**DISCUSSION**

*Comparison between Species*

RVA had a much higher diversifying selection estimate and variance of mutation rates for VP7, correlating to its huge variety of G genotypes based on VP7. As such, it may not be efficient to base future vaccines or drugs solely on a few genotypes of VP7.

RVB had higher mutation rates than RVA and RVC, especially in genes for NSP1 and NSP4. This observation was supported by high diversifying selection estimates. As both the NSP1 involved in suppressing host interferon response and the NSP4 enterotoxin are involved in host invasion, the high mutation rates and diversifying selection pressures may aid colonisation of new hosts and niches. Hence, although RVB is currently limited to Asia, we should be vigilant against its expansion to other geographical regions.

*Comparison between Proteins*

Between proteins, the structural VP proteins had lower diversifying selection and mutation rate estimates than non-structural NSP proteins. This suggests that the structural proteins are less tolerant to change or are less targeted by the immune response.

Proteins with core functions, such as the VP1 RNA polymerase and the NSP2 NTPase/helicase essential for dsRNA synthesis, also had lower diversifying selection and mutation rate estimates than those involved in host invasion, such as the NSP4 enterotoxin and the NSP1 involved in suppressing host interferon response. In particular, the high mutation rate of the gene for NSP1 may be responsible for evading innate immunity and correlates with the high incidence of the disease in young children.

Negatively-selected genes may be suitable targets for vaccines because changes to generate escape mutants are likely to be intolerable (Suzuki, 2004). Hence, this study also confirms the viability of utilising VP6 as a future vaccine and drug target across all species as its diversifying selection and mutation rate estimates were consistently the lowest.

*Zoonotic Transmission*

The main RV hosts other than humans are domesticated animals including pigs and cows. Despite close human contact with these animals, only RVA displayed both direct and reverse zoonosis. As previous studies (Martella et al., 2010) also showed evidence of zoonotic transmission for RVA, it is crucial that future RVA vaccines continue to target sequences of both human and non-human RVA strains.

Even though RVB and RVC from animals cannot infect humans directly, we should still be wary of the possibility of genetic reassortments between these species and the zoonotic RVA during co-infection events in animals. It is more likely for RVB or RVC to gain zoonotic capabilities from RVA than to evolve the function *de novo*. Therefore, phylogenies of RVB and RVC should be reviewed frequently for any RVA sequences in their genomes.

*Future Directions*

Other species RVD-RVH can also be studied. As they were omitted from this project due to insufficient sequence data, greater sampling efforts could yield useful insights, especially for human infecting species such as RVH.