) (Desselberger, 2014). NSP6 is only produced in some species of RV by using an out-of-phase open reading frame in the segment encoding for NSP5 (Mohan & Atreya, 2001). VP4 can also be cleaved by a protease such as trypsin to form VP5 and VP8 (Crawford et al., 2001). As the individual segments are numbered according to size, a segment with the same number can encode different proteins in different species or strains (Chen et al., 2002; Mlera et al., 2012; Desselberger, 2014).

*Classifications and Epidemiology*

Based on the antigenicity of VP6, RV is classified into eight different species (RVA-RVH) which differ in epidemiology (Matthijnssens et al., 2012). Of the eight, RVA, RVB and RVC are more widely studied. RVA is the most prevalent worldwide, accounting for >90% of all reported RV infections (Gentsch et al., 2005; Santos & Hoshino, 2004). RVC infections have also been reported globally, but in smaller numbers (Kuzuya et al., 2007; Schnagl et al., 2004; Araújo et al., 2011; Chang et al., 1999). In contrast, RVB infections typically occur in Asia (Tao et al., 1984; Kelkar and Zade, 2004; Saiada et al., 2010).

While RVA, RVB and RVC can be found in both humans and non-human animals, RVD, RVE, RVF and RVG are mainly restricted to animals such as birds or pigs (Estes & Kapikian, 2007). Reports on RVH infections are currently limited to pigs and humans (Jiang et al., 2008; Marthaler et al., 2014; Molinari et al., 2014). However, since most samples are obtained from patients and diseased animals, non-clinical or sub-clinical species such as RVC tend to be under-represented in prevalence estimates (Collins et al., 2008).

Additional G/P-genotyping (Glycoprotein/Protease-sensitive protein) using the antigenicities of VP7 and VP4, and genotyping based on all RV proteins, have been established for RVA (Matthijnssens et al., 2008; Matthijnssens et al., 2011) The same G-genotyping method has also been adapted for RVB and RVC (Marthaler et al., 2012; Marthaler et al., 2013).

*Immunity and Vaccines*

Children infected with RV will obtain immunity for subsequent infections (Bishop et al., 1983). For adults who have experienced repeated viral challenges, infections tend to be asymptomatic as memory B cells provide both long-term and heterotypic protection (Franco et al. 2006). This is achieved via humoral antibodies for VP4 and VP7, which are on the capsid (Offit, 1994; Feng et al., 1997; Jiang et al., 2002). These observations spurred the development of live-attenuated vaccines for RVA, including the monovalent Rotarix® (GlaxoSmithKline) in 2005 and the pentavalent RotaTeq® (Merck) in 2006 (Ruiz-Palacios et al., 2006; Vesikari et al., 2006). Both vaccines were generally successful, with vaccine efficacy of around 90% in the United States (Patel & Parashar, 2009; Tate et al., 2011a; Tate et al., 2011b; Cortese et al., 2013). They also have been found to provide herd immunity for unvaccinated children (Anderson et al., 2013; Yi and Anderson, 2013; Pollard et al., 2015).

*Significance of Study*

Despite successes with existing vaccines, a few issues affect their implementation, particularly in less-developed countries. First, RV has high mutation rates across its genome. This is due to frequent point mutations contributed by the error-prone RNA-dependent RNA polymerase (Blackhall et al., 1996), as well as re-assortments arising from co-infections of different strains (Iturriza-Gomara et al., 2001) and zoonotic transmissions (Todd et al., 2010; Steyer et al., 2008; Matthijnssens et al., 2011; Steyer et al., 2013). As a result, a rapidly evolving RV genome may render genotypic-specific vaccines less effective over time. Secondly, the efficacy of vaccines is found to be reduced in less developed countries, and plausibly requires further modifications to better adapt to different host populations and geographical locations (Lopman et al., 2012; Glass et al., 2014).

As such, phylogenetic analyses of RV may elucidate details on its future evolution, and the genotypic differences due to epidemiological factors. It will also aid the development of alternate vaccines or antivirals for RVA and other RV species. For instance, VP6 is also a potential vaccine target, as VP6-specific antibodies developed after infection were also shown to be protective (Burns et al., 1996; Jalilvand et al., 2015).

*Purpose*

Previous studies indicated that species of RV are separated into two major clades (Kindler et al., 2013). Separation between mammalian and avian RVA were also observed, along with host-specific evolution in RVB and RVC (Kindler et al., 2013). Additionally, genome constellations of RV were found to be stable, and a study on one strain of RVA only identified 6 codons under diversifying selection (Mcdonald et al., 2009; Zeller et al., 2015).

To expand the scope of current phylogenetic analysis on RV, the most sequenced VP7 gene from prevalent species – RVA, RVB and RVC – was studied. Differences in the evolution of RV between species and segments were elucidated through detecting diversifying selection and estimating mutation rates. The presence of zoonotic transmission was also analysed using reconstructed phylogenies.