Supplementary information

Emergence of novel reassortant H5N3 avian influenza viruses in Korean Mallard ducks in 2018

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Table S1. Detailed NGS analysis of KNU18-91 isolate

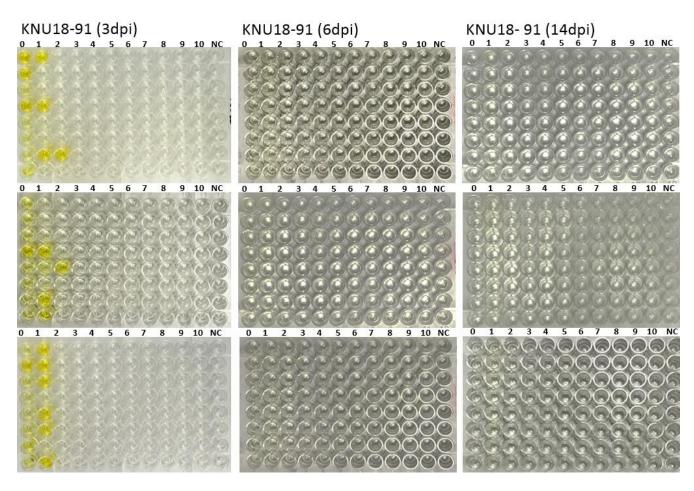
Reference					Sequenced Sample						R_ORF			S_ORF				
Segme nt#	gene nam e	desc.	Accession _ID	ref bp	# of Pre- proce ssed reads	# of Influenz a Virus extracte d reads	# of non- Influe nza Virus reads	Viru s read s %	#M_Rea ds	Uniqu e Match es (%)	S_Con bp	%Cov. (S/R)	Length	S_posi tion	E_posi tion	Length	S_posit ion	E_posit ion
1	PB2	A/greater white- fronted	KX979214	2,341	19369 890	735257	18634 633	0.03 946	24,228	4.0	2,353	98.97	2,307	1	2,307	2,088	228	2,315
2	PB1, PB1- F2	goose/Netherlands /5/2010/H5N3	КХ979754	2,341					11,483	1.9	2,315	98.89	2,274	25	2,298	2,274	18	2,291
3	PA, PA-X		KX977828	2,233					19,656	3.3	2,226	99.69	2,157	19	2,175	2,157	12	2,168
4	HA		KX978851	1,767					95,971	16.00	1,764	99.94	1,704	20	1,723	1,704	17	1,720
5	NP		KX978800	1,565					170,535	28.40	1,595	98.40	1,542	1	1,542	1,548	21	1,568
6	NA		KX979439	1,453					11,718	2.00	1,419	97.87	1,428	1	1,428	1,416	3	1,418
7	M2, M1		KX978221	1,027					249,453	41.60	1,029	99.03	762	23	784	786	1	786
8	NEP, NS1		KX977601	890					427	0.10	839	93.26	717	3	719	564	30	593

a. 12hpi	b. 24hpi	c. 36hpi
KNU18-01 H5N3 H1N1	KNU18-91 H5N3 H1N1	KNU18-91 H5N3 H1N1
3 4 5 2 3 4 5 2 3 4 MC	5 6 7 8 9 6 7 8 4 5 6 7	5 6 7 8 9 6 7 8 9 6 7 8
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d. 48hpi	e. 60hpi	6 701
KNU18-91 H5N3 H1N1	KNU18-91 H5N3 H1N1	f. 72hpi KNU18-91 H5N3 H1N1
5 6 7 8 6 7 8 9 5 6 7 8	<u>5 6 7 8 5 6 7 8 5 6 7 8</u>	5 6 7 8 5 6 7 8 4 5 6 7
(R)		
		ROCORONNA
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8.8.9.5 8 9 9 9 9 9 8		
		(5) (10 C C C C C C C C C C C C C C C C C C

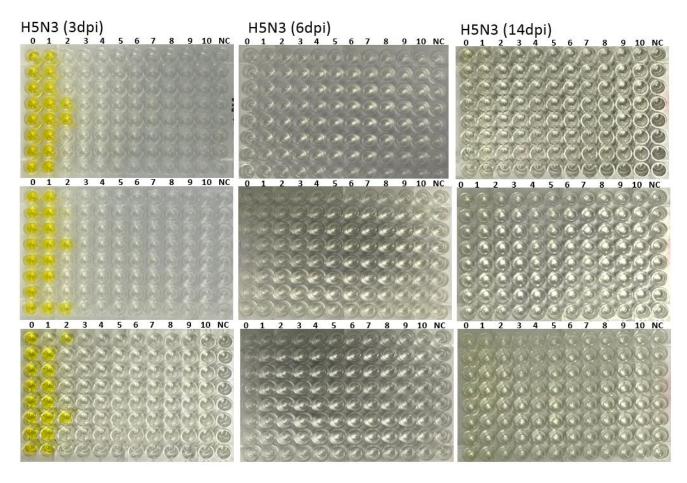
	NU	21	H5	N3		H1N1					
5	6	7	8	5	6	7	8	4	5	6	7
C	G	Ce	16	S.N.	3	5	E	0	D.	S	3)
C		C	0	-	-	0	0	0	5	3	a
C		1C	1E	0	C	6	6	-	-	3	(0)
6	60	6	E	6	6	G	6		0	5	3)
é		6	6	6	6	6	6	0	0	5	0)
é	Vic		R	6	6	6	6	0	1	3	5
2		Ca	13	100	6	6	5	S	13	0	5
		10	15	100		10	5	5	-		(m)
1.3		19	A.C.		A	2000					
	0	S	0	0	D'		3	9)	3		3)
6	0	6	0		P	0	3	9	3	3	3
C.	6	C	C	0	0	0	0	-))	3	0	3)
a	B	6	6	0	3	0	5	0	33	5	3)
	6	6	E	5	0	0	5	31	37	5	3
1		(R)	F	-	5	5	157	3)		3	a
		100	100	6	2	No.		-		3	SI
		12				1					R
	1	10	14				9	100	8	12	1.50
0	0	6	S	E	EV	0	3	3	N	38	3
Ā	i.	3			8	5	(F)		3	5	3
a			3		5	K	5	5	S.		5
2		6	1	X	X	1					-
		10 miles	1 and	K	and and	5	(A)		1		10
5		100	2		12	20	K		1		-
		100		2		12	V	No.			
	X	1			12	R	R	2			P
	100	10		0	63			20	1		4

Fig. S1. Raw ELISA data to conduct TCID₅₀ **assay.** KNU18-91, H5N3, H1N1 were infected to MDCK cells at MOI=0.01, Infected supernatants were collected at each 12hpi for 72 hours. Serial 10-fold dilutions of three different viruses' supernatant at different hpi. KNU18-91 (a-f), H5N3 (g-I), and H1N1 (m-r) were infected MDCK for 3 dpi., cell monolayers were fixed with 80% acetone and then blocked with 5% non-fat milk. Cells were washed with PBST 3 times and incubated with 0.1 µg/well of anti-influenza nucleoprotein (Medix Biochemica, Finland) at 37°C, 1 hour. Wash cells by PBST 3 times, add secondary Ab in the form of horseradish peroxidase (HRP)-conjugated rabbit antimouse IgG (Abcam, Cambridge, UK), incubated at 37°C, 1 hour. Cell then were washed with PBS-T for five times to remove nonspecific biding and 100 of 3,3',5,5'-tetra methyl benzidine (Sigma-Aldrich) substrate solution was added, it was followed by 100 µL of H2SO4 0.18M solution to stop reaction.

- 3: 10³- fold dilution of stock;
- 4: 104- fold dilution of stock;
- 5: 10⁵- fold dilution of stock;
- 6: 10⁶- fold dilution of stock;
- 7: 107- fold dilution of stock;
- 8: 10⁸- fold dilution of stock;
- 9: 109- fold dilution of stock;
- 10: 10¹⁰- fold dilution of stock;
- NC: negative control (uninfected virus);



b.





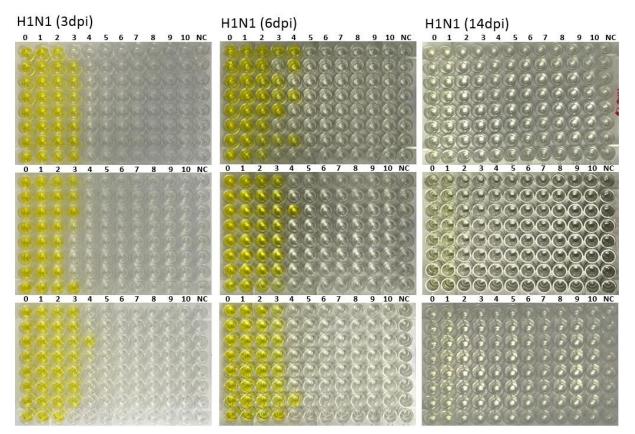


Fig. S2. Raw ELISA data to conduct TCID₅₀ assay to measure virus titer in lung. After 3, 6, 14 dpi of intranasal challenge of viruses into female Balb/c mice 8 weeks old (initial intranasal virus = 10^4 EID50/mouse). Mice lung were collected and virus titer (TCID50) was conducted by ELISA method (n=3)

- 0: 10⁰- fold dilution of stock;
- 1: 10¹- fold dilution of stock;
- 2: 10²- fold dilution of stock;
- 3: 10³- fold dilution of stock;
- 4: 104- fold dilution of stock;
- 5: 10⁵- fold dilution of stock;
- 6: 10⁶- fold dilution of stock;
- 7: 107- fold dilution of stock;
- 8: 108- fold dilution of stock;
- 9: 109- fold dilution of stock;
- 10: 10¹⁰- fold dilution of stock;
- NC: negative control (uninfected virus);

<u>Antigen:</u>	K91 virus	<u>Antigen:</u>	SYG06/2006 (H5N3)virus
HAI titer:	10 20 40 80 160 320 640 1280	HAI titer:	10 20 40 80 160 320 640 1280
<u>K91</u>		K91	
H5N3	000000000	H5N3	$\bigcirc \bigcirc $

Fig. S3. Hemagglutination assay. The mouse antisera were treated with RDE and then serially diluted with PBS, mixed with 4 hemagglutination units of a virus, and incubated for 30 min at room temperature. The HI result was obtained from the highest serum sample dilution that inhibited hemagglutination.