The Effect of Environmental Selection Pressure on the Rate of Recombination to an Advantageous Receptor Mutation in Bovine Coronavirus

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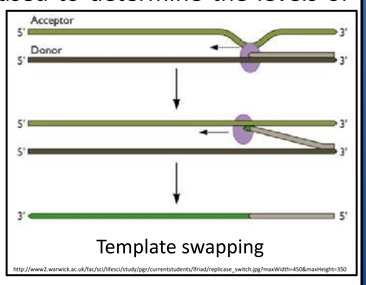
INTRODUCTION

Understanding the very nature of infectious diseases allows for the prevention of new outbreaks as well as the development of drugs and vaccines. Three fourths of newly identified human pathogens are viruses, highlighting a need to better understand these rapidly changing pathogens. At the heart of these are mostly RNA viruses, the fastest when it comes to evolution [5]. RNA viruses replicate and may recombine quickly, due mostly to their error-prone RNA-dependent RNA polymerase (RdRp) which produces mutations in each replication cycle [1]. The RdRp also leads to frame shifts due to the secondary structure of the RNA as well as template swapping, starting on the genome of one strain and "jumping" to the genome of another co-infecting strain [4]. The lack of proofreading mechanisms that many other organisms rely on to prevent lethal mutations allows RNA viruses to balance on the edge of extinction in order to fully maximize their variability [1]. All of this leads up to the production of a highly variable and responsive population that scientists have dubbed "quasispecies" [6]. These large RNA virus populations hold many different variations of their genome, maintained by the very nature of their reproduction and the ability for the collective gene pool of the population to shift to accommodate host environment. It's this very reason that of all the emerging infectious diseases identified since 1940 (about 400), more than 60% have been zoonotic [3]. In other words, in the past 75 years over 240 diseases have been able to swap hosts.

STUDY OVERVIEW

- Many of the most recent viral outbreaks have been attributed to RNA viruses that have one, or more, animal reservoirs [1]
- Bovine Coronavirus (BCoV) is a single-stranded, positive sense RNA virus
- BCoV part of the family *Coronaviridae*, which also contains the viruses that cause Severe Acute Respiratory Syndrome (SARS) and Middle Eastern Respiratory Syndrome (MERS)
- The goal of this study was to observe changes in genetic makeup of the virus' outer membrane Spike protein via recombination between two BCoV strains (Nebraska and Mebus)
- Nebraska strain contains a 12 nucleotide insert (which Mebus does not) that may cause trypsin-free cell entry because it encodes three positively charged amino acids (Arginine)
- A 1:100 mix of Nebraska to Mebus was used to infect HRT-18 cells to observe infection/replication speed
- RT-PCR and TaqMan PCR was used to determine the levels of each virus
- It was found that the presence of the Nebraska strain allowed for faster replication in trypsin-free media
- Quasispecies are a crucial part of understanding future viral outbreaks



Template swapping

METHODS

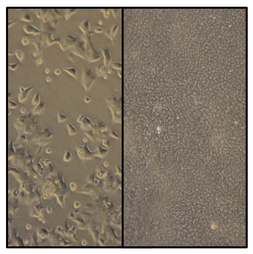


Fig. 1: Human Rectal Tumor cells (HRT-18) were grown in wells to produce monolayers (seen in the right image).

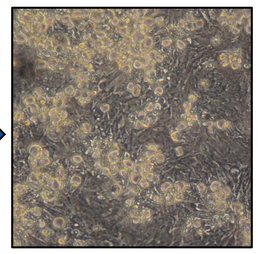


Fig. 2: Cells were infected with virus at MOI 0.001. Above image shows cytopathic effect (CPE) caused by the virus.

Fig. 3: RNA was extracted from the supernatant (using a Trizol extraction method) and converted to cDNA (using Invitrogen's SuperScript III kit)

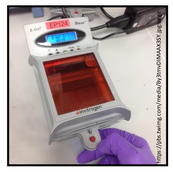


Fig. 5: cDNA amplified via the thermocycler was then run on a 4% agarose E-Gel (Invitrogen)

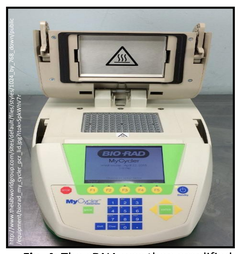


Fig. 4: The cDNA was then amplified using a thermocycler (Bio-Rad)

Fig. 6: PCR product from the thermocycler was then amplified via TaqMan PCR on the Bio-Rad iCycler to quantify the level of insert region in each sample and in each strain

RESULTS

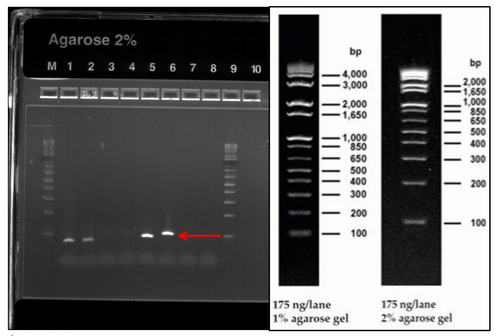


Fig. 7: Confirmation that the insert was present in the Nebraska strain and not the Mebus strain. The band in lane 6 is slightly higher than the band in lane 5, indicating that the amplified DNA in lane 6 (which is the Nebraska strain) is slightly larger.

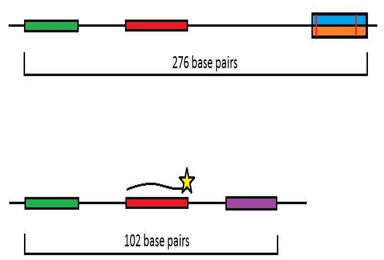


Fig. 8: Primer design was crucial in order to ensure that the data acquired would be useful to the overall experiment. The first primer distinguished between the Mebus and Nebraska strains due to two point mutations located within the reverse primers. The second primer set utilized a smaller region of the already amplified region to determine if the insert sequence was present and at what levels. This was done using a TaqMan probe that bound specifically to the insert region.

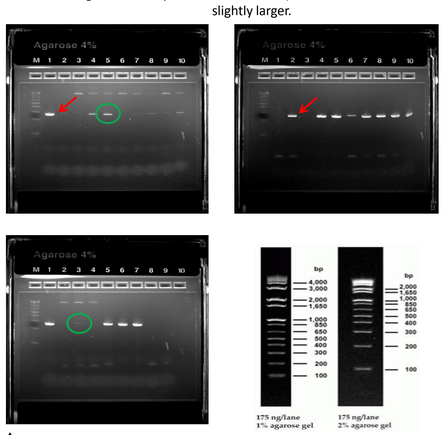
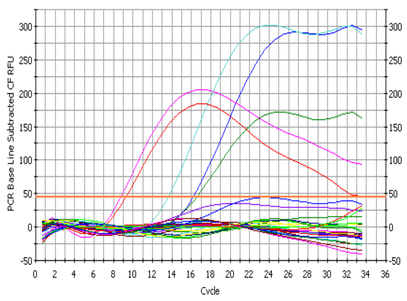


Fig. 9: After the PCR was run, the products were run out onto 4% agarose gel. Around 80% of the bands matched what was expected of each primer set and the specific conditions each sample was incubated in. The red arrows indicate that the primer sets allowed for distinction between Mebus and Nebraska. The green circles indicate that the Nebraska strain was better adapted to trypsin free environments

Fig. 10: TaqMan PCR results were unable to confirm template swapping and the acquisition of the insert region by the Mebus strain. The Nebraska strains showed that they contained the insert and are the only samples to show values above the threshold.



CONCLUSION & FUTURE DIRECTIONS

The experiment confirmed the hypothesis that the BCoV strain Nebraska is able to utilize a trypsin-free environment more easily than the Mebus strain, due to the 12 nucleotide insert found within its Spike receptor. The intensity of the bands in Figure 9 indicated that the levels of Nebraska had risen past the initial 1:100 ratio (more so than the trypsin incubated culture). However, PCR was unable to confirm the evidence of template swapping occurring between the two strains. Template swapping is know to happen regularly with single-stranded, positive sense RNA viruses. It is possible that given time constraints or the lack of an environment that fully favored the Nebraska strain, template swapping was unable to happen. This experiment was still able to illustrate the amazing ability of an RNA virus to adapt to a new environment, much like the adaptation required to jump from host species to host species. A greater understanding of viral quasispecies is needed if we want to better identify, treat and prevent new emerging viruses. Further experiments should look to isolate the cause and effect of viral template swapping.

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