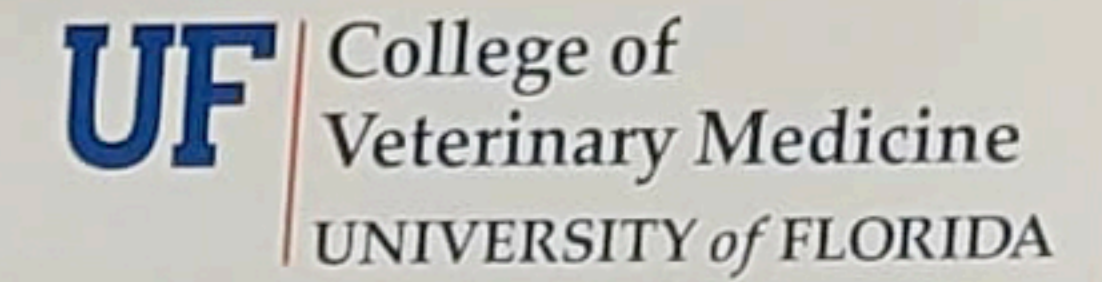




Genome Sequencing of Rotavirus A from a Florida Racing Pigeon (*Columba livia domestica*)



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Introduction

- Pigeons (*Columba livia domestica*) are susceptible to a variety of parasitic, bacterial, and viral diseases.
- The genus *Rotavirus* (family *Reoviridae*) includes nine species, rotavirus A-D and F-J.
- Pigeons infected with rotavirus A (RVA) have been reported in the USA (California), Australia, Denmark, Belgium, Hungary, and Germany.
- The RVA genome is composed of 11 segments of double-stranded RNA, which encode six structural (VP1-4, VP6-VP7) and six non-structural (NSP1-6) proteins.
- A 2-year-old Florida racing pigeon was submitted for necropsy to the Bronson Animal Disease Diagnostic Laboratories. The gross pathology examination showed that the intestine was distended, flaccid, and congested.
- Frozen intestinal tissue was sent to the Wildlife and Aquatic Veterinary Disease Laboratory in Gainesville, FL, for viral discovery using a next-generation sequencing approach.

Objectives

- Determine the genetic and phylogenetic relationship of the rotavirus A found in a racing pigeon in Florida.

Materials and Methods

- Full genome sequencing of RVA was accomplished using a next-generation sequencing approach.
- The 5' end of the coding sequences of segments 2 and 4 were determined using a Rapid Amplification for cDNA End PCR Kit and Sanger sequencing.
- Gaps within segments 2 and 3 were closed by RT-PCR followed by Sanger sequencing.
- The genotype of the RVA was determined using the Rotavirus Classification Tool.
- A Maximum Likelihood phylogenetic analysis of the VP1 segment was performed in IQ-TREE.
- Genetic analyses of the VP4 and VP7 segments were performed using the Sequence Demarcation Tool.

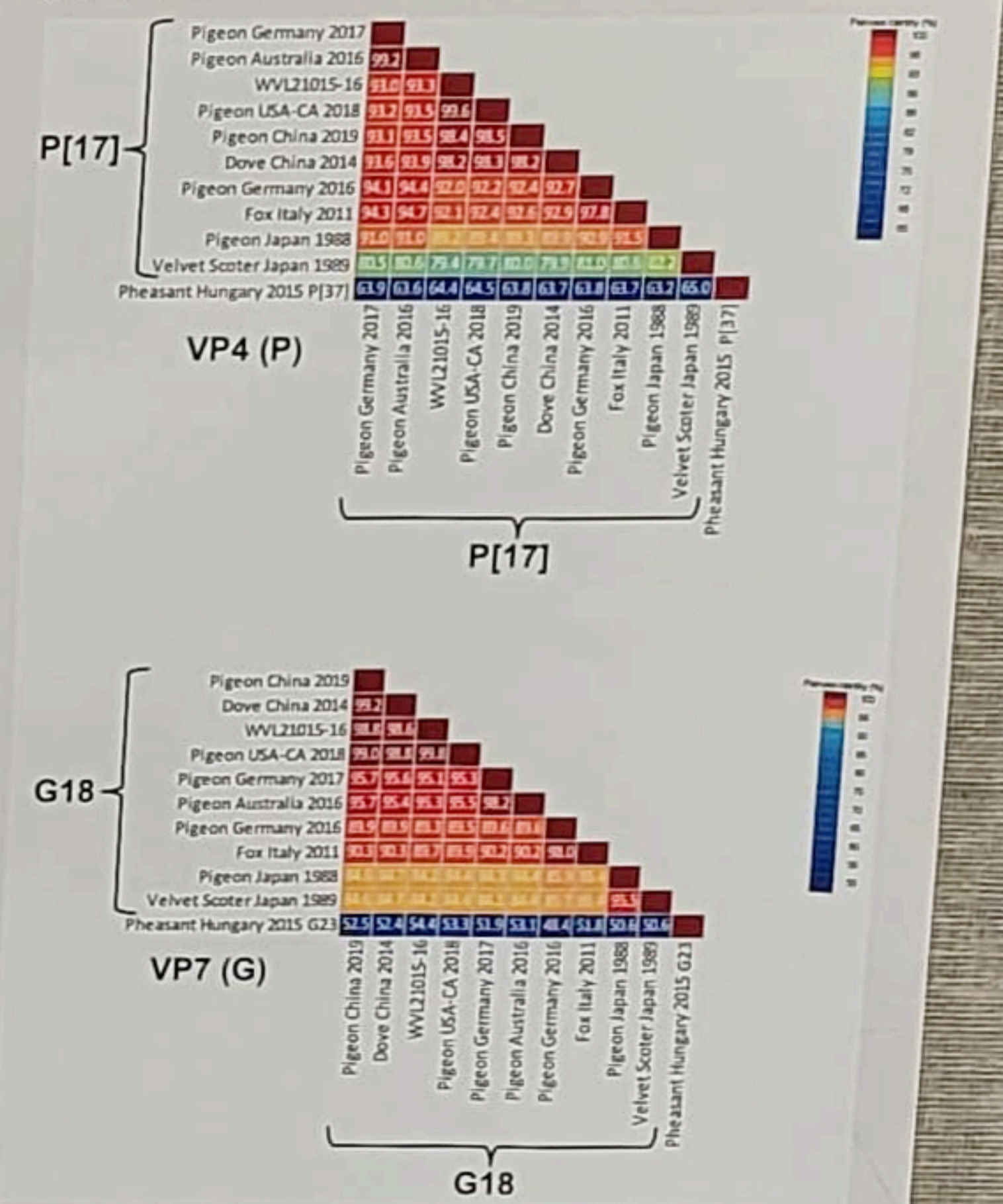
Results

- The total length of the complete coding sequences of the 11 RVA segments was 17,794 bp, with a G+C content of 34.98%.
- BLASTN searches of all 11 genomic RNA segments showed the highest nucleotide (nt) identity (99.55-99.89%) to RVA/Pigeon-wt/USA/K1802315/2018/G18P[17] isolated from a dead racing pigeon in California.
- The genotype of the Florida racing pigeon RVA was determined to be G18P[17]-I4-R4-C4-M4-A4-T4-N4-E19-H4.
- Phylogenetic analysis of the conserved VP1 segment showed that the RVA from Florida and California were grouped together (Figure 1).
- The pairwise comparison analyses showed that both the Florida RVA VP4 and VP7 segments are nearly identical to California RVA (99.6% and 99.8%, respectively) and closely related to RVA samples from China (Figure 2).

Figure 1. Maximum Likelihood cladograms depicting the relationship of the rotavirus A, identified from a Florida racing pigeon (highlighted in red) to other rotaviruses based on nucleotide sequence alignments of the VP1 genes. All nodes with black circles are supported by bootstrap values of >80%. The trees were rooted in non-RVA samples.



Figure 2. Pairwise nucleotide sequence comparisons of VP4 and VP7 performed against the closest relatives to the obtained RVA sample.



Conclusions

- Our study confirms that RVA was present in a population of Florida racing pigeons.

Future Study

- Determine the distribution of pigeon RVA G18P[17] in Florida and its potential impact on the global racing pigeon industry.