

# Serologic Evidence of Exposure to Highly Pathogenic Avian Influenza H5 Viruses in Migratory Shorebirds, Australia

## Appendix

**Appendix Table 1.** Hemagglutinin inhibition assay demonstrating fold differences between homologous and heterologous H5 clade antigens to the corresponding reference H5 clade ferret antibodies used in this study\*

Antigen	Antibody						
	1.1.1 <i>Cambodia</i>	2.1.3.2a <i>Indonesia</i>	2.3.2.1b <i>Barn Swallow</i>	2.3.2.1c <i>Viet Nam</i>	2.3.4 <i>Guizhou</i>	2.3.4.4 <i>Gyrfalcon</i>	2.3.4.4 <i>Hubei</i>
1.1.1 Cambodia	<b>1,280</b>	<20	<20	<20	40	<20	<20
2.1.3.2a Indonesia	<20	<b>640</b>	<20	<20	<20	<20	<20
2.3.2.1b BarnSwallow	<20	<20	<b>1,280</b>	320	<20	<20	<20
2.3.2.1c VietNam	<20	<20	160	<b>320</b>	<20	<20	<20
2.3.4.2 Guizhou	40	<20	<20	<20	<b>640</b>	<20	<20
2.3.4.4 Gyrfalcon	<20	<20	<20	<20	<20	<b>160</b>	<20
2.3.4.4 Hubei	<20	<20	<20	<20	<20	<20	<b>80</b>

\*1.1.1 Cambodia, A/Cambodia/X0810301/2013(H5N1); 2.1.3.2a Indonesia, A/Indonesia/NIHRD11771/2011(H5N1); 2.3.2.1b Barn Swallow, A/barn swallow/Hong Kong/D10-1161/2010(H5N1); 2.3.2.1c Viet Nam, A/duck/Vietnam/NCVD-1584/2012(H5N1); 2.3.4 Guizhou, A/Guizhou/1/2013(H5N1); 2.3.4.4 Gyrfalcon, A/gyrfalcon/Washington/41088-6/2014(H5N8); 2.3.4.4 Hubei, A/Hubei/29578/2016(H5N6). Bold indicates homologous antigen-antibody titer.

**Appendix Table 2.** Summary of red-necked stint serum samples assayed for avian influenza H5 viruses, Australia, 2010–2018\*

Year†	No. collected	ELISA–positive‡	ELISA–inconclusive§	Tested by HI assay¶	HPAI H5–positive#	HPAI H5 prevalence, % (95% CI)
2011–12	129	27	8	25	1	0.7 (0.04–4.9)
2012–13	175	55	0	51	0	<0.001
2013–14	274	47	1	43	2	0.7 (0.1–2.9)
2014–15	280	75	0	67	5	1.8 (0.6–4.3)
2015–16	261	15	12	23	2	0.7 (0.1–3.1)
2016–17	177	25	10	30	8	4.5 (2.1–9.0)
2017–18	235	51	16	68	5	2.1 (0.8–5.2)
TOTAL	1,531	295	47	307	23	1.5 (1.0–2.3)

\*CI, confidence interval; HI, hemagglutinin inhibition; HPAI, highly pathogenic avian influenza.

†Austral summer, October–April, when the species is in Australia.

‡Sample/negative ratio <0.5, as defined by manufacturer (IDEXX, <https://www.idexx.com>).

§Sample/negative ratio between 0.5–0.6, demonstrated by Brown et al. (1) and Shriner et al. (2) to indicate a positive result in wild bird samples.

¶Includes samples for which the ELISA result was positive or inconclusive, and sufficient volume to run at least 3 antigens.

#Positive for ≥1 of 7 HPAI H5 antigens used in this study.

**Appendix Table 3.** Summary of Pacific black duck serum samples assayed for avian influenza H5 viruses, Australia, 2010–2018\*

Year	No. Collected	ELISA–positive†	ELISA–		Tested by HI assay	HPAI H5–positive§	LPAI H5–positive¶
			inconclusive‡	assay			
2011	114	60	13	72	0	9	
2012	92	65	13	74	0	4	
2013	75	48	7	44	0	1	
2014	21	12	2	14	0	1	
2015	0	0	0	0	0	0	
2016	20	8	0	8	0	0	
2017	11	2	0	2	0	0	
2018	61	20	7	26	0	1	
<b>Total</b>	<b>394</b>	<b>215</b>	<b>42</b>	<b>20</b>	<b>0</b>	<b>16</b>	

\*HI, hemagglutinin inhibition; HPAI, highly pathogenic avian influenza; LPAI, low pathogenic avian influenza.

†Sample/negative ratio <0.5, as defined by manufacturer (IDEXX, <https://www.idexx.com>).

‡Sample/negative ratio between 0.5–0.6, demonstrated by Brown et al. (1) and Shriner et al. (2) to indicate a positive result in wild bird samples.

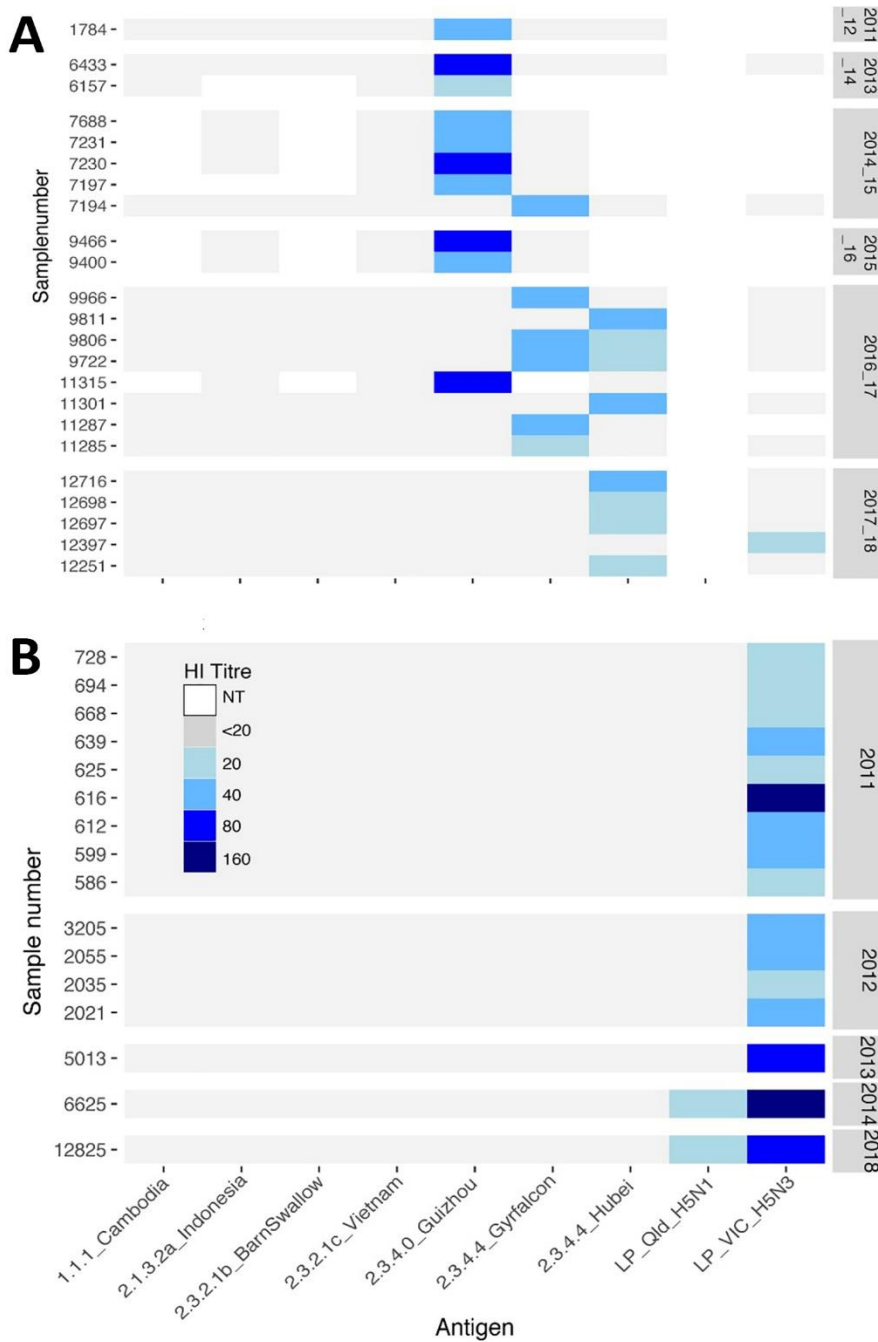
§Positive for any of 7 HPAI H5 antigens used in this study.

¶Positive for either of the 2 LPAI H5 antigens used in this study.

**Appendix Table 4.** Temporal patterns of circulation of avian influenza H5 virus clades, globally\*

H5 clade	Year										Total
	2010	2011	2012	2013	2014	2015	2016	2017	2018		
1	2										2
<b>1.1.1</b>	<b>13</b>	<b>1</b>	<b>2</b>								<b>16</b>
1.1.2	14	16	29	39							98
1.1-like	1										1
2.1.3.1	3	2						1			6
2.1.3.2	11										11
2.1.3.2a	16	22	4	2	1						45
<b>2.1.3.2b</b>	<b>13</b>	<b>10</b>	<b>1</b>				<b>1</b>				<b>25</b>
2.1.3.2-like	7										7
2.1.3.3	2										2
2.2.1	46	11	1	1	1		1				61
2.2.1.1	10	2				2	2				16
2.2.1.1a	43	6			1		1				51
2.2.1.2	20	99	42	51	63	104	28	1			408
2.2.2	5										5
2.2.2.1	17	15									32
2.3.2.1	1										1
2.3.2.1a	4	50	147	43	32	24	37	61	11		409
<b>2.3.2.1b</b>	<b>7</b>	<b>19</b>	<b>4</b>	<b>4</b>							<b>34</b>
<b>2.3.2.1c</b>	<b>51</b>	<b>114</b>	<b>51</b>	<b>135</b>	<b>89</b>	<b>241</b>	<b>66</b>	<b>9</b>	<b>16</b>		<b>772</b>
<b>2.3.4</b>	<b>19</b>	<b>5</b>	<b>3</b>								<b>27</b>
2.3.4.1	6										6
<b>2.3.4.2</b>	<b>15</b>	<b>3</b>									<b>18</b>
<b>2.3.4.4</b>	<b>7</b>	<b>6</b>	<b>7</b>	<b>56</b>	<b>233</b>	<b>403</b>	<b>117</b>	<b>85</b>	<b>6</b>		<b>920</b>
7.2	4	2	7	9	8	2					32
Am-nonGsGD	27	106	42	76	34	25	80	27			417
EA-nonGsGD	12	31	4	4	10	8	4				73
<b>Total</b>	<b>376</b>	<b>520</b>	<b>344</b>	<b>420</b>	<b>472</b>	<b>809</b>	<b>337</b>	<b>184</b>	<b>33</b>		<b>3,495</b>

\*Lineage determined by assessments of sequences deposited in GenBank (<https://www.ncbi.nlm.nih.gov/genbank>). We downloaded metadata for all avian influenza H5 virus hemagglutinin sequences from 2010–2018 (n = 3,495) curated in the Influenza Research Database (<https://www.fludb.org>). Bold indicates clades included in this study.



**Appendix Figure.** Heatmap of serum samples positive for either highly pathogenic or low pathogenic avian influenza H5 virus antigens used in this study. A) Samples from red-necked stints. Year refers to the austral summer (October–April), when this species is in Australia. B) Samples for Pacific black ducks. White indicates serum samples not tested; gray indicates a titer <20, which was the starting titer of this assay; and blue indicates samples containing neutralizing antibodies, shades vary by hemagglutinin inhibition titer (20–160). Sample numbers are ordered by collection year on the y-axis. Antigens used in this study are on the x-axis and abbreviated with relevant clade information.

## References

1. Brown JD, Stallknecht DE, Berghaus RD, Luttrell MP, Velek K, Kistler W, et al. Evaluation of a commercial blocking enzyme-linked immunosorbent assay to detect avian influenza virus antibodies in multiple experimentally infected avian species. *Clin Vaccine Immunol.* 2009;16:824–9. [PubMed https://doi.org/10.1128/CVI.00084-09](https://doi.org/10.1128/CVI.00084-09)
2. Shriner SA, VanDalen KK, Root JJ, Sullivan HJ. Evaluation and optimization of a commercial blocking ELISA for detecting antibodies to influenza A virus for research and surveillance of mallards. *J Virol Methods.* 2016;228:130–4. [PubMed https://doi.org/10.1016/j.jviromet.2015.11.021](https://doi.org/10.1016/j.jviromet.2015.11.021)