

Nath BK, et al. Lesions and viral loads in racing pigeons naturally coinfecting with pigeon circovirus and columbid alphaherpesvirus 1 in Australia

Supplemental Table 1. Viral copy numbers of pigeon circovirus (PiCV) and columbid alphaherpesvirus 1 (CoHV1) in tissues from 4 dead birds and swab samples from 46 other birds in the flock.

Bird	Sample	Clinical status	PiCV copy number/ μ L	CoHV1 copy number/ μ L
1	Liver	Dead	1.05×10^8	1.72×10^2
	Oropharynx	Dead	2.62×10^7	4.52×10^4
	Bursa	Dead	2.54×10^8	3.38×10^2
2	Liver	Dead	2.38×10^{11}	7.03×10^3
	Oropharynx	Dead	2.84×10^9	2.68×10^6
	Bursa	Dead	2.37×10^8	3.18×10^3
3	liver	Dead	1.82×10^9	2.66×10^6
	bursa	Dead	5.05×10^7	4.28×10^4
	Oropharynx	Dead	1.33×10^8	2.46×10^6
4	Bursa	Dead	2.10×10^9	3.64×10^2
	Liver	Dead	1.37×10^{10}	4.48×10^2
	Oropharynx	Dead	5.29×10^8	1.89×10^4
5	Oro-cloacal swab	Healthy	5.90×10^1	ND
6	Oro-cloacal swab	Healthy	7.31×10^3	ND
7	Oro-cloacal swab	Healthy	4.48×10^2	2.40×10^1
8	Oro-cloacal swab	Healthy	2.98×10^3	ND
9	Oro-cloacal swab	Healthy	1.06×10^3	ND
10	Oro-cloacal swab	Healthy	9.40×10^1	ND
11	Oro-cloacal swab	Healthy	8.61×10^3	ND
12	Oro-cloacal swab	Healthy	2.97×10^2	ND
13	Oro-cloacal swab	Healthy	4.31×10^2	ND
14	Oro-cloacal swab	Healthy	2.06×10^3	ND
15	Oro-cloacal swab	Healthy	1.80×10^2	4.50×10^1
16	Oro-cloacal swab	Healthy	2.88×10^3	1.51×10^2
17	Oro-cloacal swab	Healthy	2.75×10^2	ND
18	Oro-cloacal swab	Healthy	3.34×10^2	ND
19	Oro-cloacal swab	Healthy	5.19×10^2	ND
20	Oro-cloacal swab	Healthy	2.08×10^3	ND
21	Oro-cloacal swab	Healthy	1.66×10^2	2.30×10^1
22	Oro-cloacal swab	Healthy	4.89×10^2	3.00×10^1
23	Oro-cloacal swab	Healthy	3.85×10^2	ND

Lesions in pigeons coinfectd with PiCV and CoHV1

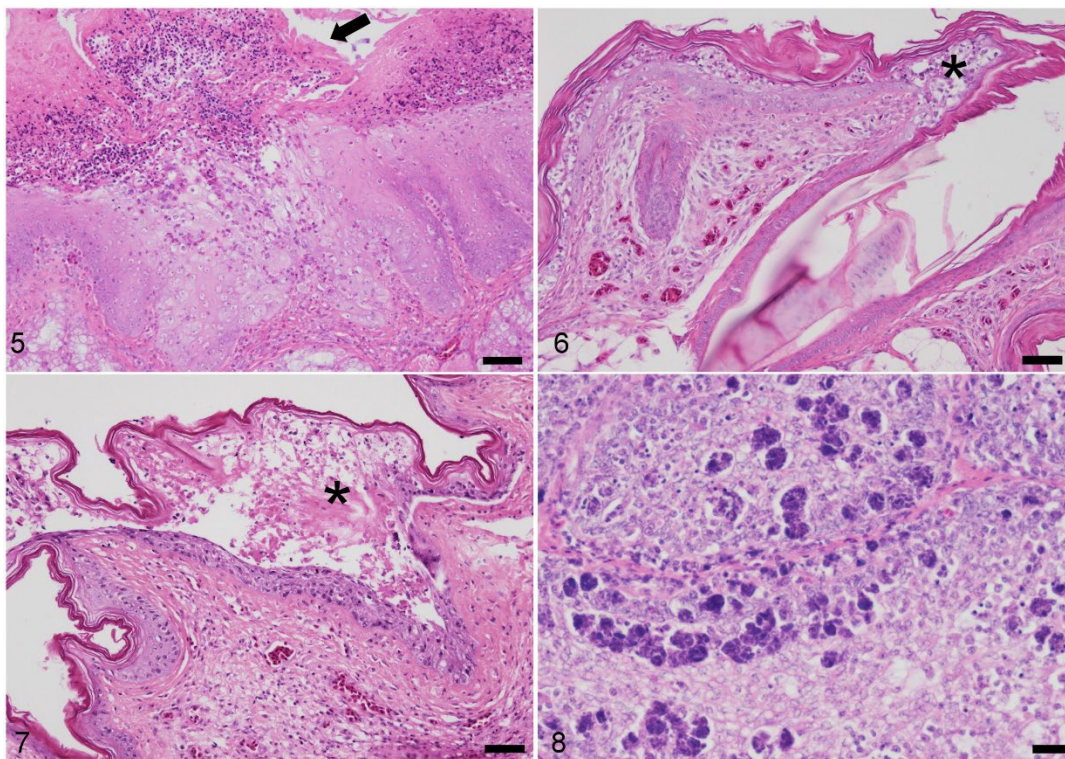
24	Oro-cloacal swab	Healthy	1.43×10^3	ND
25	Oro-cloacal swab	Healthy	2.10×10^2	ND
26	Oro-cloacal swab	Healthy	2.89×10^2	ND
27	Oro-cloacal swab	Affected	1.02×10^7	3.30×10^1
28	Oro-cloacal swab	Healthy	7.05×10^3	5.20×10^1
29	Oro-cloacal swab	Affected	2.80×10^8	ND
30	Oro-cloacal swab	Healthy recovered	ND	ND
31	Oro-cloacal swab	Affected	8.98×10^8	1.86×10^5
32	Oro-cloacal swab	Affected	1.74×10^5	4.57×10^3
33	Oro-cloacal swab	Affected	8.69×10^7	1.40×10^1
34	Oro-cloacal swab	Healthy recovered	6.60×10^1	6.15×10^2
35	Oro-cloacal swab	Healthy	8.26×10^4	8.17×10^2
36	Oro-cloacal swab	Affected	3.43×10^7	2.731×10^3
37	Oro-cloacal swab	Healthy	1.97×10^2	8.00×10^1
38	Oro-cloacal swab	Affected	2.77×10^6	8.00×10^1
39	Oro-cloacal swab	Healthy	7.34×10^2	6.68×10^2
40	Oro-cloacal swab	Healthy adult*	6.96×10^3	ND
41	Oro-cloacal swab	Healthy adult*	1.09×10^4	ND
42	Oro-cloacal swab	Healthy adult*	1.04×10^3	ND
43	Oro-cloacal swab	Healthy adult*	ND	ND
44	Oro-cloacal swab	Healthy adult*	7.09×10^6	7.00×10^1
45	Oro-cloacal swab	Healthy	8.36×10^4	8.27×10^2
46	Oro-cloacal swab	Healthy	7.44×10^2	6.78×10^2
47	Oro-cloacal swab	Healthy squab	3.00×10^1	ND
48	Oro-cloacal swab	Healthy squab	1.00×10^1	ND
49	Oro-cloacal swab	Healthy squab	1.80×10^1	1.10×10^1
50	Oro-cloacal swab	Healthy squab	3.40×10^1	1.70×10^1

ND = not detected.

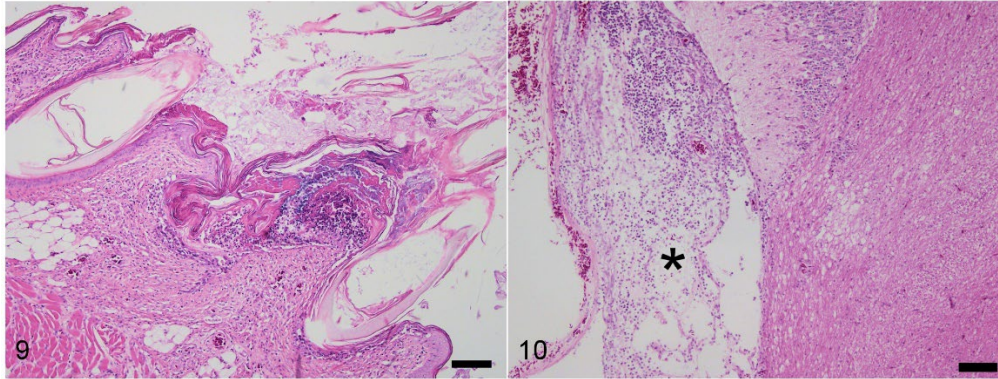
* Suspect case zero.



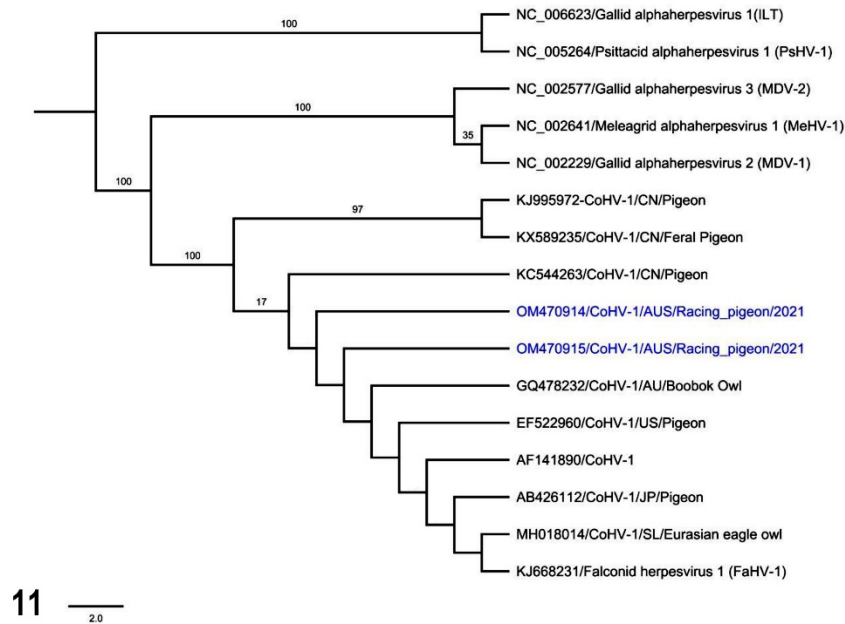
Supplemental Figures 1–4. Clinical signs in young rock pigeons (*Columba livia*) naturally coinfectd with PiCV and CoHV1. **Supplemental Figure 1.** Diffuse conjunctivitis. **Supplemental Figure 2.** Severe diffuse subcutaneous facial edema. **Supplemental Figure 3.** Peri-cloacal hyperkeratosis. **Supplemental Figure 4.** Fibrinosuppurative pharyngitis.



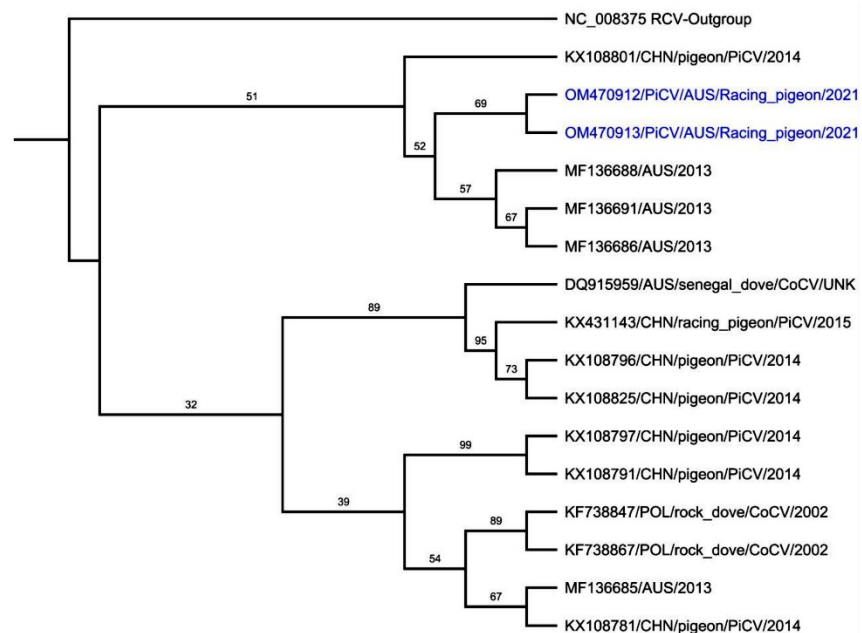
Supplemental Figures 5–8. Histopathologic findings in young rock pigeons naturally coinfecting with PiCV and CoHV1. **Supplemental Figure 5.** Laryngeal mucosa with thick serocellular exudate of heterophils (arrow) and disruption of epithelium. Bar = 50 μ m. **Supplemental Figure 6.** Peri-cloacal skin and feather follicle infundibulum with diffuse ballooning degeneration of keratinocytes (*) and mixed inflammatory cells in the dermis. Bar = 50 μ m. **Supplemental Figure 7.** Vesicle formation in peri-cloacal skin with subcorneal pustule (*) containing degenerate heterophils. Bar = 50 μ m. **Supplemental Figure 8.** Bursa of Fabricius with basophilic botryoid intracytoplasmic inclusion bodies indicative of PiCV infection. Bar = 20 μ m.



Supplemental Figures 9, 10. Histopathologic findings in young rock pigeons naturally coinfecting with PiCV and CoHV1. **Supplemental Figure 9.** Peri-cloacal skin with foci of intense heterophilic inflammation. Bar = 100 μ m. **Supplemental Figure 10.** Severe thickening of the meninges with edema, hemorrhage, and a diffuse infiltrate of macrophages and heterophils (*). Bar = 100 μ m.



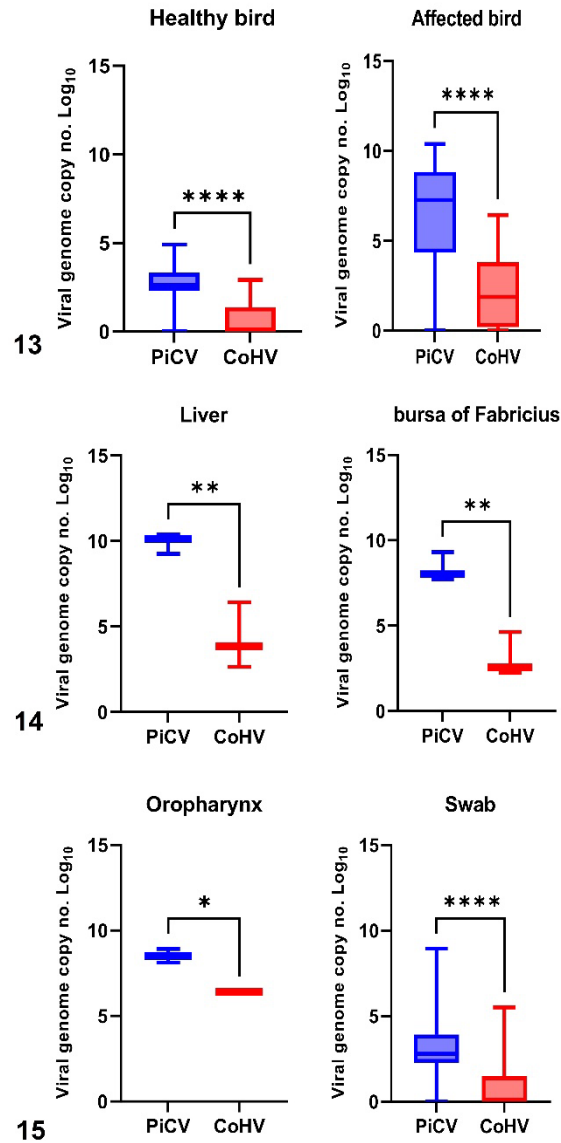
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Supplemental Figures 11, 12. Phylogenetic analysis of CoHV1 and PiCV sequences.

Supplemental Figure 11. Maximum likelihood (ML) phylogenetic tree of avian herpesviruses DNA dependent DNA polymerase (*DDDP*) gene. **Supplemental Figure 12.** ML phylogenetic tree of PiCV replication-associated (*Rep*) gene. Our sequences are highlighted in blue.



Supplemental Figures 13–15. Association between PiCV and CoHV1 copy numbers.

Supplemental Figure 13. Comparison of viral loads in oro-cloacal swabs from healthy birds and clinically affected birds. **Supplemental Figure 14.** Viral loads in liver and bursa of Fabricius from birds collected during postmortem examination. **Supplemental Figure 15.** Comparison of viral loads in oropharyngeal swab collected from 4 dead birds during postmortem examination with combined oro-cloacal swabs collected from healthy and affected birds.

Supplemental Link 1. Cloaca including bursa of Fabricius and adjacent skin demonstrating circoviral inclusion bodies in the bursa and skin as well as subcorneal pustules with eosinophilic intranuclear herpesvirus-like inclusion bodies within keratinocytes.

<https://slice.edu.au/s/73c9031b>

Supplemental Link 2. Section through brain and middle ear demonstrating bilateral tympanitis and diffuse granulomatous meningitis. <https://slice.edu.au/s/6511e3a9>