Evolution of coronaviruses in captive ruminants

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Background

- Coronaviruses are single stranded, positive sense RNA viruses of the family Coronaviridae
- Transmitted from animals to humans, causing disease outbreaks e.g. Severe Acute Respiratory Syndrome in 2002
- Cell tropism is mediated by the Spike (S) protein[1]
- The S1 subunit is the target of neutralizing antibodies
- Mutations in the S1 subunit are associated with host switch events *in vitro*[1]
- Bovine coronavirus (BCoV) infection in captive ruminants from the provides an *in vivo* model of S1 gene evolution in a relatively closed population

Hypothesis: Bovine coronavirus variants previously associated with host switch events and escape from neutralizing antibodies *in vitro* are the most abundant variants *in vivo* during primary infection of captive ruminants in a closed herd

Specific Objectives

- Determine the genotypes of Bovine coronavirus isolates circulating in the state of Missouri
- Evaluate intrahost heterogeneity of the S1 gene following infection of captive ruminants (antelopes, kudu, giraffe)

Methods

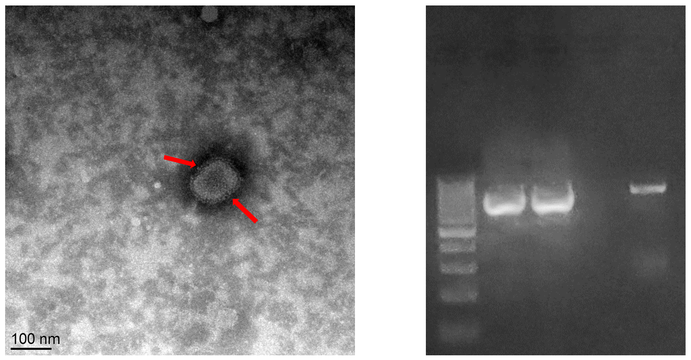

Figure 1. Laboratory diagnosis of BCoV. Electron microscopy of fecal homogenates was used to identify viral particles with the characteristic "corona" of BCoV. Arrows indicate viral Spike protein, which resembles club shaped projections off the viral particle surface. Diagnostic RT-PCR with primer pairs specific for BCoV was used to amplify an 800bp fragment of the nucleocapsid gene.

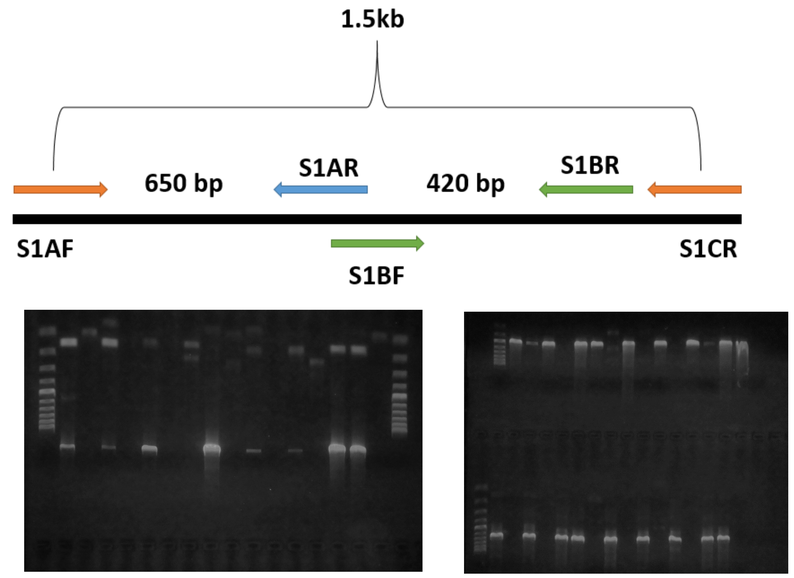

Figure 2. Amplification of the S1 gene. Primer pairs S1AF and S1CR (orange) were used to amplify a 1.5kbp fragment of the S gene (left gel). A semi-nested PCR using S1AF and S1AR (blue) amplified a 650bp fragment and a nested PCR with S1BF and S1BR (green) amplified a 420bp fragment (right gel). Fragments were purified and sequenced using PCR primers.

Results

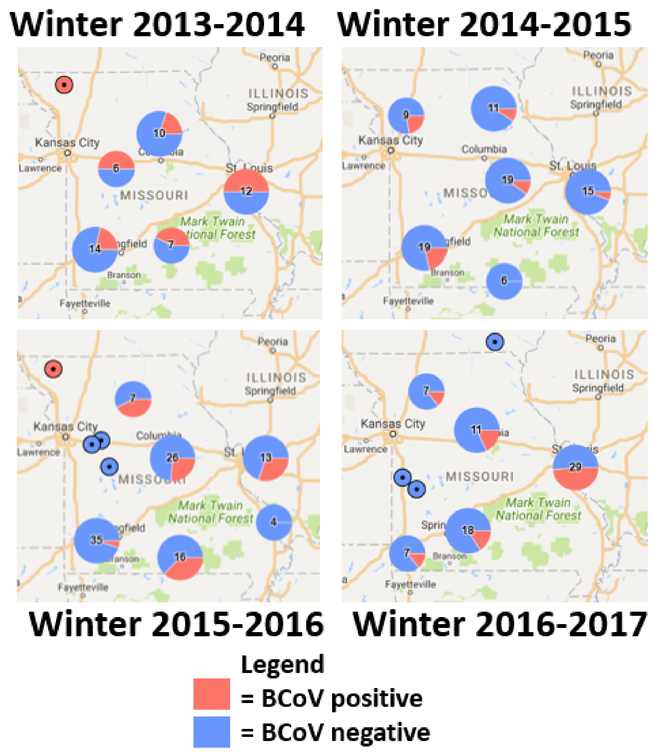

Figure 3. Distribution of samples processed at the VMDL for BCoV diagnosis (2013-2017). Samples were selected for sequencing to represent different zip codes and seasons in Missouri.

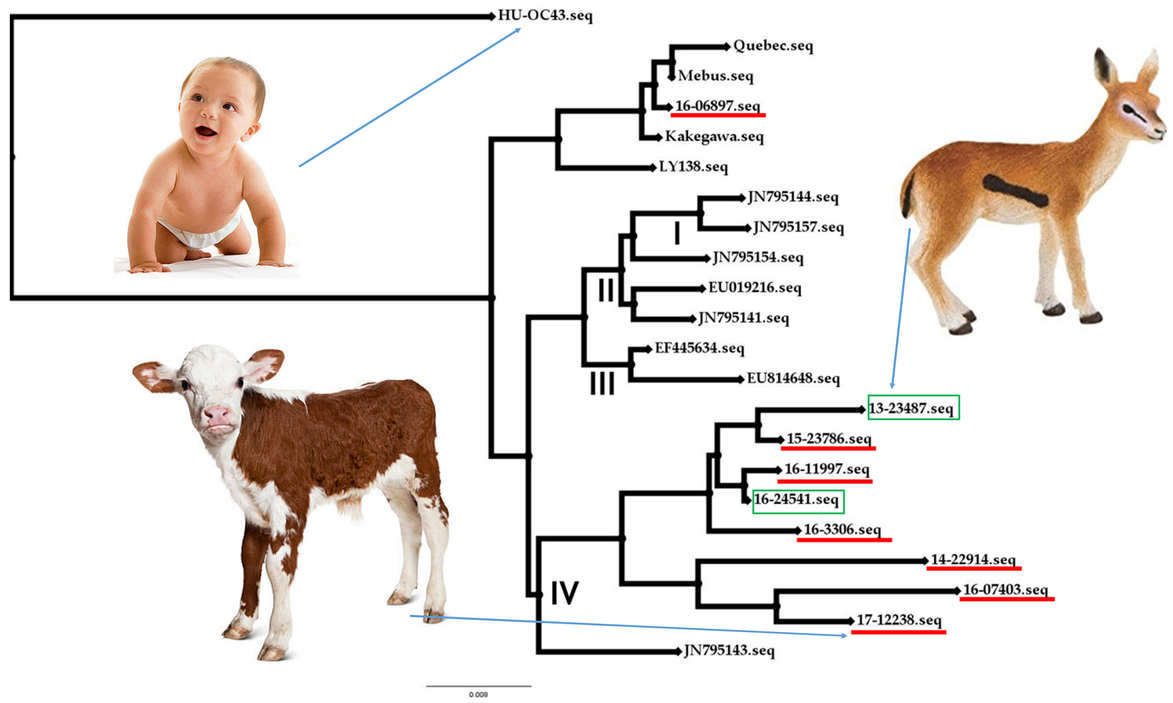

Figure 4. Genotypes of BCoV in Missouri (2013-2017). A maximum likelihood analysis and majority consensus tree showed that most of the isolates from Missouri belonged to genotype IV. Isolate 16-06897 is similar to the Mebus and Quebec strains (vaccine strains). Isolates from the captive ruminants are in green boxes. Isolates from Missouri cattle are underlined in red.

Conclusions

- Isolates of BCoV in Missouri between 2013 and 2017 belong to genotype IV, irrespective of the time of isolation, the ruminant species from which they were isolated, and the location of sample collection
- Ongoing study: deep sequencing is being used to probe intra-host variation of BCoV in newly infected captive ruminants

References

1) Borucki, Monica K. *et al.*(2013). The Role of Viral Population Diversity in Adaptation of Bovine Coronavirus to New Host Environments. PLoS ONE 8(1).

Acknowledgements

- Student support was provided from the University of Missouri Office of Research