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Report to Pennsylvania Soybean Promotion Board

Title: Genome characterization of coronaviruses from PA calves and benefits of soybean in reducing calf mortality
Project #: R 2017-03/OSP192683
Final report: October 3, 2018
Principal Investigator: Dr Suresh Kuchipudi

In this multi-year project, we aimed to identify bovine coronavirus (BCoV) strains circulating in Pennsylvania, evaluate the effect of soybean consumption by bovine calves on susceptibility to BCoV infection, and optimize laboratory conditions to grow BCoV since it is a challenging virus to cultivate *in vitro*. Results of the consumption/susceptibility study have been reported previously (October 2017). In this report we describe the isolation and identification of the first BCoV field strains characterized in Pennsylvania cattle and detail the optimized methods for growing BCoV in the laboratory.

BCoV Field Strains:

Whole-genome sequencing of ten BCoV isolates was conducted, as described in previous report. From the sequences, in-depth analysis of sequencing reads allowed reconstruction of whole or partial genomes of 9 of the 10 isolates.

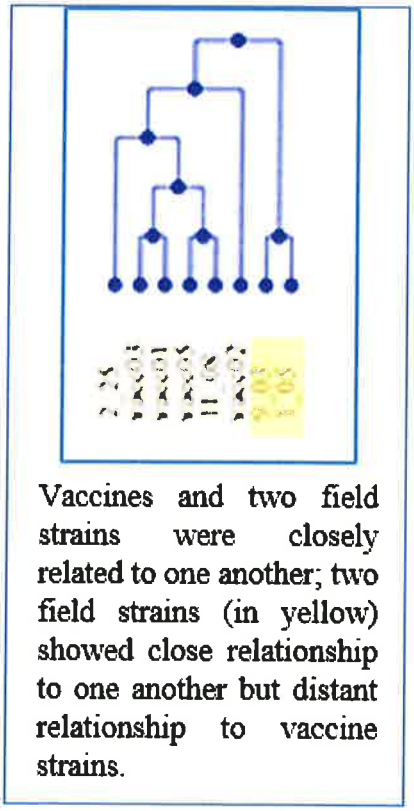
Sample ID	% of Genome Constructed	Median copies of Genome Constructed	Number of Mutations Identified
4-17-03	99.95	73.2	520
7-16-34	12.00	1	3
7-16-04	0.66	1	not possible
4-17-07	50.80	1.1	75
4-17-08	99.90	23.7	505
7-16-15	39.92	1	36
7-16-23	99.98	1785.1	474
4-17-25	99.15	17.32	484
7-16-25	86.35	2.56	299
7-16-26	76.09	2.04	231

For the first time, both partial and complete genome sequences of BCoV from Pennsylvania were identified. From these results, variation in the BCoV strains circulating in PA could be mapped on the genetic level. The sequences of the four complete genomes (>99% genome constructed) were deposited into the National Center for Biotechnology Information GenBank database and were published in the journal *Genome Announcements*.

The genome of BCoV is approximately 30,850 nucleotides in length, and for 7 of the 10 isolates at least 50% of this length was identified, allowing moderate comparison with vaccine and reference strains. The "depth-of-coverage" (median number of times each nucleotide is represented) for the four fully-sequenced genomes ranged from 17 to 1827, which are all sufficient for determining single nucleotide polymorphisms. Single nucleotide polymorphisms (SNP's, pronounced "snips") indicate individual positions of the genome which are unique to the genome of that virus isolate. Closely related virus isolates contain similar SNP's, whereas distantly related virus isolates will not have the same SNP's. Unique SNP patterns can be used to distinguish between wild field virus isolates and vaccine virus strains. In our data set of 7 samples with reliable depth-of-coverage and at least 50% complete genome sequence, between 75-505 SNP's distinct from the reference strain were identified.

BCoV from five commercially available vaccines were also sequenced, with 4 of 5 producing ≥97.9% full-genome length with depth-of coverage ≥13. At least 49 (up to 377) unique SNP's were identified in the vaccine strains. The sequence of a fifth vaccine produced only 20.65% genome length, and it was not possible to identify SNP's from this vaccine strain. The SNP pattern of these vaccine strains provides new data to guide understanding of the relationship between the field isolates and the vaccines being used in Pennsylvania cattle in 2016-2017. When SNP patterns from vaccine and field strains were compared, a unique pattern of 11 SNP's was identified which was distinct between field strains and vaccine strains. This information can help to develop new diagnostic assays to examine if animals with BCoV have a vaccine strain or a field strain.

Furthermore, from the four complete genome sequence SNP profiles, it was determined that some circulating strains are closely related to the major commercially available vaccines targeting BCoV (65-94% identical), but half of the sequenced strains showed only 27-53% similarity in SNP profile compared with the vaccine strains (see Figure). This result indicates the presence of newly emerging strains which could be less likely to be effectively controlled by the existing vaccines. A disproportionate number of SNP's that differed between at least one vaccine and one field strain were found in the Spike protein coding region. The Spike protein of BCoV is the major antigen of the virus, serving as the primary target for antibodies produced in response to vaccination or natural infection. Our results suggest that the spike protein is changing to evade vaccine protection. Combined with the finding of genomes with an increasing divergence from the vaccine strains, monitoring of the genetic makeup of bovine coronaviruses in PA is relevant for informed future vaccine development to target the circulating strains.



Vaccines and two field strains were closely related to one another; two field strains (in yellow) showed close relationship to one another but distant relationship to vaccine strains.

BCoV Cell Culture:

Three cell lines were tested for the ability to grow bovine coronavirus, based on cells used in the scientific literature. The cell lines were HRT-18G (human colon), HCT 116 (human colon), and MDBK (bovine kidney). Through trials of these three cell types with four different growth media formulations, HCT 116 with UltraCULTURE™ serum-free media containing TPCK-treated trypsin

was found to be the best choice for culturing field strains. This was in contrast to growing the standard laboratory reference strain; the reference strain grew best with HRT-18G cells with DMEM media with 5% fetal bovine serum and insulin-transferrin-sodium selenite media supplement. It was very important to include the field strains in this experiment since the results revealed a major difference in virus cultivation between the standard laboratory strains and the field strains, which has not been described previously in the scientific literature.

The superiority of HCT 116 and HRT-18G cell lines may be related to the fact that both are derived from colon cells; BCoV can cause infection in intestinal cells of cattle, which is why calf diarrhea is frequently associated with BCoV infection. The optimized culture system will serve as a significantly improved method for isolating naturally occurring field strains of BCoV in cell culture.

Sincerely,

Suresh Kuchipudi

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