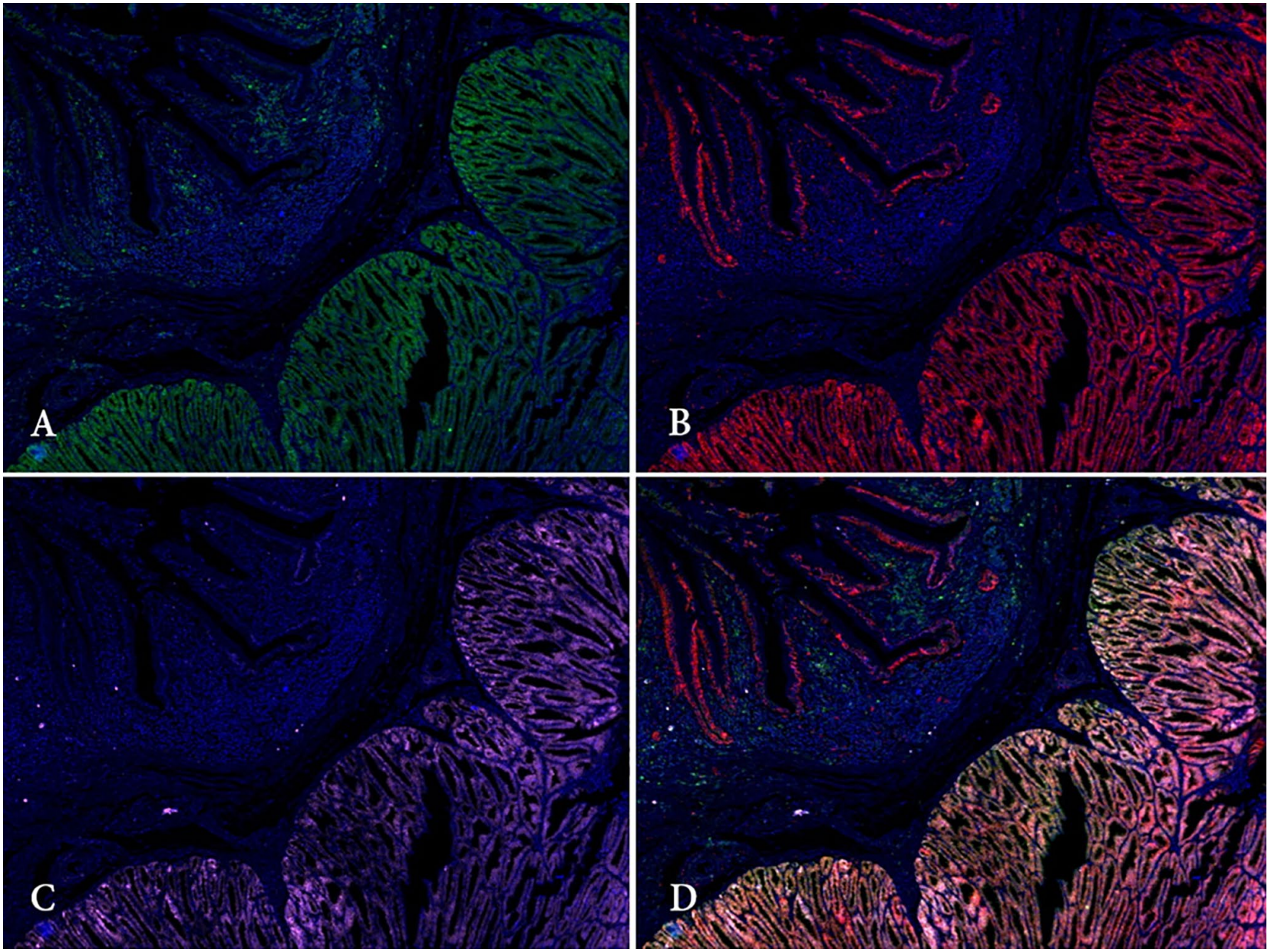
JVDI: Supplementary material

Liu H, et al. Histologic findings and viral antigen distribution in natural coinfection of layer hens with subgroup J avian leukosis virus, Marek's disease virus, and reticuloendotheliosis virus

Supplementary Table 1. Primers used for detection of subgroup J avian leukosis virus (ALV-J), Marek's disease virus (MDV), and reticuloendotheliosis virus (REV).

Name	Sequence	Length (bp)
ALV-J-*gp85*	5-GGAGTTCATCTATTGCAACAACCGA-3 5-GCGCCTGCTAACGGTGGTGACC-3	924
ALV-A-*env*	5-GGTTGGTCTAGACAGGAAGC-3 5-CATTGCCACAGCGGTAC-3	180
ALV-B-*env*	5-CATACGATAGTCCGGCTG-3 5-CCCCACACATCCTGACA-3	260
MDV-*meq*	5-CGCGAATTCTACAGGTGTAAAGAGATG-3 5-TAACTCGAGTGCTGAGAGTCACAATGC-3	1,058
REV-ITR	5-CATACTGGAGCCAATGGTT-3 5-AATGTTGTAGCGAAGTACT-3	300
BLSV-ORF3	5-GCTTGATTTACAACCCACC-3 5- GGCATCAGTAACCCCAAT-3	375

Supplementary Figure 1. Alignments for the amino acid mutations sites in the Meq protein. CVI988: 6 amino acid sites at residues 71(S), 80(D), 115(V), 139(T), 176(P), 217(P). Cases 1–3: 6 amino acid mutations at residues 71(A), 80(Y), 115(A), 139(A), 176(R), 217(A).


A
B
C
D

Supplementary Figure 2. Fluorescence multiplex immunohistochemistry localization of subgroup J avian leukosis virus (ALV-J), Marek's disease virus (MDV), and reticuloendotheliosis virus (REV) antigens in chicken proventriculus. A–C. Anti-MDV *meq* (1:200), anti–ALV-J *gp85* (1:150), and anti-REV *gp90* (1:200) mouse monoclonal antibodies were respectively used as the primary antibodies. Horseradish peroxidase–conjugated goat anti-mouse IgG was used as the secondary antibody. **A.** Fluorescein isothiocyanate–conjugated tyramide reagent (MDV staining, green fluorescence), **B.** Cy3-conjugated tyramide reagent (ALV-J staining, red fluorescence), and **C.** Cy5-conjugated tyramide reagent (REV staining, pink fluorescence) were used as fluorescent signals for detection of target antigens in tissues and cells. Nuclei were counterstained with DAPI (blue fluorescence). **D.** Antigens are co-localized by image overlay (merged images).