

Evaluation of Cross-Genotype Neutralization of Avian Reoviruses by Vaccine-Induced Antibodies

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Introduction

- Avian reoviruses (ARVs) cause high economic losses in poultry¹.
- They are classified into seven antigenically distinct cluster groups (CGs), all of which are present in Canada².
- ARVs are well-studied in some parts of North America but have not been characterized in the Atlantic provinces.
- Commercial vaccines are currently based solely on CG1 antigens¹.

Methods

ARV isolate characterization

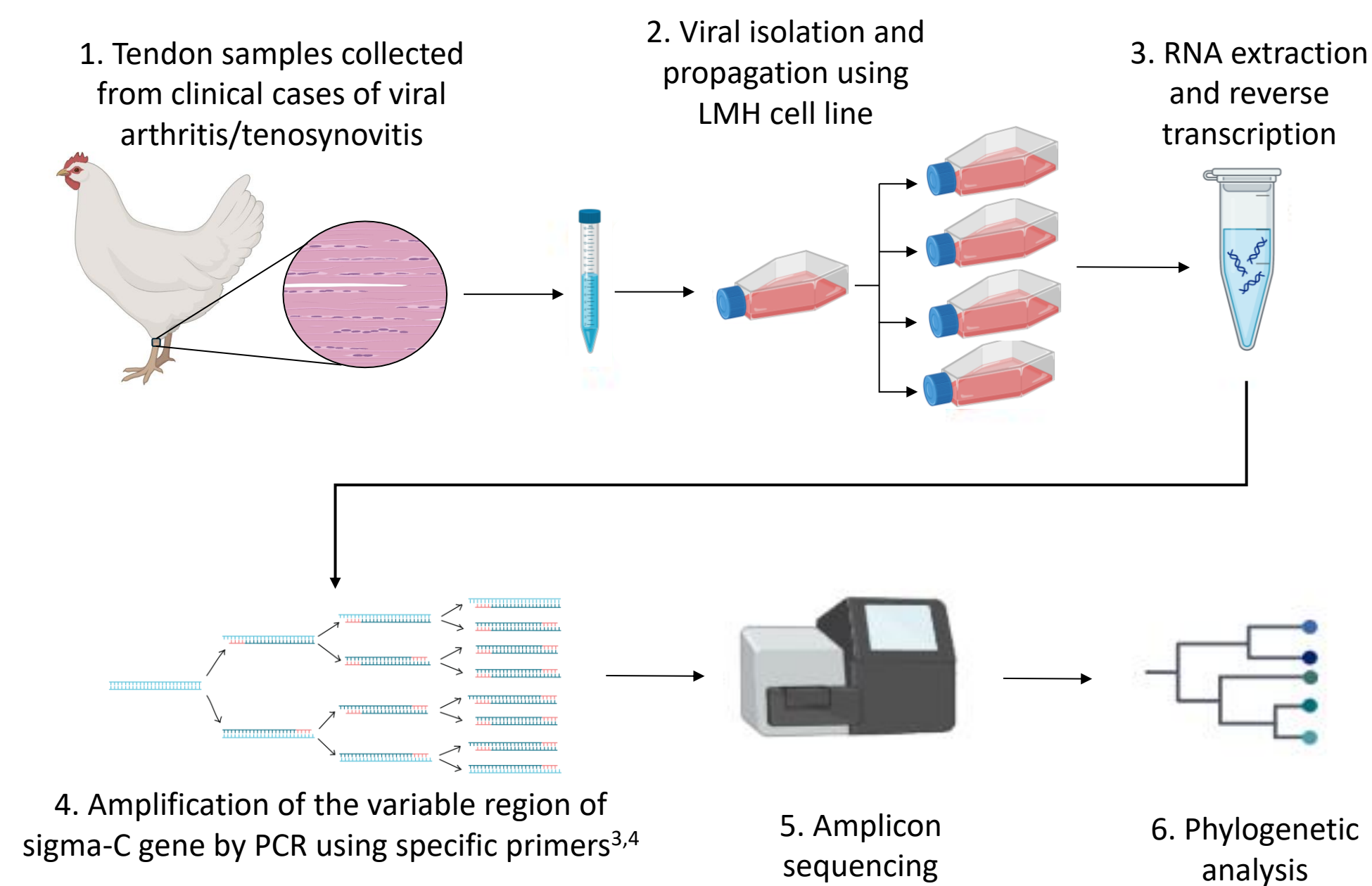


Figure 1: Method of genetic characterization of ARVs isolated from clinical cases of arthritis/tenosynovitis across Atlantic Canada. Tendon tissue from affected birds was homogenized and filtered through a 0.22-micron filter. The filtrates were used to inoculate LMH cells for virus isolation. Viral nucleic acid was extracted, and the sigma-C gene was amplified from each isolate by PCR. Sigma-C amplicons were sequenced and used for phylogenetic clustering of isolates.

Viral Neutralization Assays

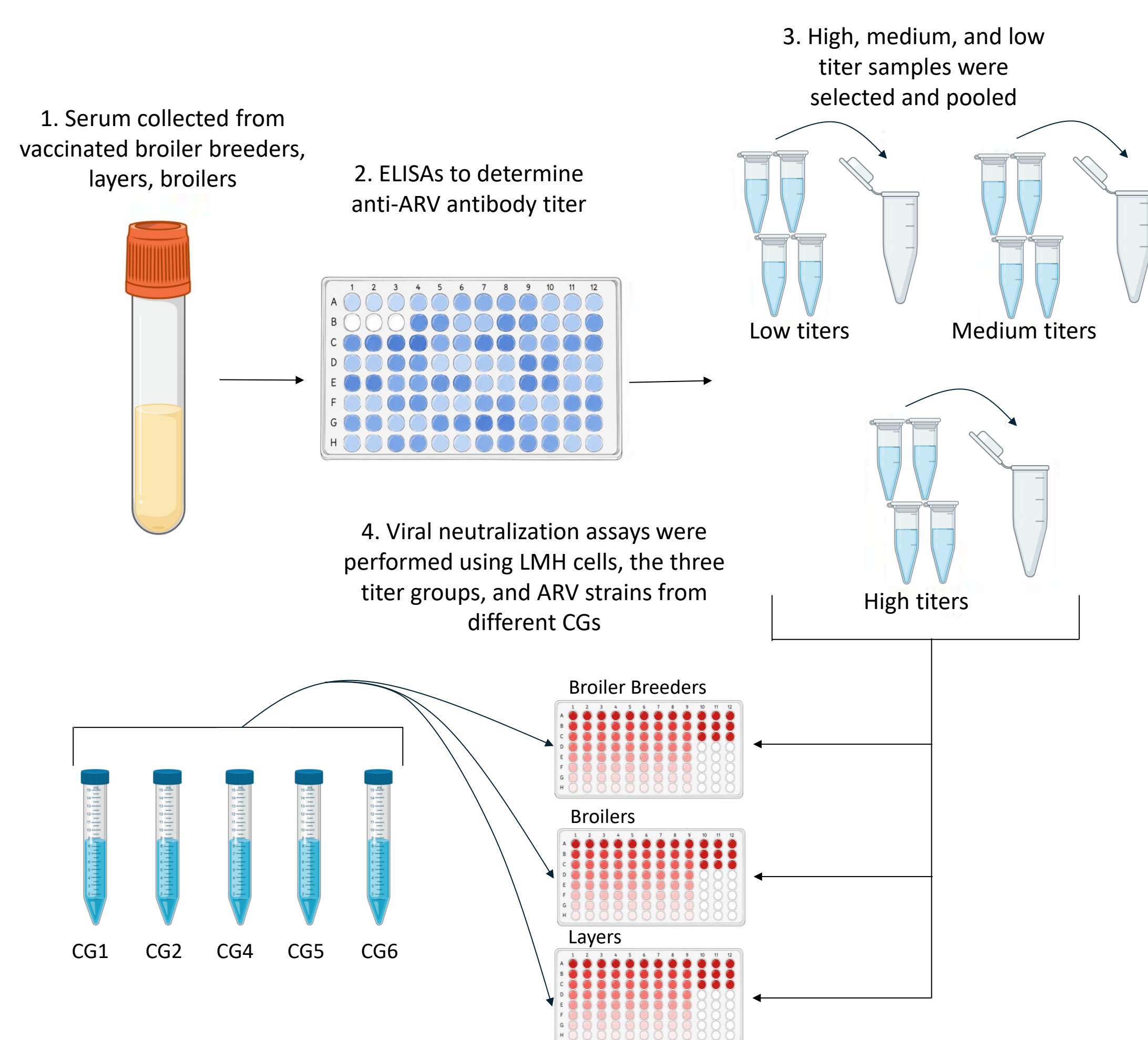


Figure 2: Methodology to assess the neutralizing activity of commercial vaccine-induced antibodies against different ARV cluster groups. Serum was collected from vaccinated broiler breeders and layers, and from broilers from vaccinated parent flocks. ELISAs were performed using the IDEXX Avian Reovirus Antibody Test Kit to determine the anti-ARV antibody titer in each sample. Samples representative of high, medium, and low titers were selected and pooled (see Table 1). Viral neutralization assays were performed to compare neutralizing activity of different antibody titers against different CGs.

Results

Phylogenetic analysis

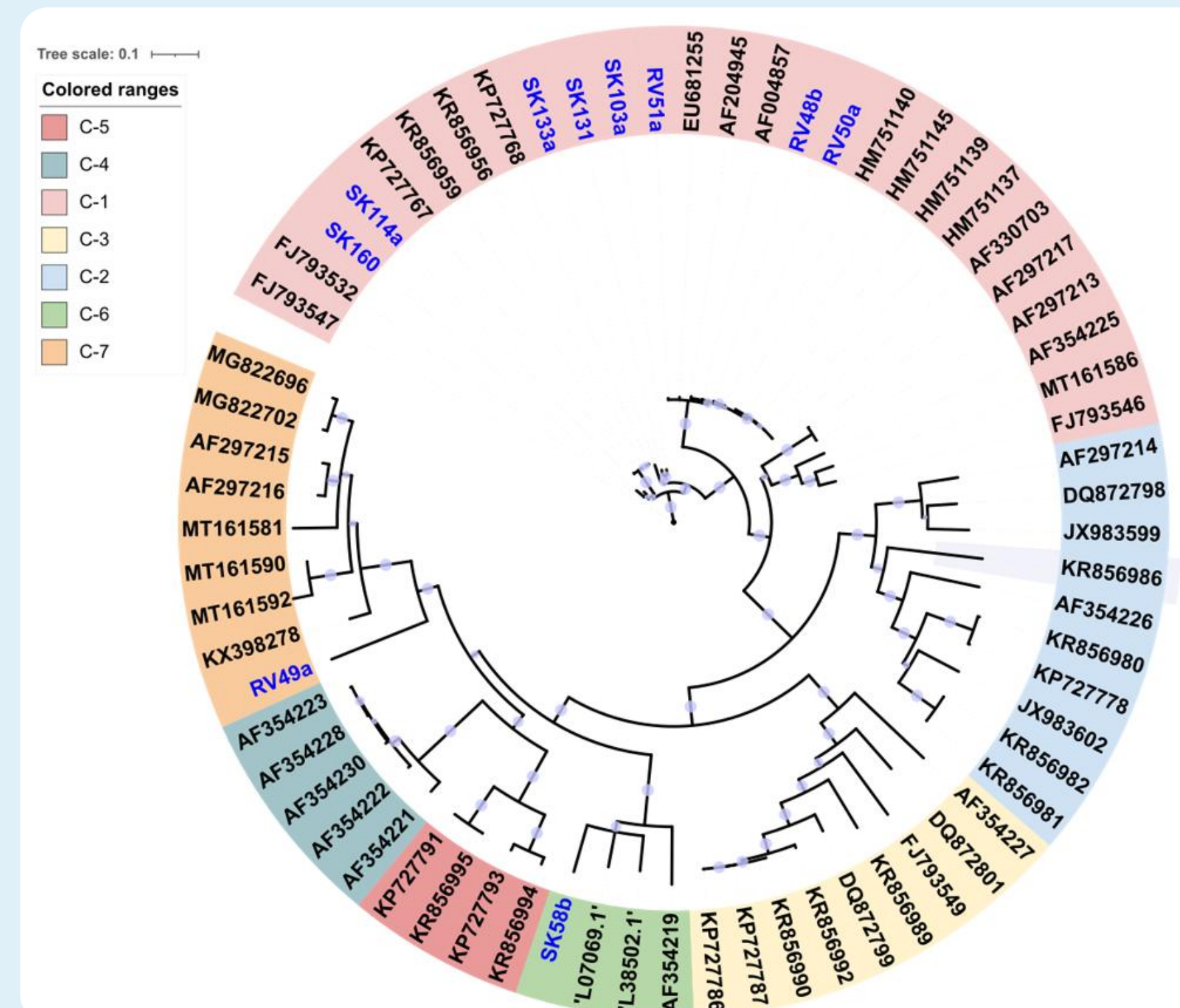


Figure 5: Phylogenetic tree of ARV isolates based on the most variable Sigma-C gene. The tree was constructed using 58 Sigma-C reference sequences from GenBank and 10 of our isolates (labelled in blue). Evolutionary relationships were inferred using the Tamura-Nei genetic distance model by a Neighbour-Joining method with 1000 bootstrap replicates in GeneiousPrime software (Dotmatrix). The tree was visualized and annotated using the Interactive Tree of Life (ITOL) online platform. The scale bar represents the number of nucleotide substitutions per site.

ELISA titers

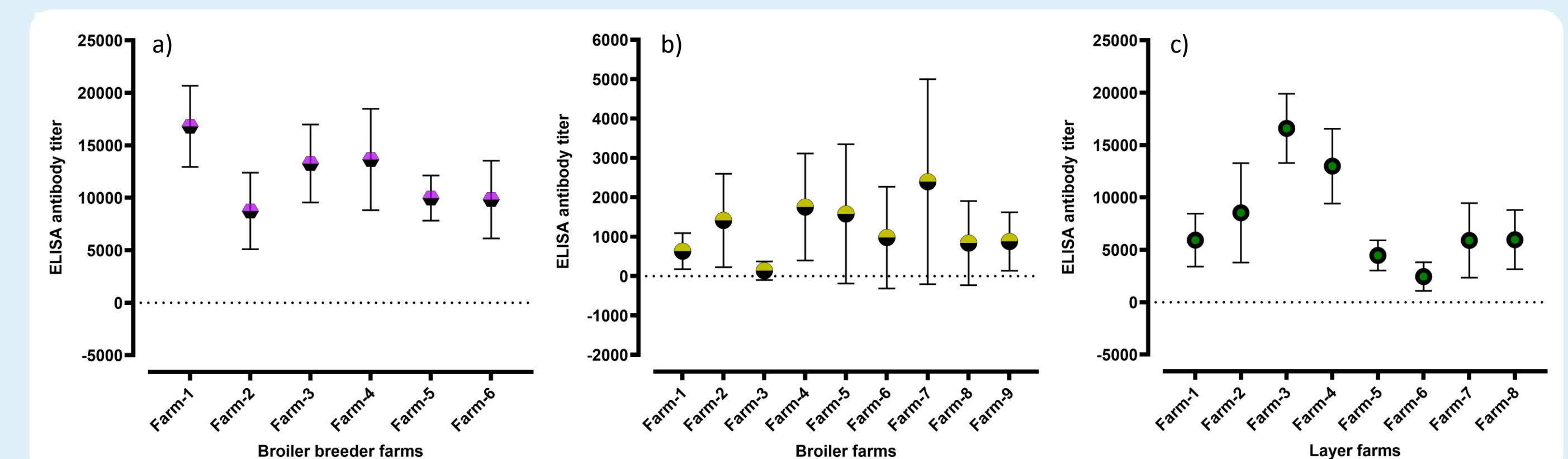


Figure 3: Anti-ARV antibody titers measured by the IDEXX Avian Reovirus Antibody Test Kit. Graphs (a), (b), and (c) show mean antibody titer $\pm$ one standard deviation for broiler breeder, broiler, and layer flocks, respectively.

Table 1: Ranges of anti-ARV antibody titers included in high, medium, and low titer serum pools

Antibody titers	Broiler Breeders	Broilers	Layers
High	18,563 - 23,555	6,122 - 7,741	12,903 - 13,905
Medium	13,690 - 15,045	1,109 - 1,143	6,784 - 8,009
Low	7,000 - 8,691	523 - 562	2,807 - 2,873

Viral neutralization

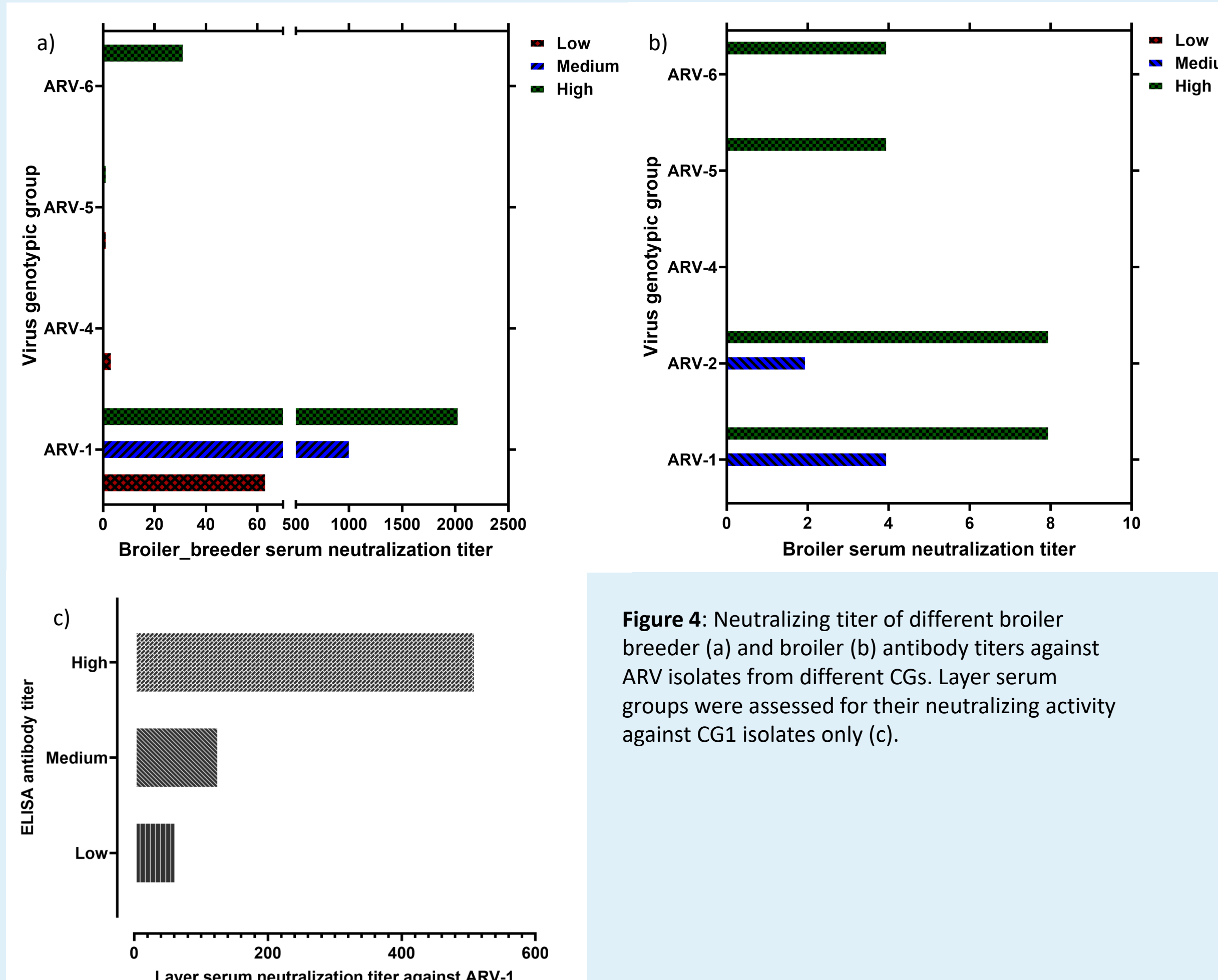


Figure 4: Neutralizing titer of different broiler breeder (a) and broiler (b) antibody titers against ARV isolates from different CGs. Layer serum groups were assessed for their neutralizing activity against CG1 isolates only (c).

Conclusions

- Multiple CGs are present in Atlantic Canada.
- Anti-ARV vaccination programs in Atlantic Canada are effective in inducing a strong immune response.
- High-titer serum shows greater neutralizing activity against CG1 isolates compared to medium- or low-titer serum.
- Neutralizing activity of vaccine-induced antibodies against non-CG1 ARV strains is negligible or zero, indicating poor cross-protection.
- Future work will focus on increased molecular surveillance and the development of multivalent vaccines based on the nature of circulating ARVs.

References

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