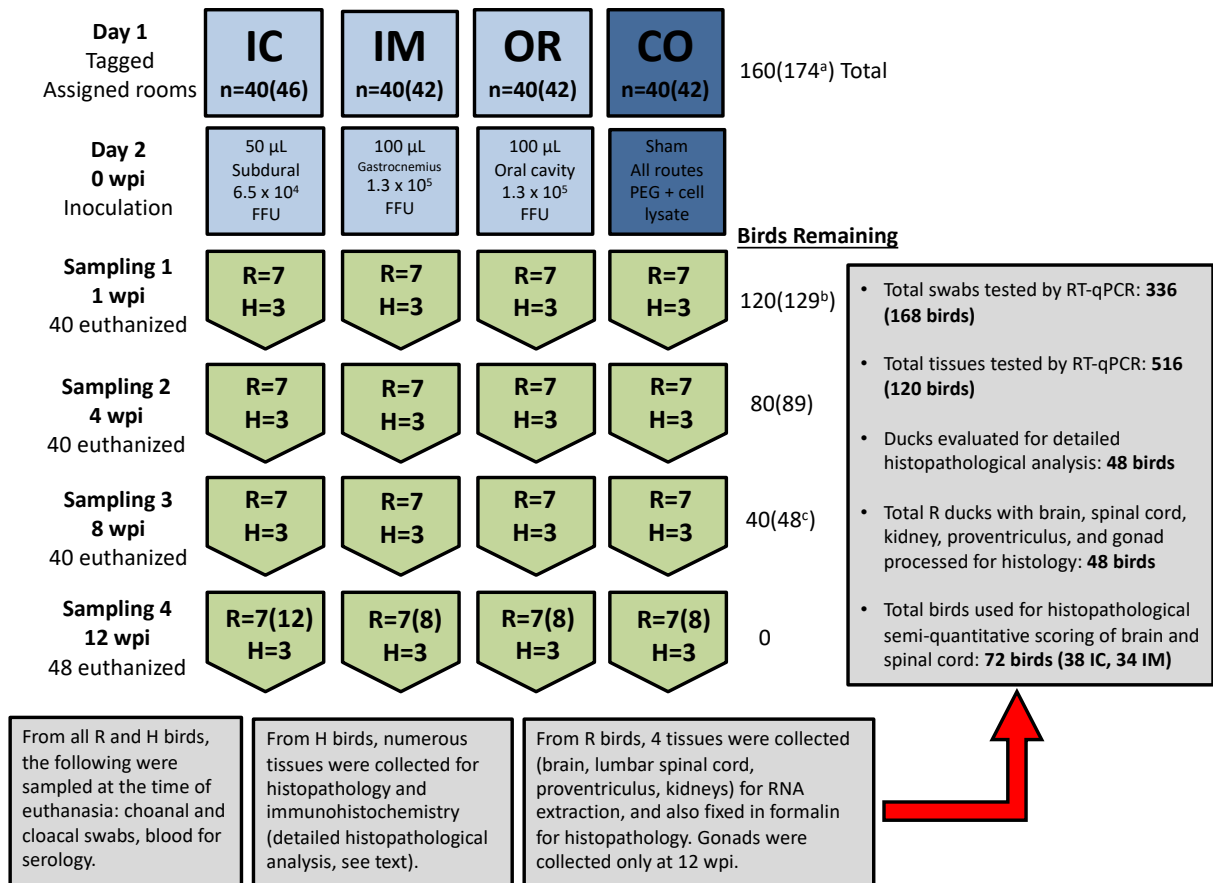


SUPPLEMENTARY MATERIAL:

Pathogenesis of Aquatic Bird Bornavirus in Muscovy Ducks

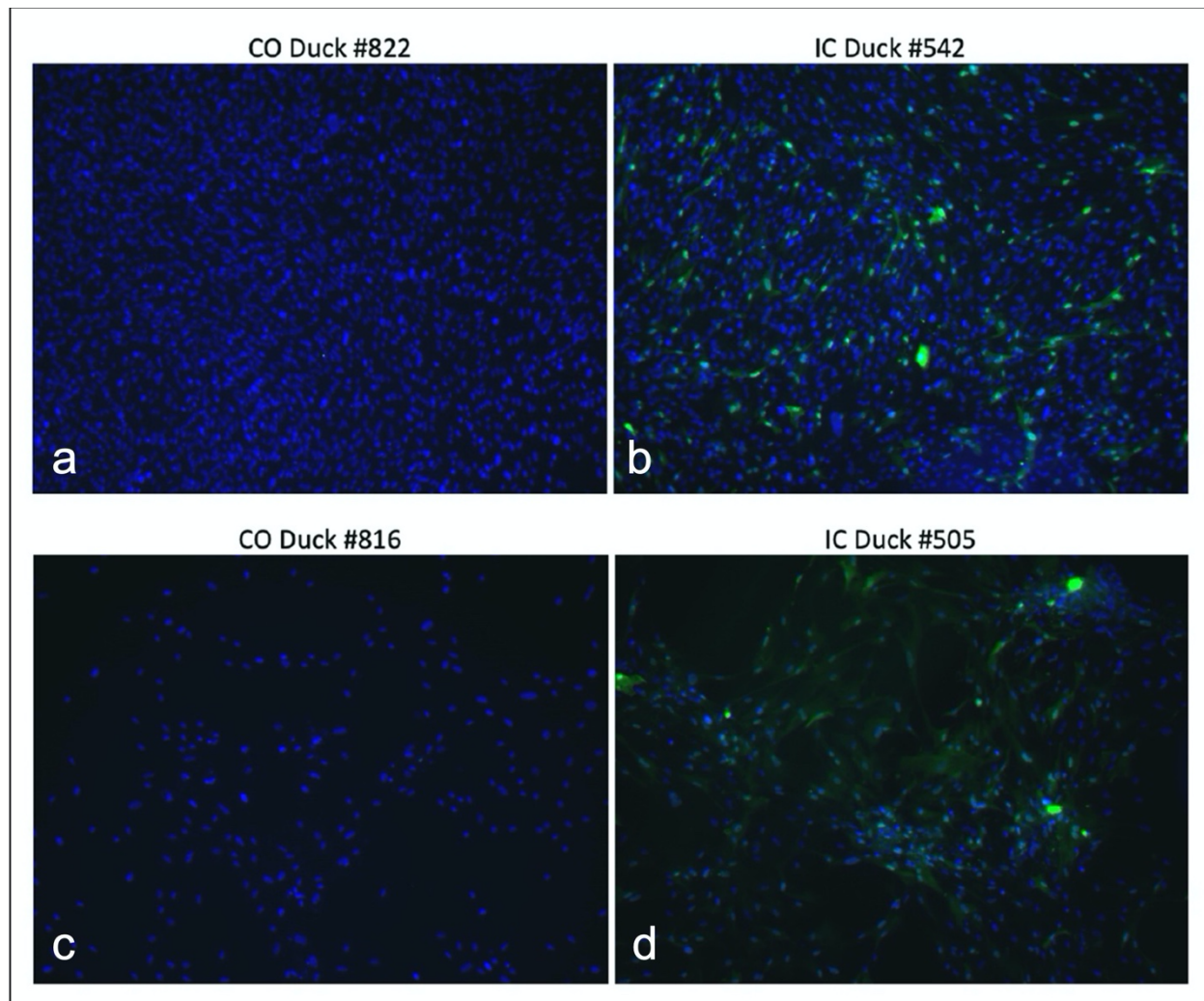
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Supplementary Figure S1. Schematic representation of the experimental plan for infection of Muscovy ducks with ABBV-1. Reported are the virus inoculum, the number of birds in each experimental groups (IC, intracranial; IM, intramuscular; PO, oral; CO, control), euthanasia schedule, and the sampling protocol for downstream testing. “H”, indicates birds that were sampled for detailed pathology assessment; “R”, indicates birds sampled for virus titration by RT-qPCR. Numbers in parenthesis indicate the actual number of ducks used and remaining at each time point, based on unexpected deaths and extra birds. See text for detailed description.

^aTwo ducklings were euthanized at two days of age, prior to inoculation, to be used as early control birds. ^bTwo ducklings died immediately after inoculation and 1 died unexpectedly at 3 days post infection (dpi). ^cOne duckling died unexpectedly at 46 dpi.



Supplementary Figure S2. (a, b) Isolation of ABBV-1 from the brain of an experimentally infected Muscovy duck at 12 wpi. Immunofluorescence assay for ABBV-1 N antigen in control (CO) duck #822 and intracranial (IC) duck #542 at passage 3. The brain from the IC duck yielded ABBV-1 in CCI-141 after three passages, as shown by nuclear signal in scattered cells. No signal is appreciated in the brain of the CO duck. (c, d) Isolation of ABBV-1 from the kidney of an experimentally infected Muscovy duck at 12 wpi. Immunofluorescence assay for ABBV-1 N antigen in control (CO) duck #816 and intracranial (IC) duck #505 at passage 3. The kidney from the IC duck yielded ABBV-1 in CCI-141 after three passages, as shown by nuclear signal in scattered cells. No signal is appreciated in the kidney of the CO duck.

Supplementary Table 1. Pairwise comparisons between proportions of Muscovy ducks presenting with inflammation of the nervous system, after intracranial (IC) or intramuscular (IM) infection with aquatic bird birnavirus-1 (ABBV-1).

	Cerebrum IC	Optic lobe IC	Brainstem IC	Cerebellum IC	Spinal cord IC	Brain meninges IC	Spinal meninges IC	Peripheral nerves IC	Cerebrum IM	Optic lobe IM	Brainstem IM	Cerebellum IM	Spinal cord IM	Brain meninges IM	Spinal meninges IM
Optic lobe IC	1														
Brainstem IC	1	1													
Cerebellum IC	1	1	1												
Spinal cord IC	0.948	1	1	1											
Brain meninges IC	1	1	1	1	1										
Spinal meninges IC	<0.001	1	1	0.271	1	0.002									
Peripheral nerves IC	0.327	1	1	1	1	0.845	1								
Cerebrum IM	0.003	1	1	1	1	0.015	1	1							
Optic lobe IM	0.001	1	1	0.441	1	0.007	1	1	1						
Brainstem IM	0.011	1	1	1	1	0.042	1	1	1	1					
Cerebellum IM	<0.001	1	1	0.109	0.676	<0.001	1	1	1	1	1				
Spinal cord IM	0.024	1	1	1	1	0.09	1	1	1	1	1	1			
Brain meninges IM	<0.001	0.065	0.065	0.001	0.007	<0.001	1	1	1	1	1	1	0.582		
Spinal meninges IM	<0.001	0.972	0.972	0.042	0.328	<0.001	1	1	1	1	1	1	1	1	
Peripheral nerves IM	0.001	1	1	0.441	1	0.007	1	1	1	1	1	1	1	1	1

Cells in green are statistically significant ($p < 0.05$).

False discovery rate was adjusted using Hommel's multiple comparison tests

Supplementary Table 2. Pairwise comparisons between proportions of Muscovy ducks presenting with infection in multiple tissues / swabs, after intracranial (IC) or intramuscular (IM) infection with aquatic bird birnavirus-1 (ABBV-1).

	Brain IC	Spinal cord IC	Proventriculus IC	Kidney IC	Cloacal swab IC	Choanal swab IC	Brain IM	Spinal cord IM	Proventriculus IM	Kidney IM	Cloacal swab IM
Spinal cord IC	0.901										
Proventriculus IC	0.03	1									
Kidney IC	0.014	1	1								
Cloacal swab IC	<.001	<.001	<.001	<.001							
Choanal swab IC	<.001	<.001	<.001	<.001	1						
Brain IM	0.01	1	1	1	<.001	0.002					
Spinal cord IM	<.001	0.299	1	1	<.001	0.03	1				
Proventriculus IM	<.001	0.006	0.412	0.412	0.11	0.99	0.93	1			
Kidney IM	<.001	0.014	0.684	0.686	0.044	0.629	1	1	1		
Cloacal swab IM	<.001	<.001	<.001	<.001	1	1	<.001	0.002	0.18	0.076	
Choanal swab IM	<.001	<.001	<.001	<.001	1	1	<.001	<.001	0.044	0.018	1

Cells in green are statistically significant ($p < 0.05$).

False discovery rate was adjusted using Hommel's multiple comparison tests