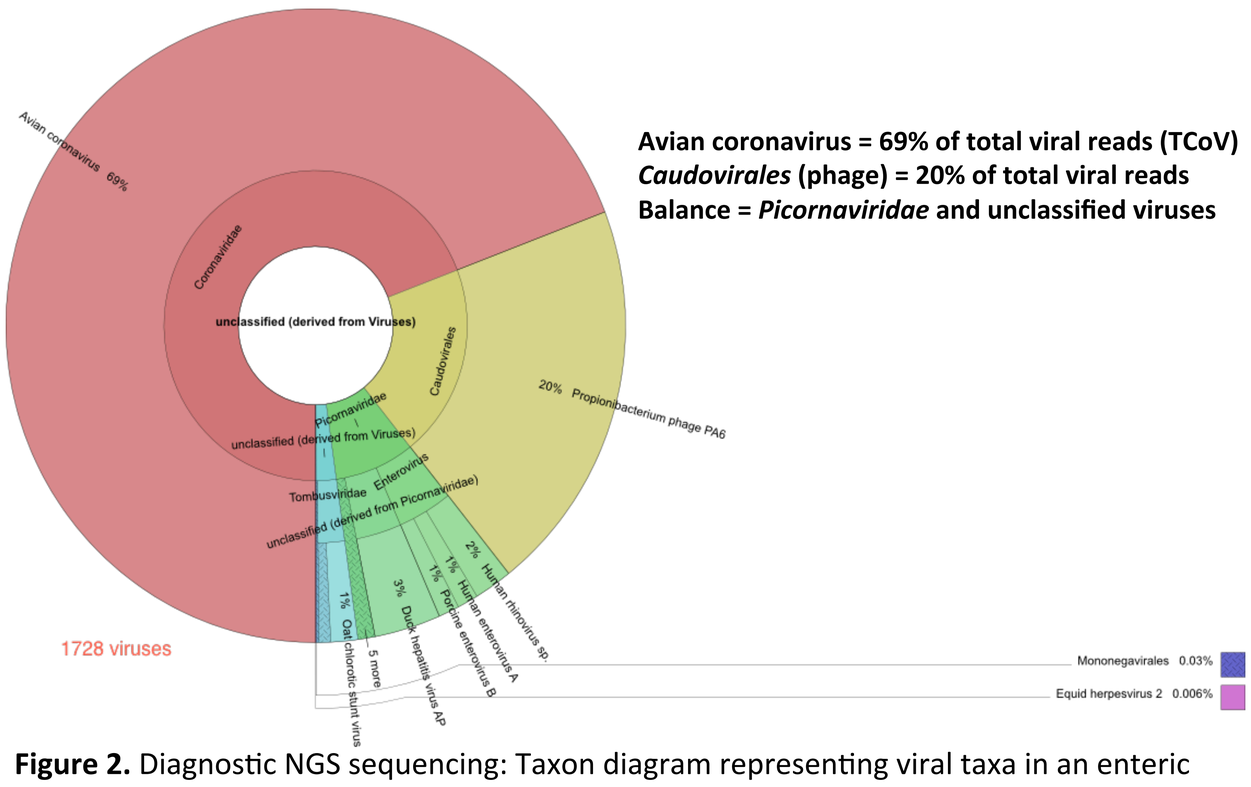
Investigating the poultry enteric virome: selection of candidate agents and genes for targeted interventions

J. Michael Day and Qingzhong Yu
U.S. National Poultry Research Center, Southeast Poultry Research Laboratory (SEPRL)
USDA/ARS Athens, Georgia, U.S.A.

Michael.Day@ars.usda.gov

Abstract

Viral infections of the avian gastrointestinal tract negatively impact poultry health and performance. A better understanding of the complex viral community present in the poultry gut would allow the design and application of early interventions to reduce the viral load in order to improve gut health and reduce the incidence of enteric syndromes and secondary infections that will make the full transition to antibiotic-free poultry production more problematic. Our metagenomic investigations of the enteric viral community in turkeys and chickens have revealed numerous novel viruses that may play roles in the performance problems and enteric syndromes observed in the field. Some of these newly described viruses remain largely uncharacterized and may be present in some United States geographic regions and not others, and there may be unique and varied genotypes, pathotypes, and geographical isolates circulating in poultry flocks as well. With the exception of certain autogenous vaccines, no specific therapeutic agents currently exist to aid in the control and prevention of poultry enteric viral infections. Part of our current strategy to combat early viral infection in poultry is the development an enterotropic vaccine platform that will lead to highly efficacious vaccines to control enteric diseases of poultry. To this end, we have constructed infectious clones of an enterotropic **Newcastle disease virus (NDV)** vaccine strain expressing the major antigenic spike glycoproteins of a **turkey enteric coronavirus (TCoV)** field strain initially detected in our laboratory using diagnostic high-throughput sequencing. The recombinant viruses, rV4/TCoV-S1 and rV4/TCoV-S2, were rescued using reverse genetics technology and the expression of the TCoV S1 and S2 spike glycoprotein subunits was confirmed *in vitro*, and their safety and stability was assessed *in vivo*. This serves as a proof-of-concept for the use of viral metagenomic data to inform the design of recombinant vaccines targeting specific enteric viruses associated with enteric disease in poultry.



Avian coronavirus = 69% of total viral reads (TCoV)
***Caudovirales* (phage) = 20% of total viral reads**
Balance = *Picornaviridae* and unclassified viruses

Figure 2. Diagnostic NGS sequencing: Taxon diagram representing viral taxa in an enteric sample collected from a turkey flock with severe enteritis (North Carolina, U.S.A., 2012). Data collected from an RNA enteric virus metagenomic analysis (Roche 454 GS FLX platform). Krona diagram created in MG-RAST.

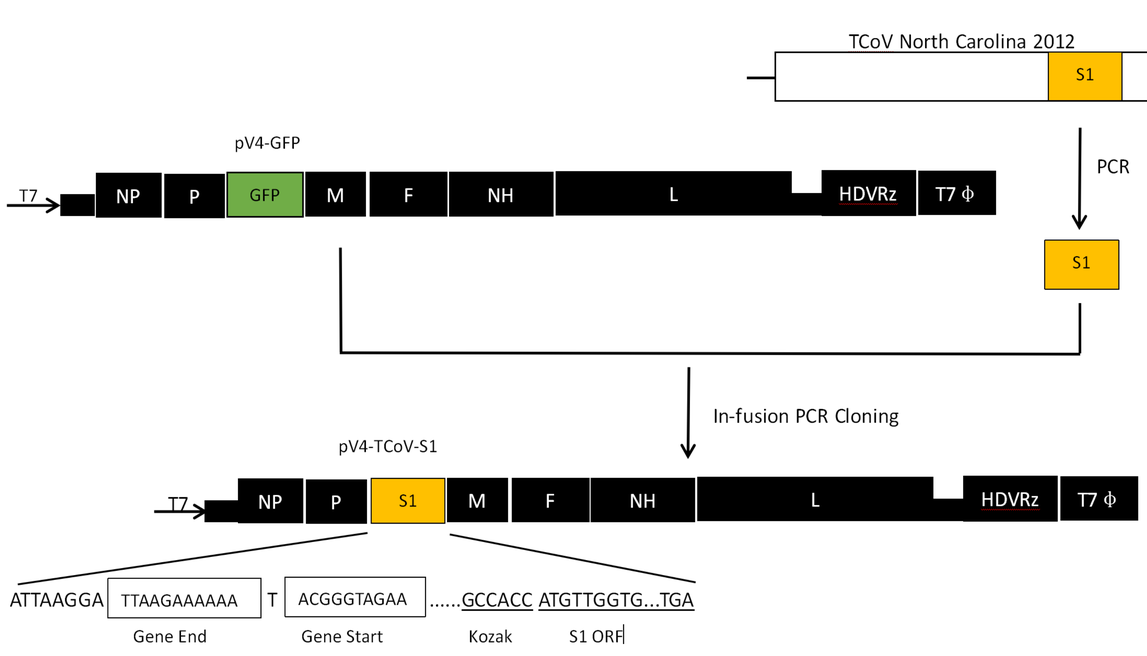


Figure 3. Generation of the pV4-TCoV-S1 infectious clone

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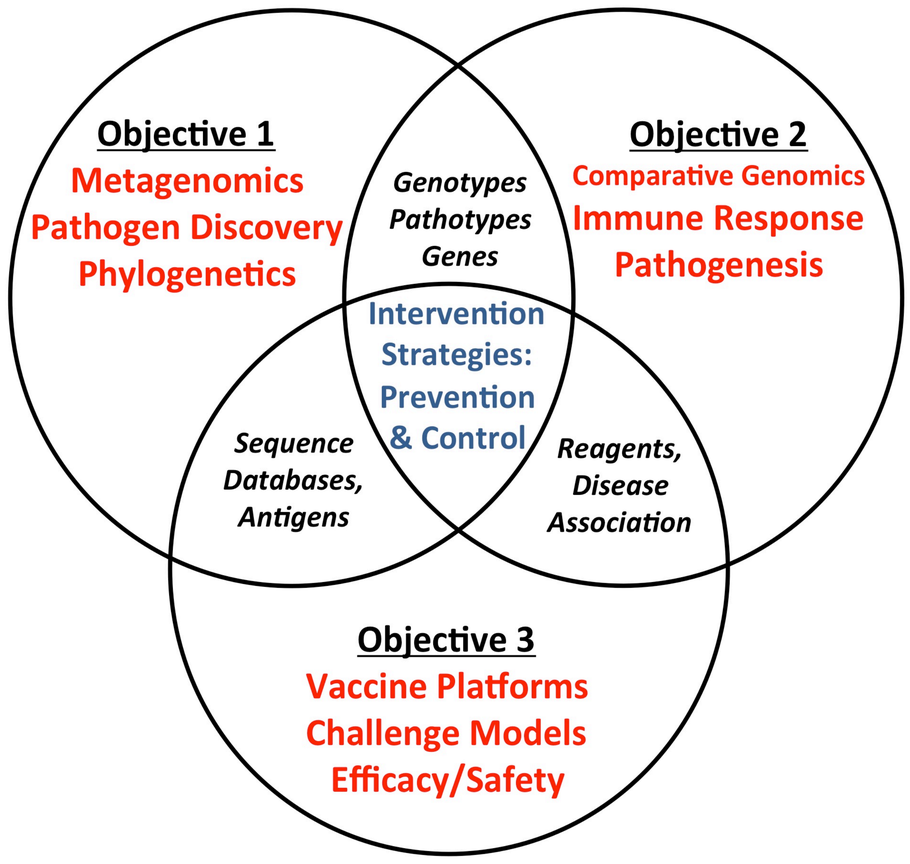

Figure 1. Poultry enteric virus discovery and characterization at SEPRL, including targeted interventions to control known and novel viruses in the field

Approach and Methods

- Develop an enterotropic NDV vaccine (V4 strain-based) infectious clone with green fluorescent protein (GFP) as a reporter
- Generate an NDV (V4 strain-based) recombinant virus expressing the major antigenic spike glycoprotein (S1 subunit) of TCoV for use as a vaccine
- Biological assessments of the recombinant viruses, including determining the mean death time (MDT) in embryoated eggs; the intracerebral pathogenicity index (ICPI); hemagglutinin titer (HA); the egg infectious dose (EID_{50}); and the tissue infectious dose ($TCID_{50}$) in DF-1 cells
- Detection of TCoV-S1 protein expression in DF-1 cells by IFA (using hyperimmune sera from TCoV-infected commercial poults)

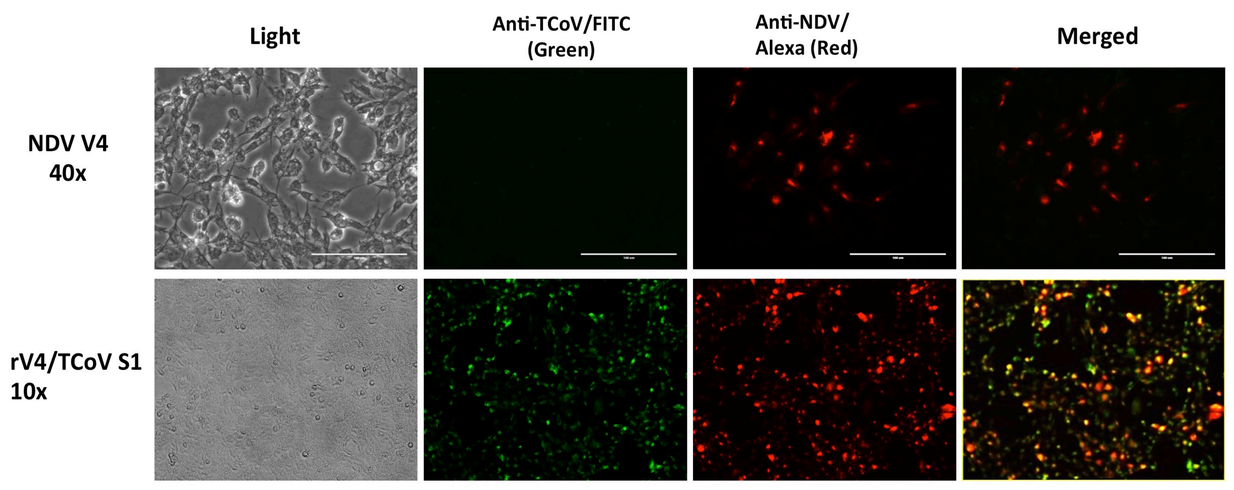

Figure 4. Detection of TCoV-S1 protein expression by IFA.
Primary antibodies: Anti-NDV HN Mab (mice) + anti-TCoV turkey sera
Secondary antibodies: Anti-mouse IgG conjugated with Alexa anti-Turkey IgG

Conclusions

- Successfully developed an NDV V4 strain-based live vaccine vector expressing TCoV-S1 protein as a bivalent vaccine candidate
- rV4/TCoV-S1 maintained similar biological properties compared with parental V4 virus
- TCoV-S1 maintained antigenicity that can be recognized by anti-TCoV turkey sera