Surveillance and characterization of Eurasian Avian-like H1N1 swine influenza viruses in China

- Yang Huanliang
- Professor
- China

Harbin Veterinary Research Institute,
Chinese Academy of Agricultural Sciences

3 - 4 April 2024

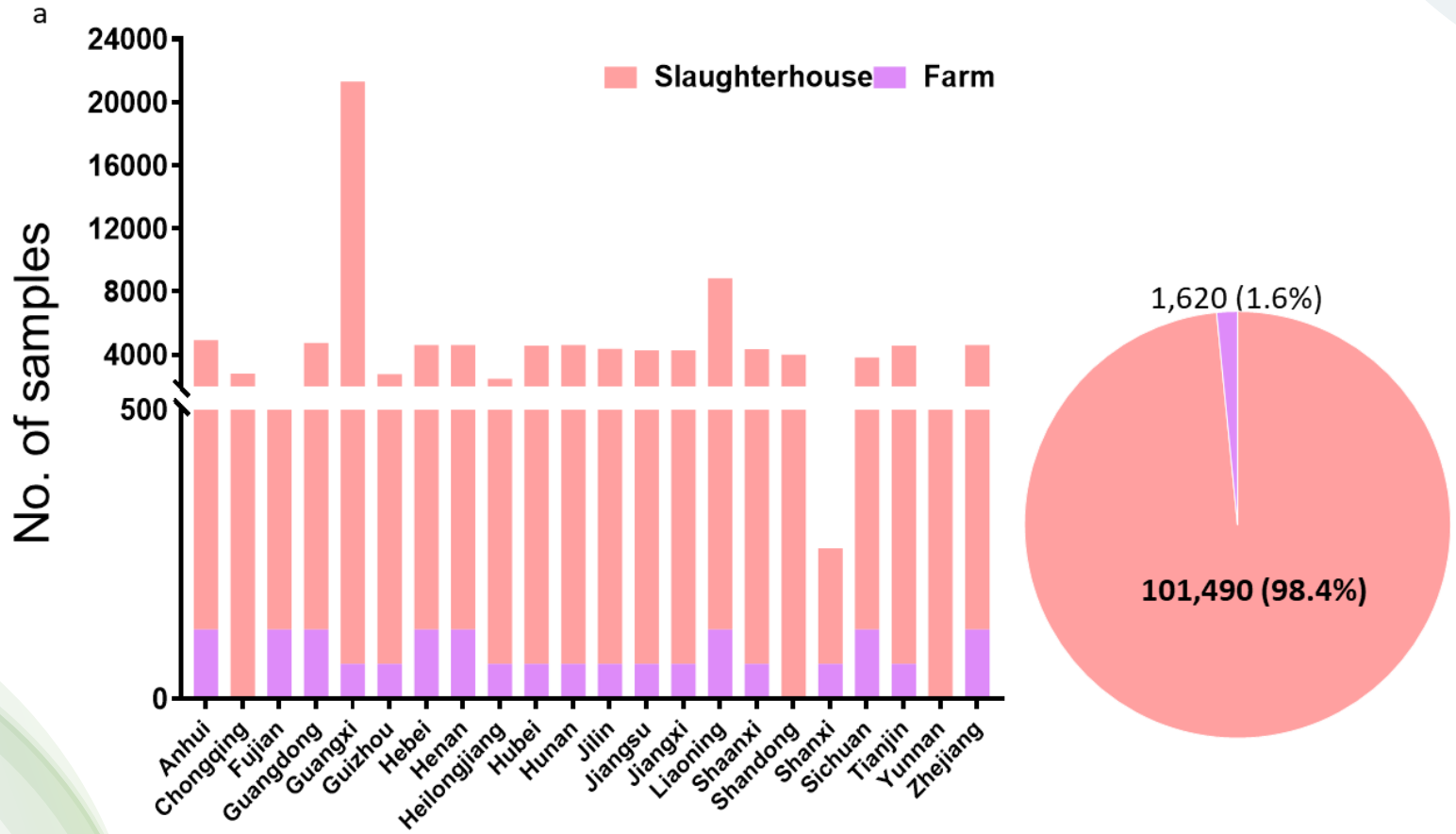
Index

Section 1 Swine influenza surveillance in China

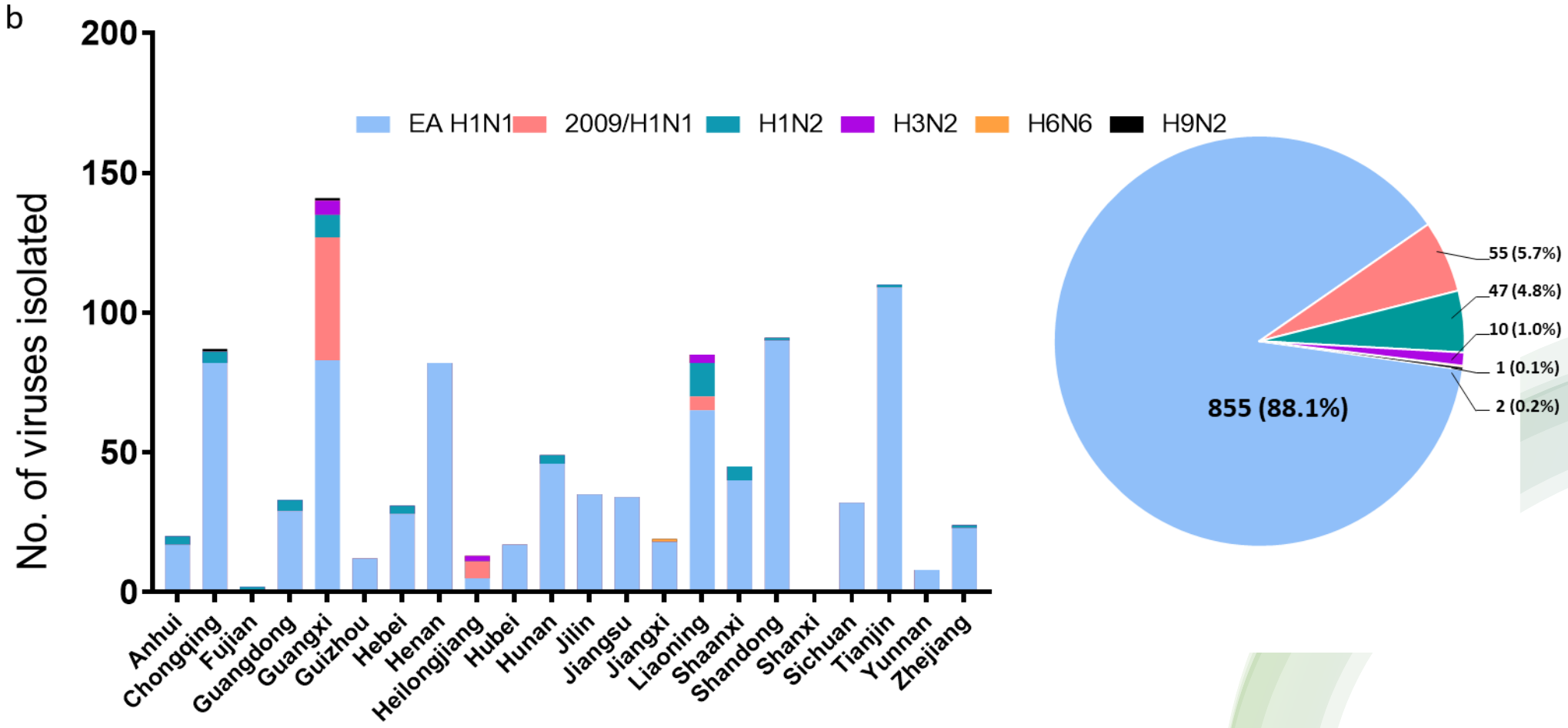
We found that EA H1N1 viruses are predominant swine influenza viruses in pigs in China.

Will the EA H1N1 viruses become more lethal and more efficient in transmission during their circulation in Nature?

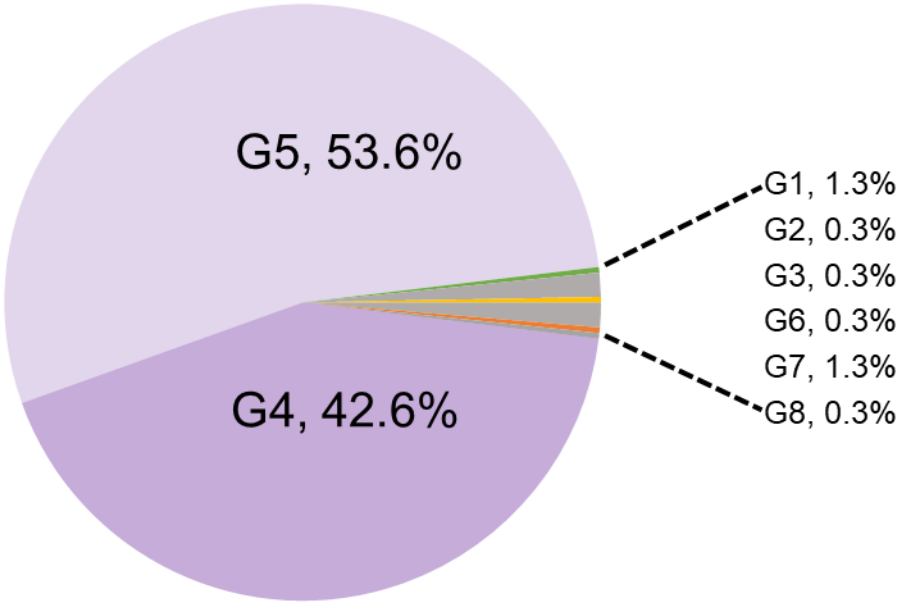
Sample collection and virus isolation from 2013 to 2019

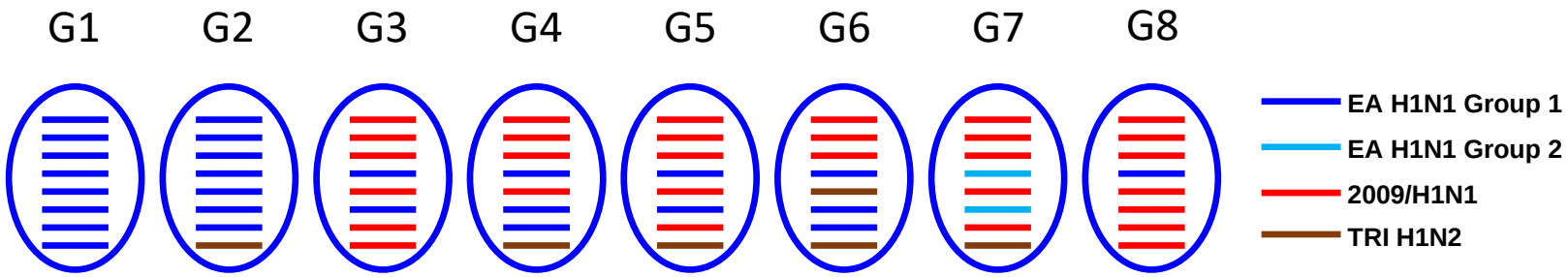


Sample collection and virus isolation from 2013 to 2019（970 viruses, 855 of the 910 H1N1 strains carried the HA gene of EA H1N1 viruses）



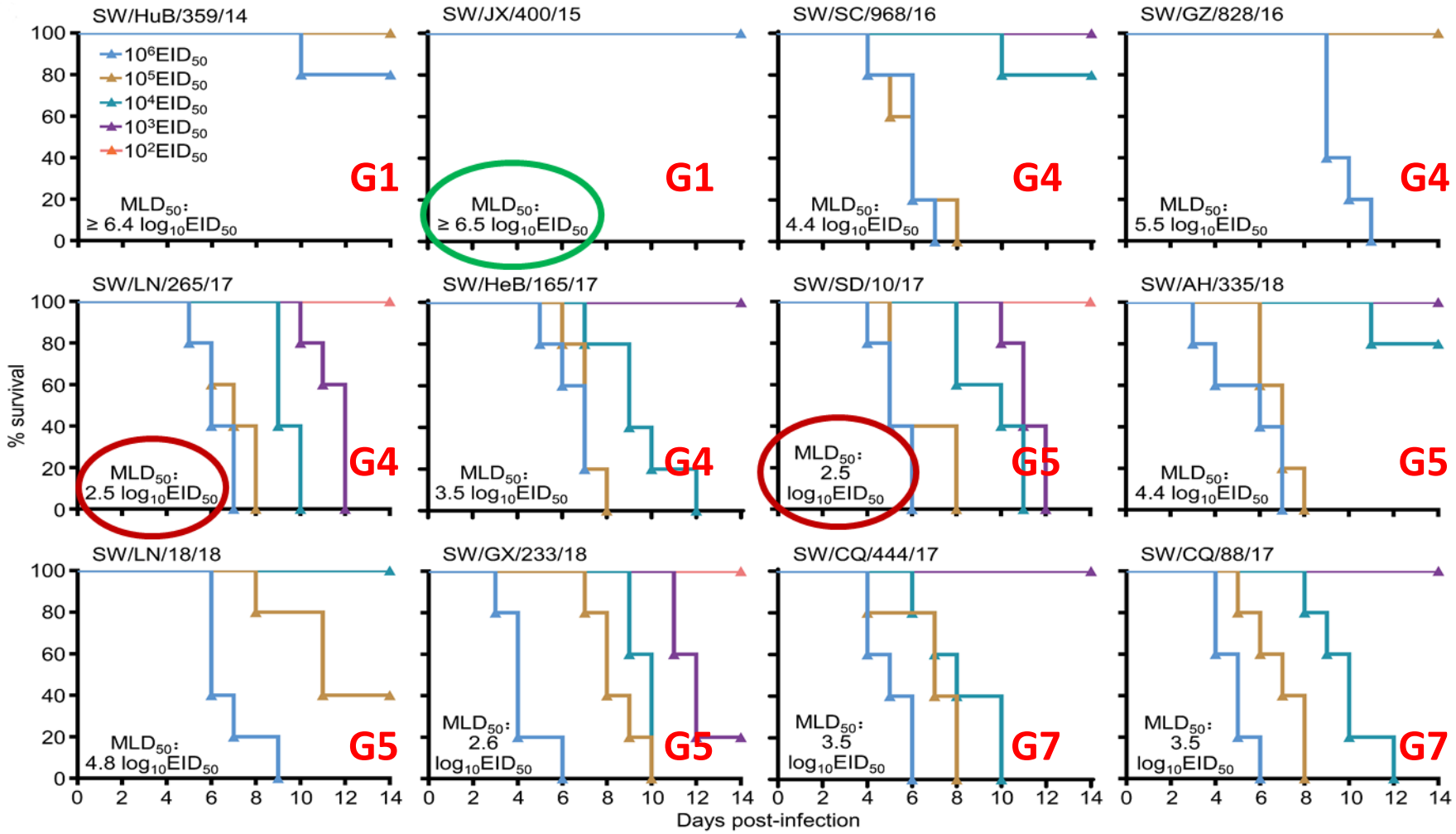
We fully sequenced the genome of 319 representative viruses, the viruses were divided into eight different genotypes (G1 to G8) based on their gene constellations.





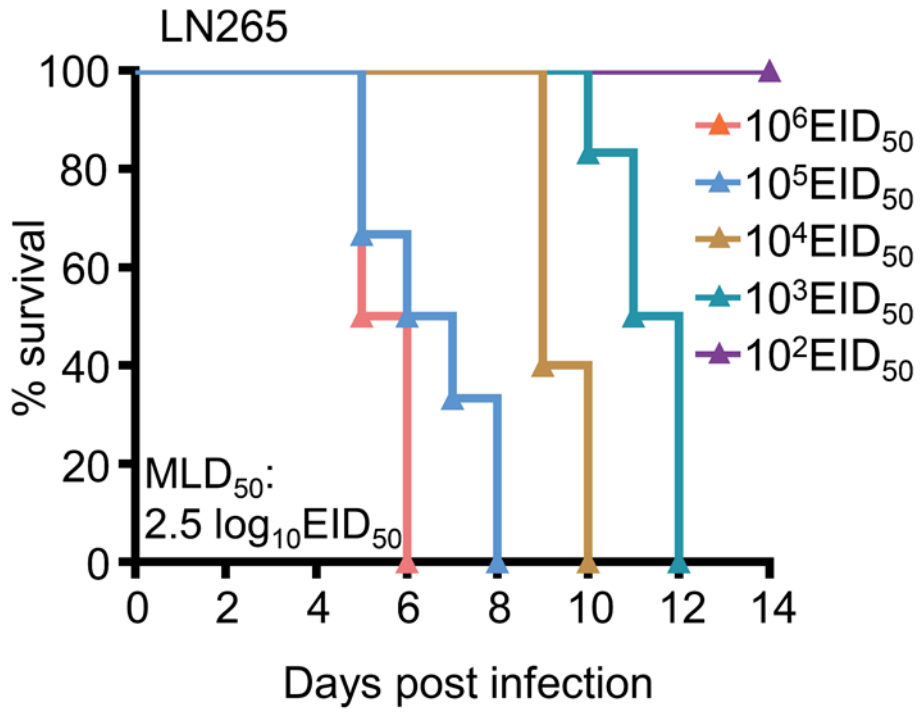
Section 2 Characterization of Eurasian Avian-like H1N1 swine influenza viruses

Some of the viruses isolated in this period showed increased pathogenicity in mice with up to a 10,000-fold, based on the MLD_{50} values



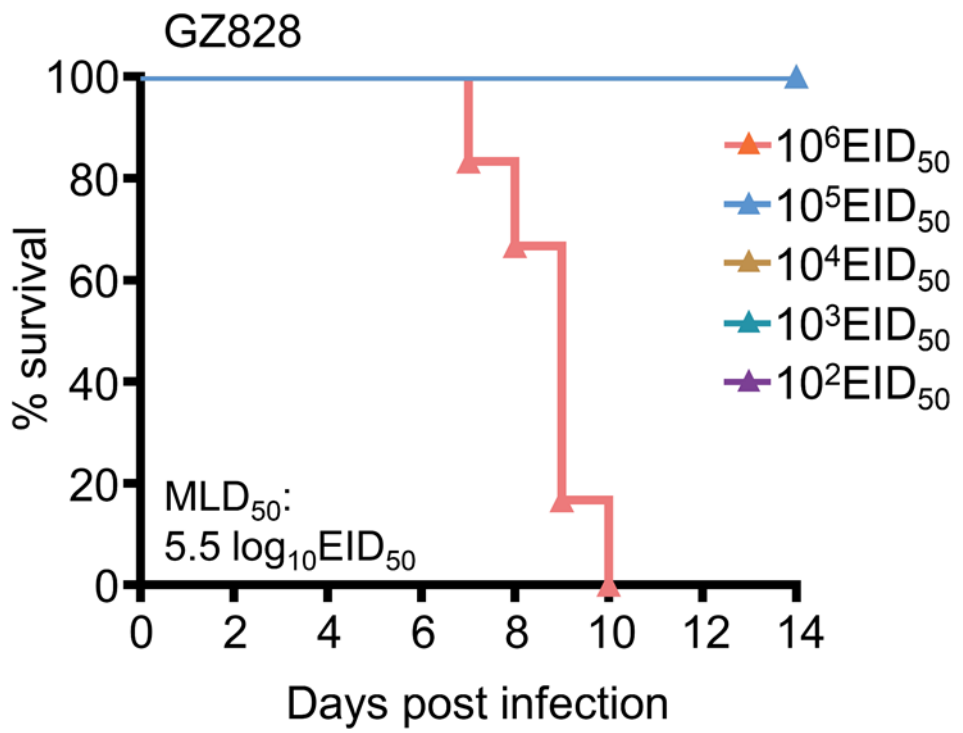
LN265

MLD_{50}=2.5$log_{10}EID_{50}$



GZ828

MLD_{50}=5.5$log_{10}EID_{50}$

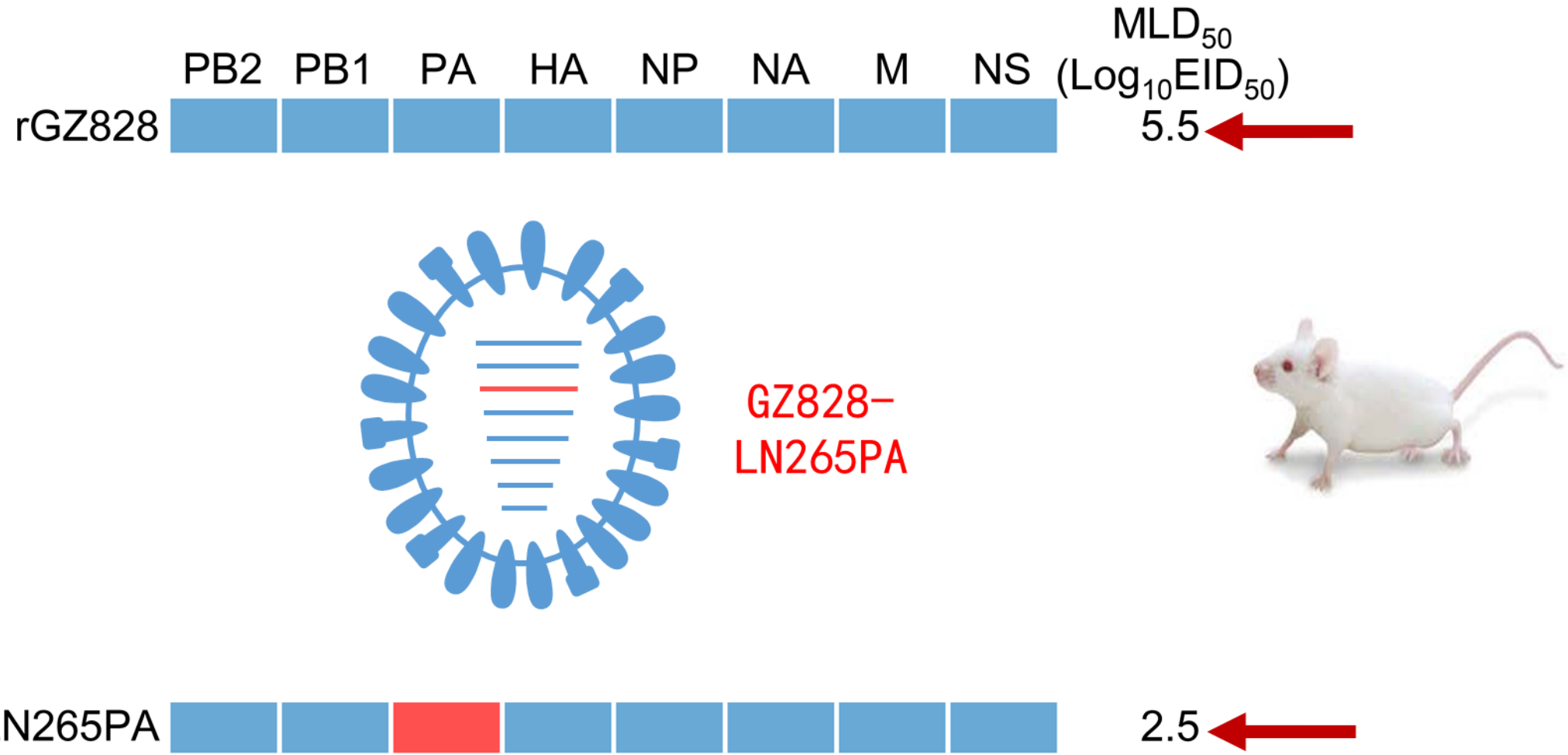


Meng F., et al, *PNAS*, 2022

The PA gene of GZ828 significantly decreases the pathogenicity of LN265

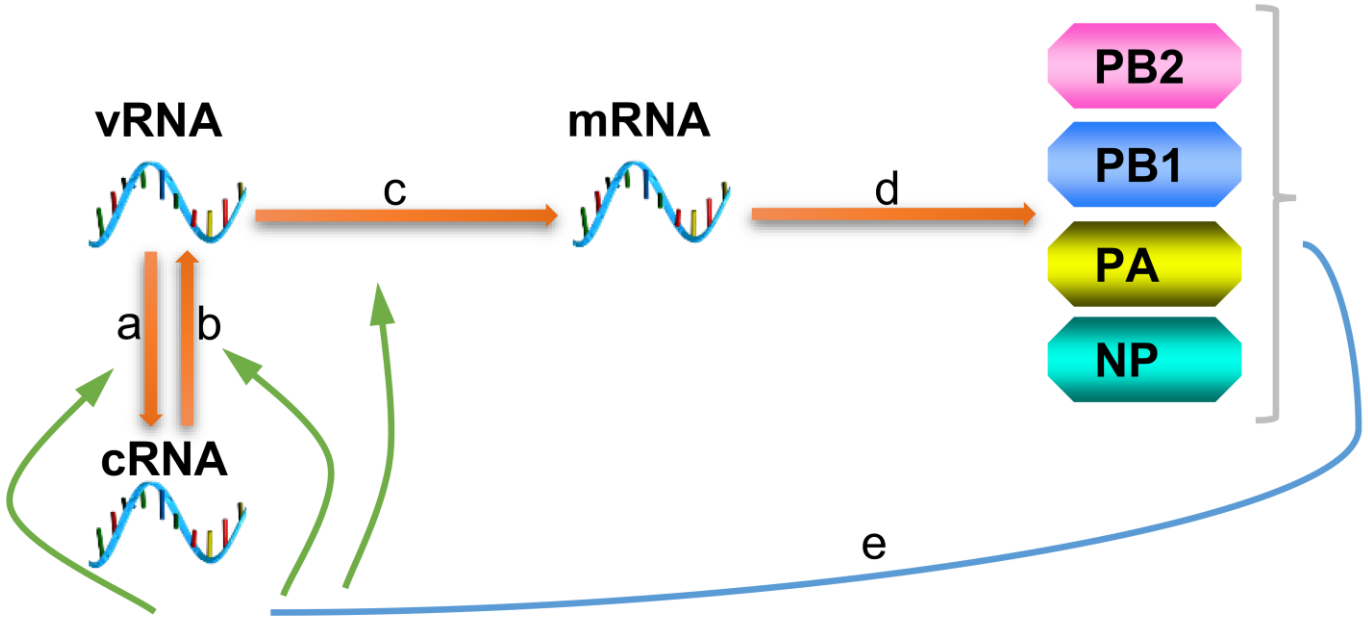
	PB2	PB1	PA	HA	NP	NA	M	NS	MLD_{50} ($Log_{10}EID_{50}$)
rGZ828									5.5
rLN265									2.5
rLN265-GZ828PB2									2.0
rLN265-GZ828PB1									2.5
rLN265-GZ828PA									6.5
rLN265-GZ828HA									2.3
rLN265-GZ828NP									2.0
rLN265-GZ828NA									2.5
rLN265-GZ828M									2.3
rLN265-GZ828NS									2.8

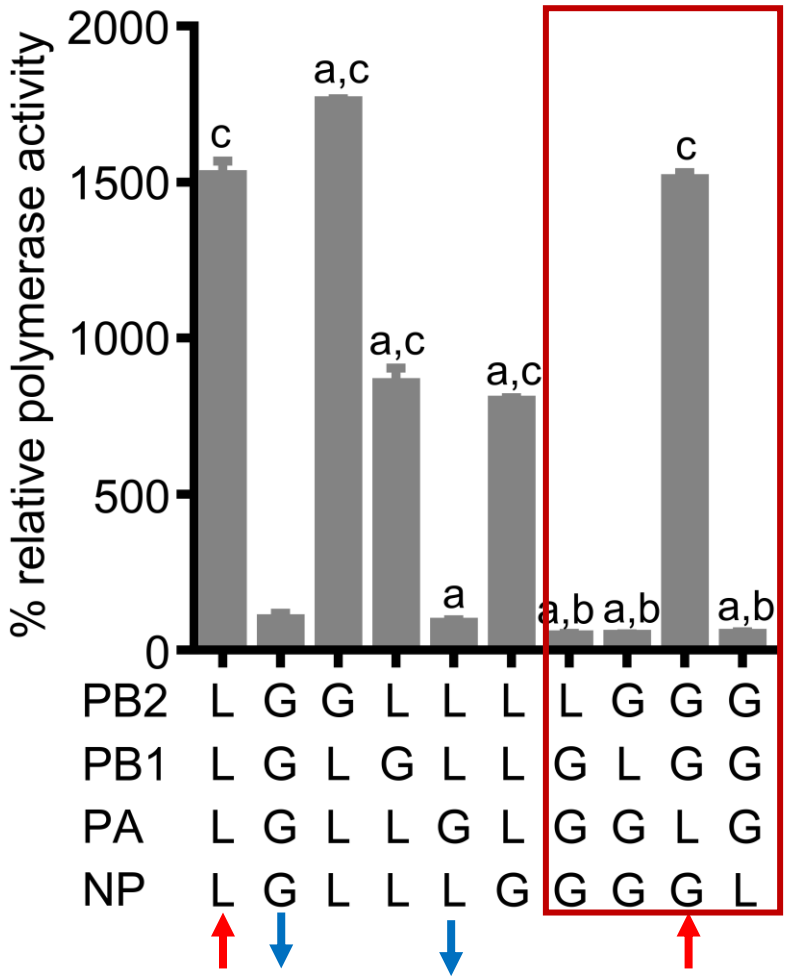
The PA gene of LN265 significantly increases the pathogenicity of GZ828



Meng F., et al, *PNAS*, 2022

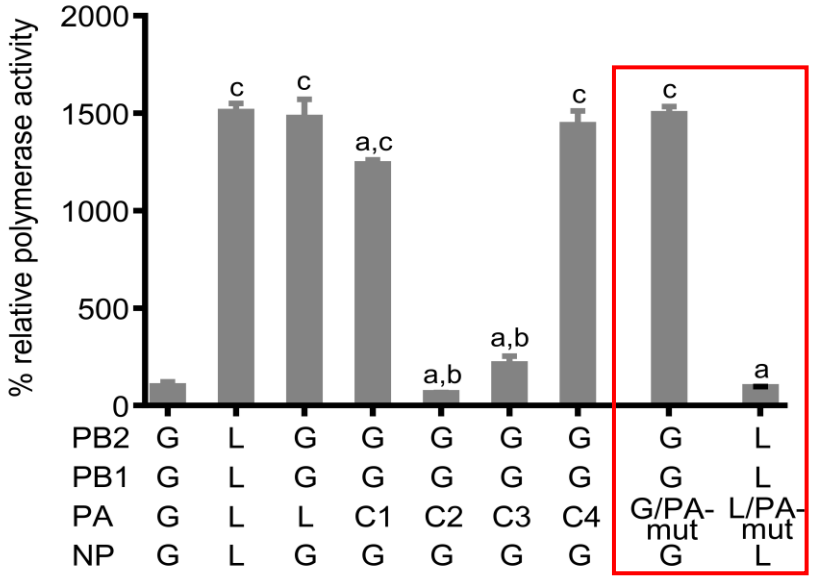
- LN265 and GZ828 differ in their polymerase activity.
- The PA gene of GZ828 significantly decreases the polymerase activity of LN265.
- The PA gene of LN265 significantly increases the polymerase activity of GZ828.



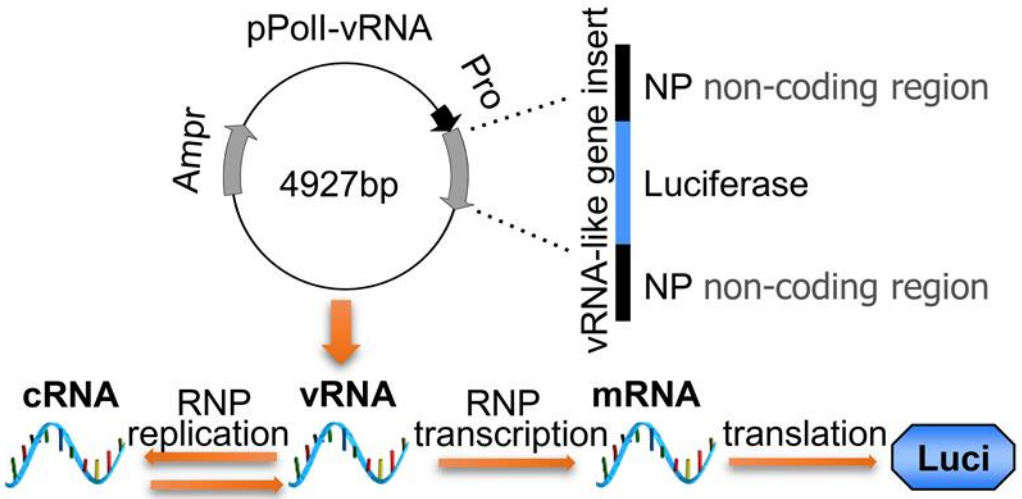


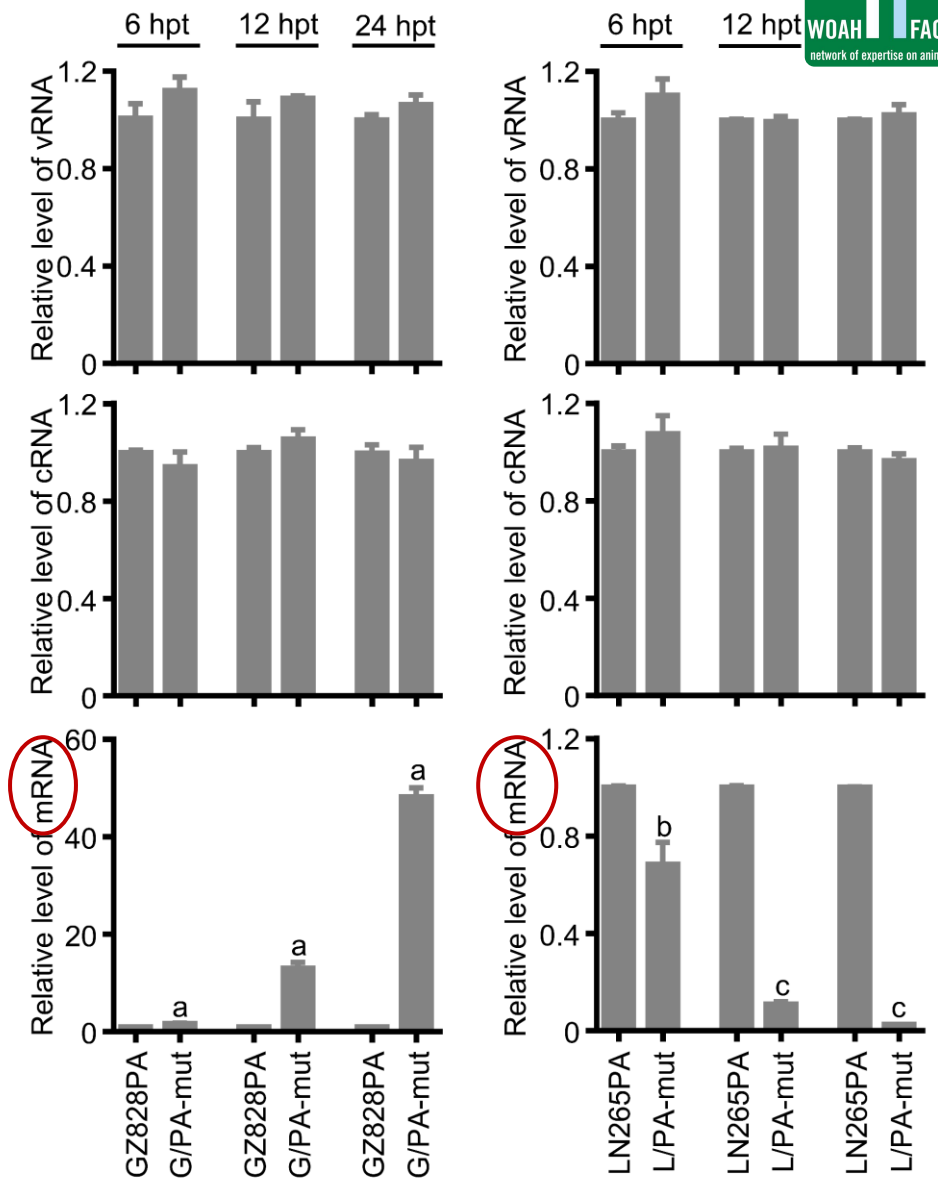
Four amino acids (I100V, K321N, V330I, and T639A) in PA collectively contribute to the difference in the polymerase activity of the GZ828 and LN265.

	100	120	228	256	321	330	359	362	364	437	531	639	643
						331						619	
LN265PA	I	I	N	K	K	V	N	K	S	H	R	T	K
GZ828PA	V	V	S	Q	N	I	S	R	N	I	G	A	R
C1	I	I	N	K	K	V	S	R	N	I	G	A	R
C2	V	V	S	Q	N	I	N	K	S	H	R	A	R
C3	V	V	S	Q	N	I	S	R	N	I	G	T	K
C4	I	I	N	K	K	V	S	R	N	I	G	T	K
G/PA-mut	*I*	V	S	Q	*K*	*V*	S	R	N	I	G	*T*	R
L/PA-mut	*V*	I	N	K	*N*	*I*	N	K	S	H	R	*A*	K



Further studies indicated that the four amino acid substitutions in PA contribute to the viral mRNA transcription, but not the vRNA or cRNA replication

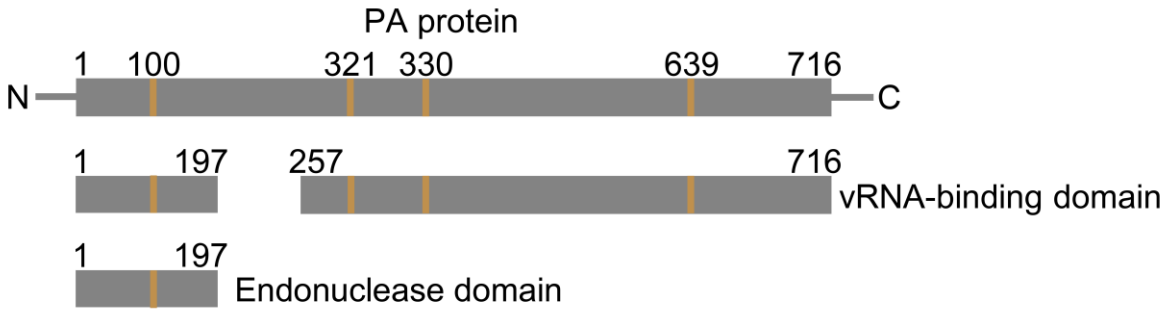


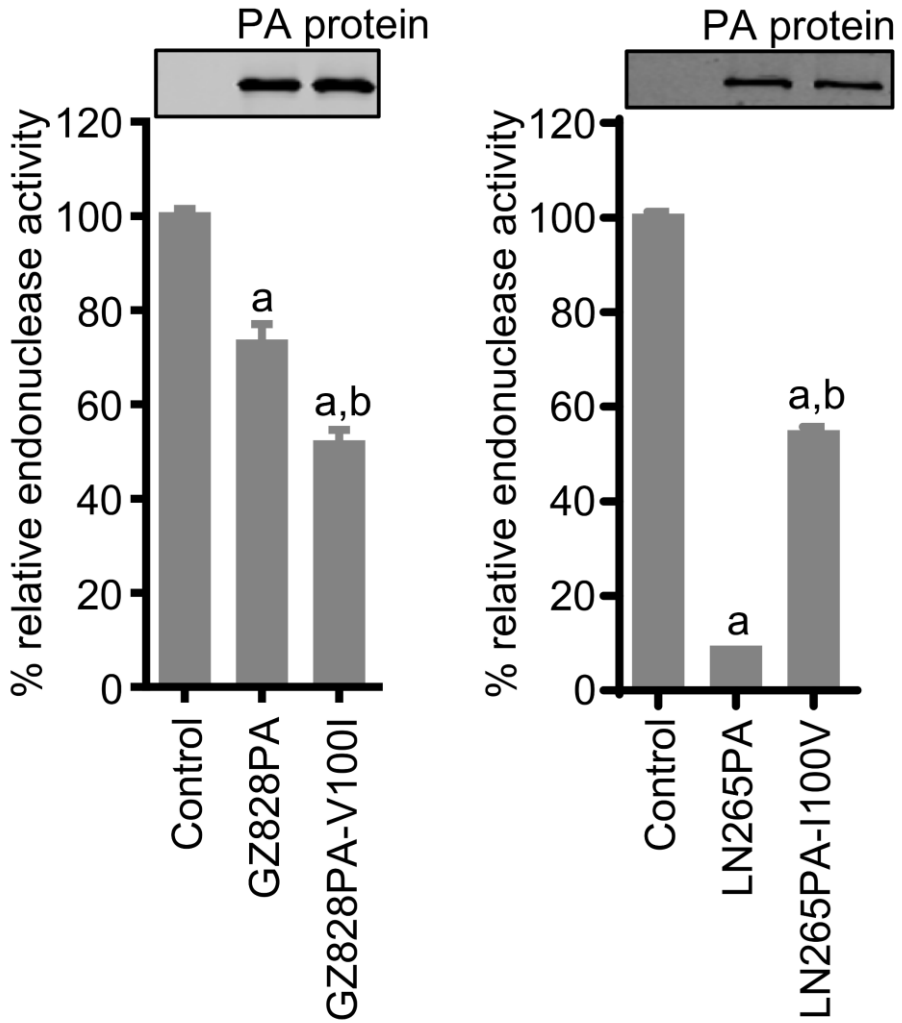


The amino acid at position 100 in PA affects its endonuclease activity

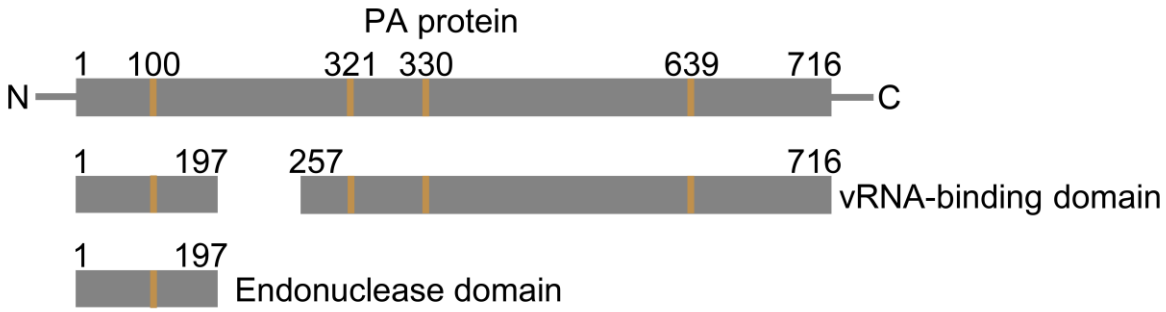
V100I: increased

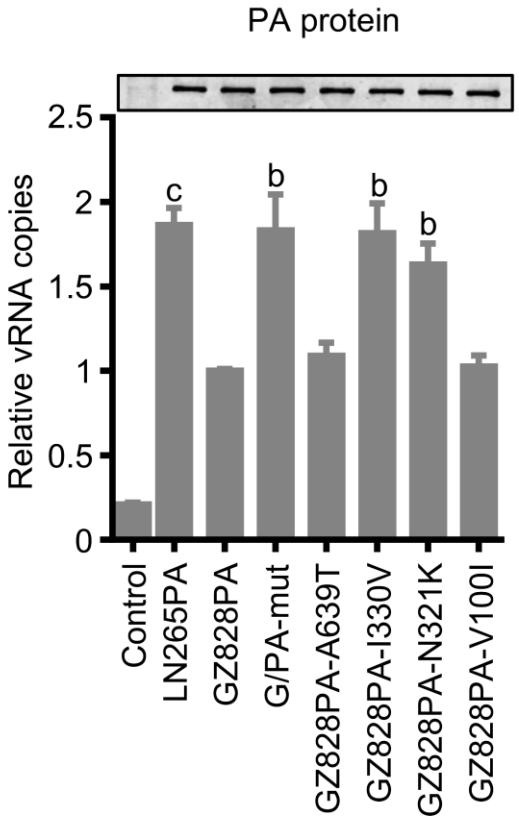
I100V: decreased

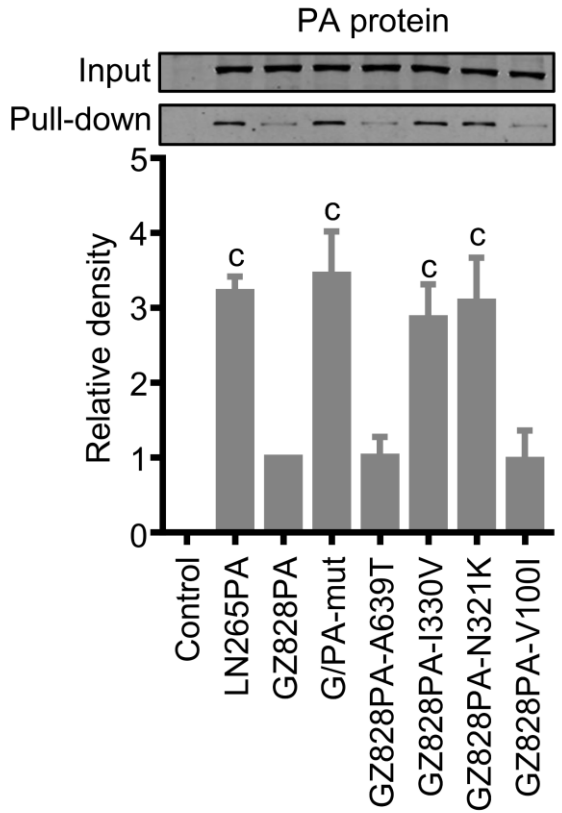




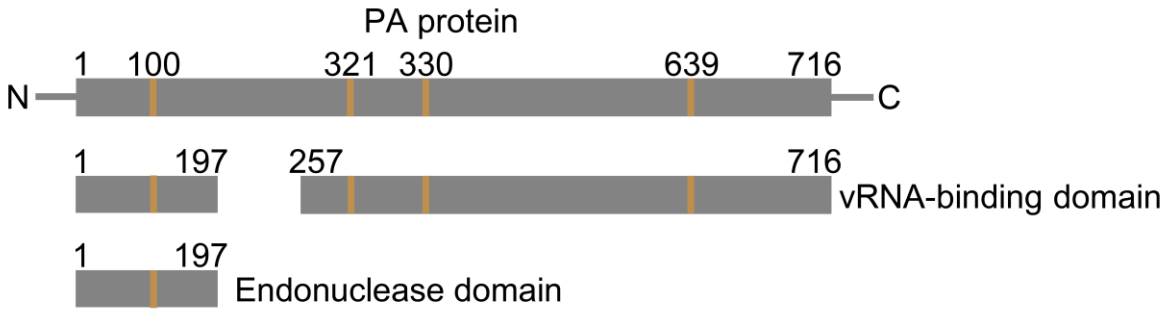
The substitutions N321K and I330V in the PA of GZ828 increase its vRNA-binding ability

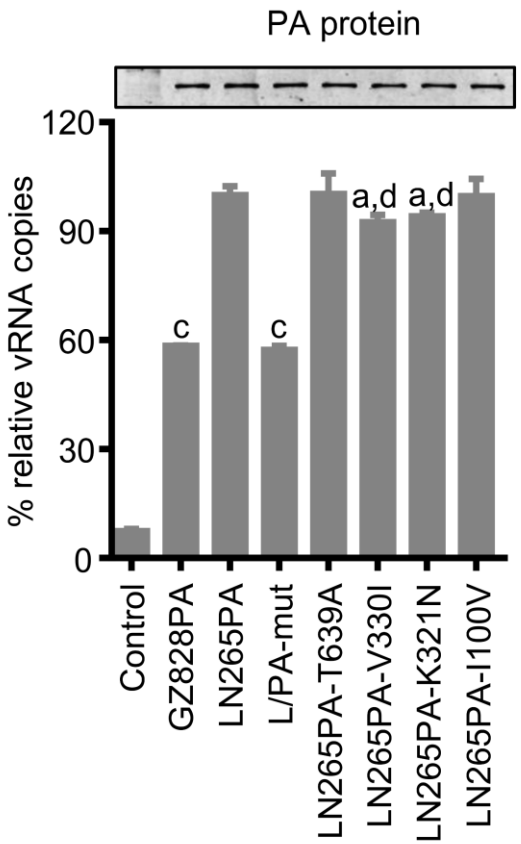


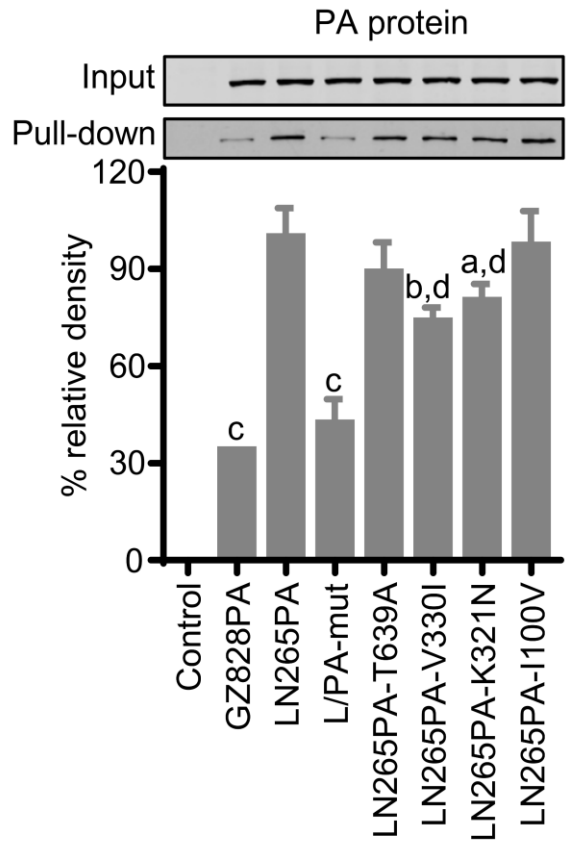




The substitutions K321N and V330I in the PA of LN265 decrease its vRNA-binding ability







The four amino acid substitutions (I100V, K321N, V330I, and T639A) in PA collectively attenuated the LN265 virus by over 7900-fold

Virus	Other genes	PA 100	PA 321	PA 330	PA 639	MLD_{50} ($Log_{10}EID_{50}$)	Fold attenuated
rLN265	LN265	I	K	V	T	2.5	/
rLN265-PA/I100V	LN265	V	K	V	T	4.5	100
rLN265-PA/K321N	LN265	I	N	V	T	3.7	16
rLN265-PA/V330I	LN265	I	K	I	T	3.5	10
rLN265-PA/T639A	LN265	I	K	V	A	4.3	63
rLN265-PA/mut	LN265	V	N	I	A	6.4	7943

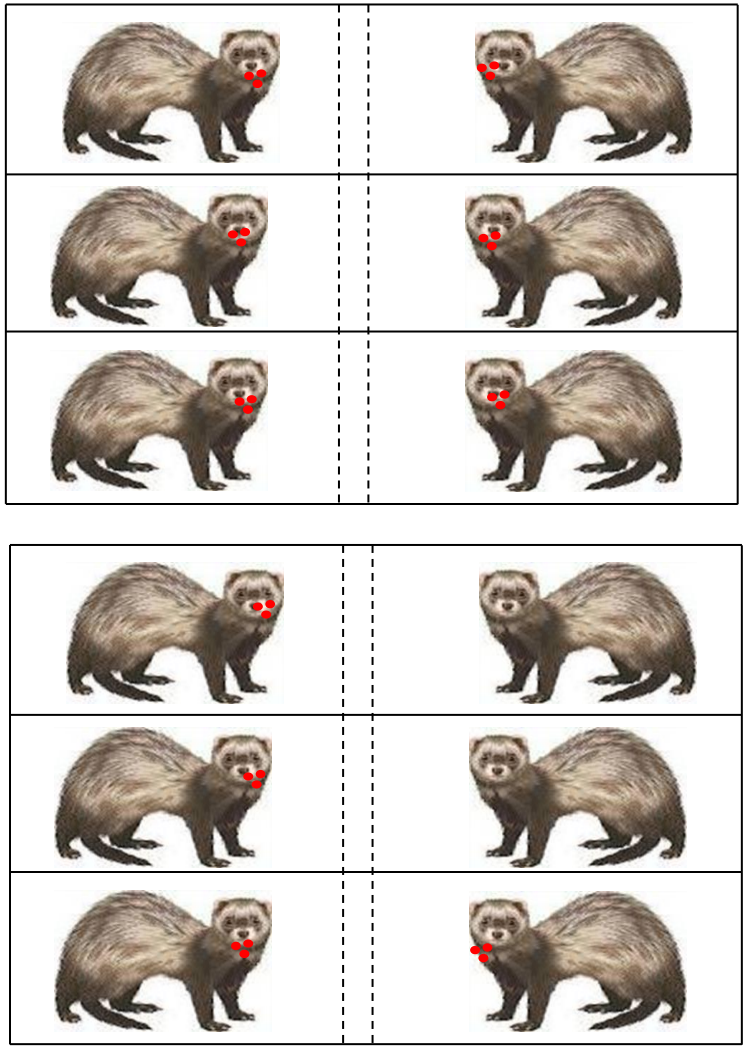
The four amino acid substitutions (V100I, N321K, I330V, and A639T) in PA collectively increased the pathogenicity of GZ828 by 1000-fold

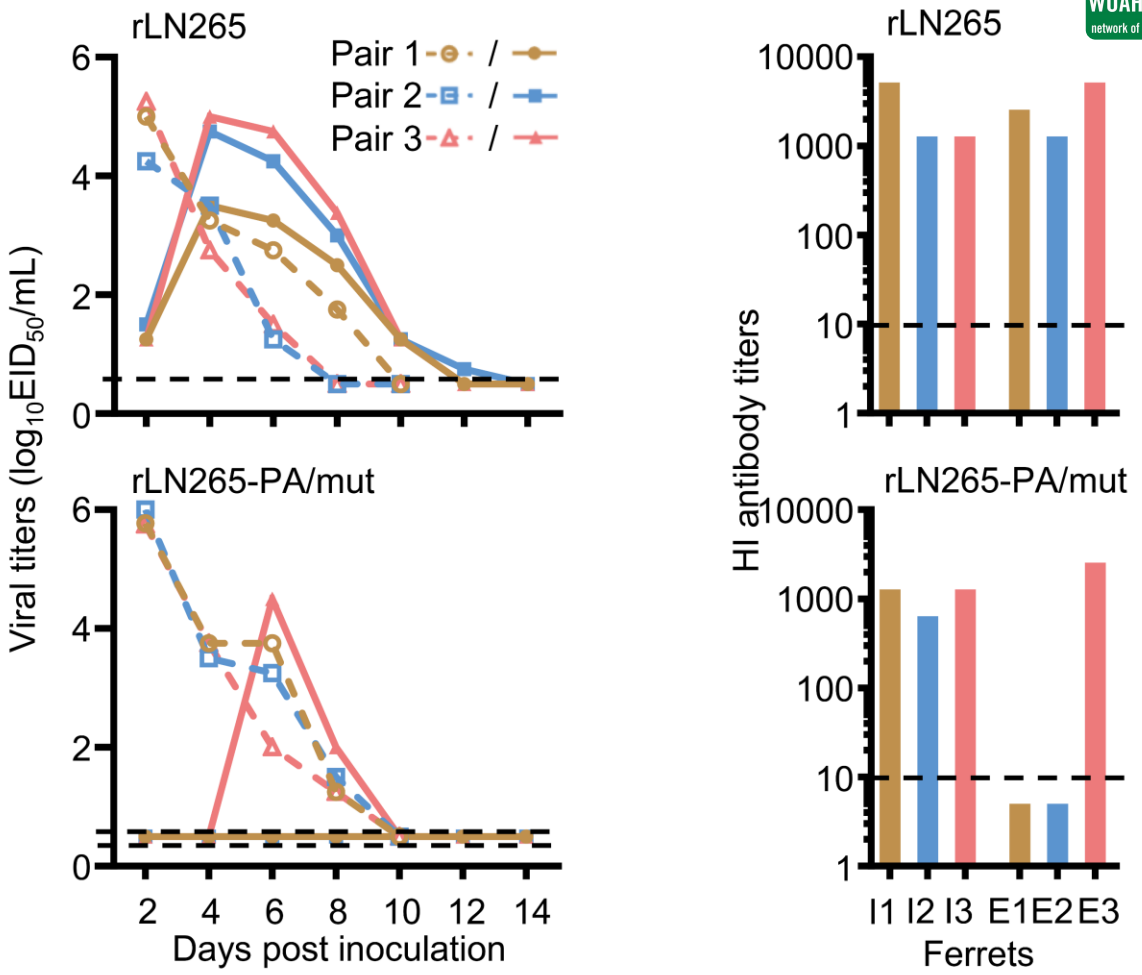
Virus	Other genes	PA 100	PA 321	PA 330	PA 639	MLD_{50} ($Log_{10}EID_{50}$)	Fold of virulence increased
rGZ828	GZ828	V	N	I	A	5.5	/
rGZ828-PA/V100I	GZ828	I	N	I	A	3.6	79
rGZ828-PA/N321K	GZ828	V	K	I	A	4.3	16
rGZ828-PA/I330V	GZ828	V	N	V	A	4.8	5
rGZ828-PA/A639T	GZ828	V	N	I	T	4.0	32
rGZ828-PA/mut	GZ828	I	K	V	T	2.5	1000

The substitution of the four-amino acid in PA alter the transmission of EA H1N1 virus

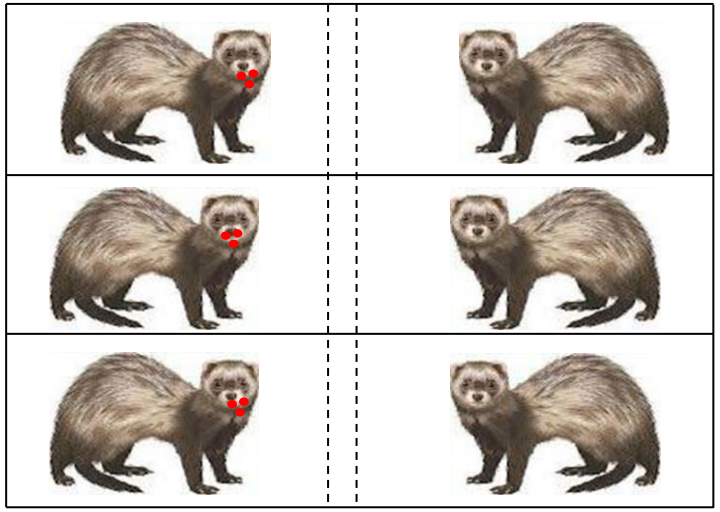
rLN265

rLN265-PA/mut

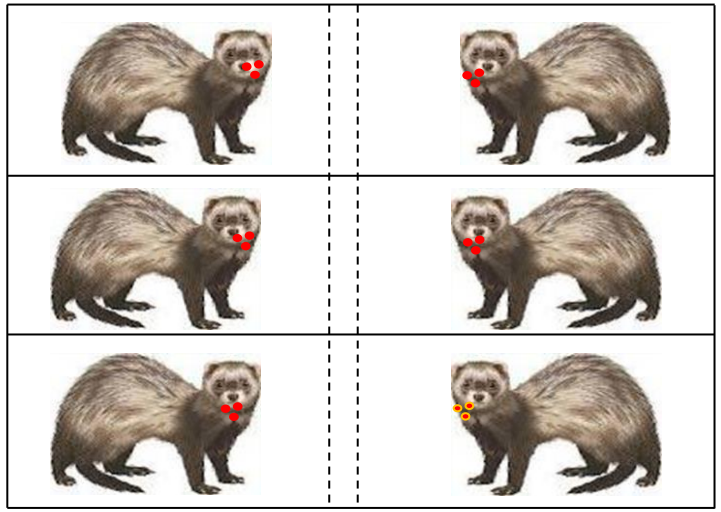


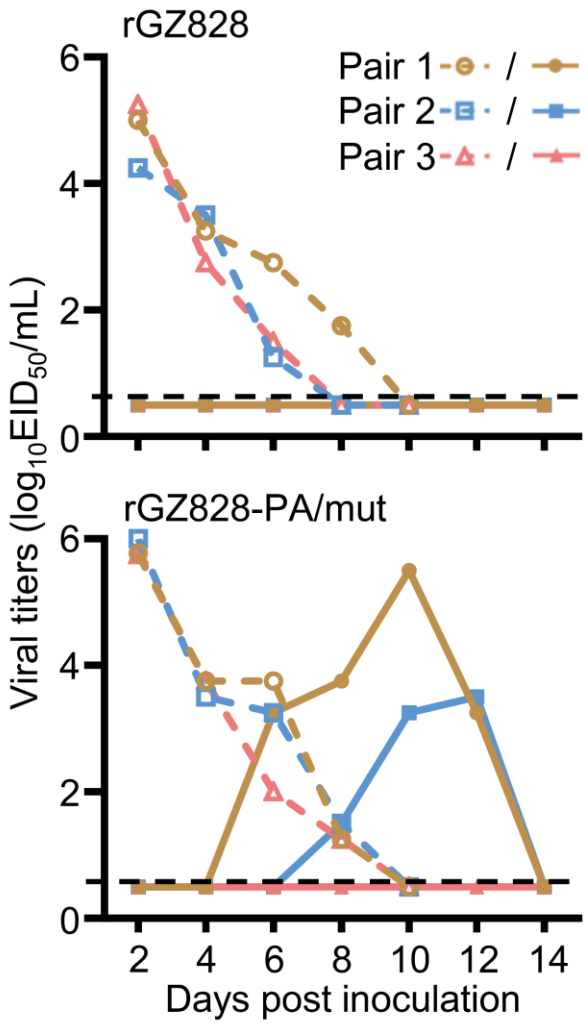


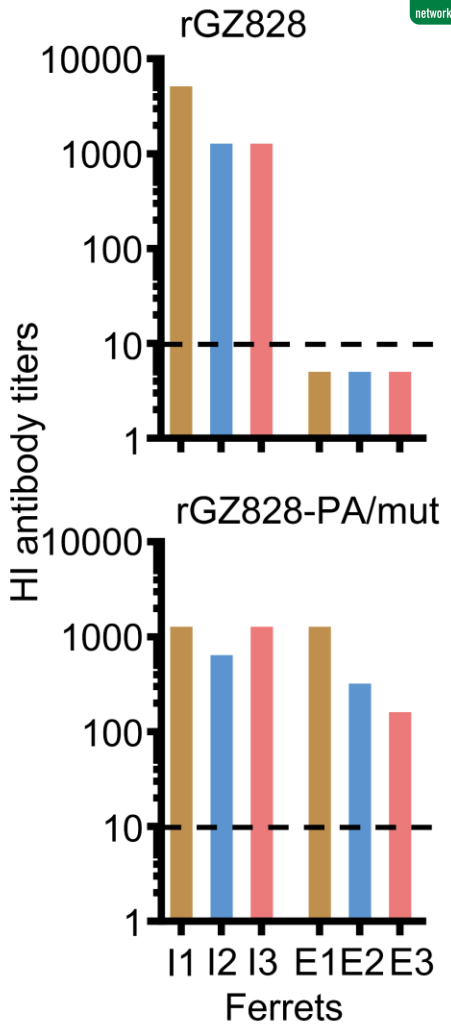
rGZ828



rGZ828-PA/mut



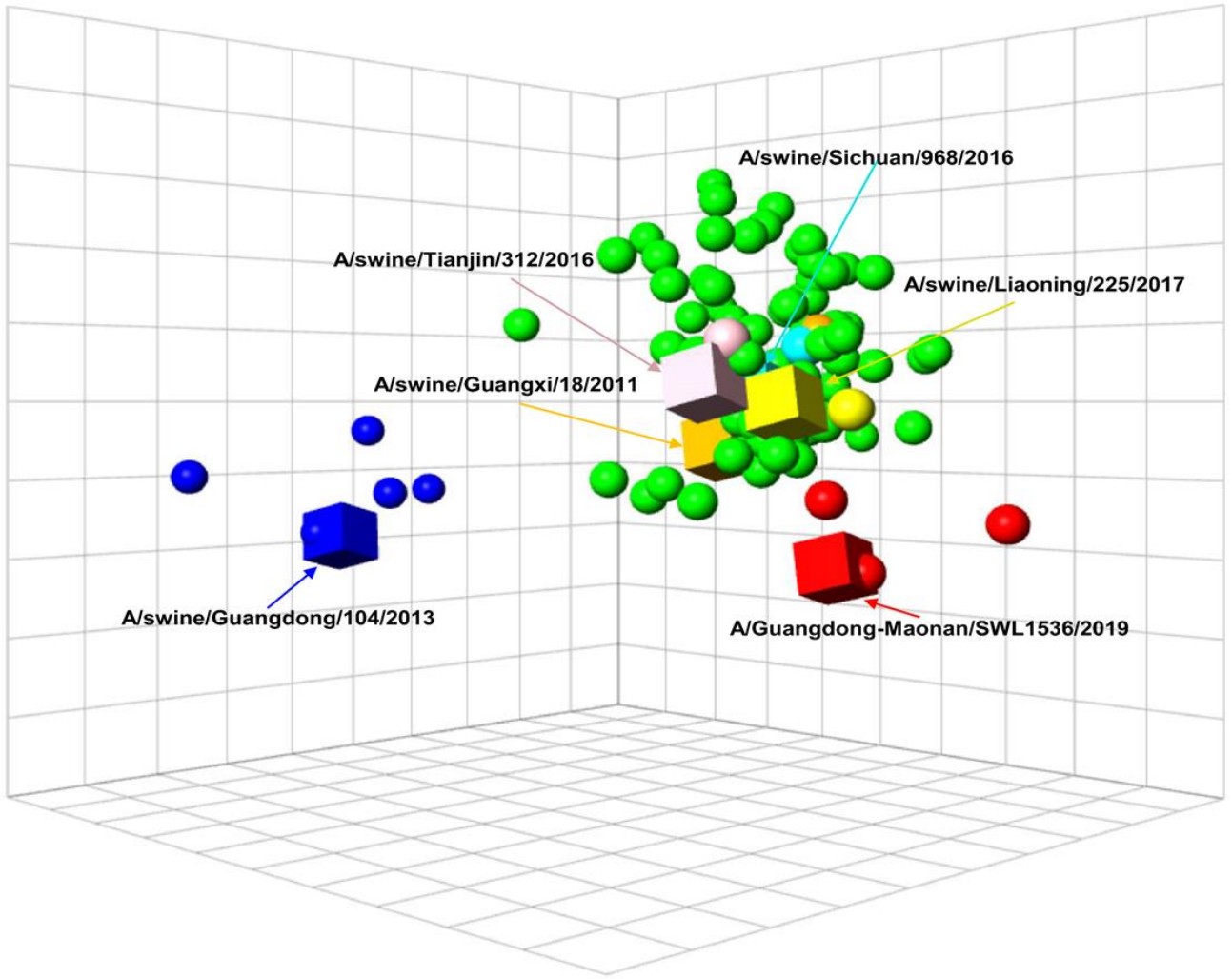




EA H1N1 viruses caused human cases in multiple European countries and China

Country	Number of human cases	Year
Switzerland	4	1986, 2002, 2009
The Netherlands	5	1986, 1993, 2016, 2019, 2020
Spain	1	2008
Italy	1	2016
Germany	2	2020, 2022
China	23	2011—2016, 2019—2022

More than two-thirds of the EA H1N1 viruses reacted poorly or did not react with antiserum against human H1 vaccine strain



Acknowledgments

Dr. Chen Hualan **Dr. Meng Fei**

Thank you

Summary

We collected nasal swabs from 103,110 pigs in China between October 2013 and December 2019, and isolated 855 EA H1N1 viruses. Genomic analysis of 319 representative viruses revealed that these EA H1N1 viruses formed eight different genotypes through reassortment with viruses of other lineages circulating in humans and pigs, and two of these genotypes (G4 and G5) were widely distributed in pigs. Some of the reassortant EA H1N1 viruses isolated in this period showed increased pathogenicity in mice. Accumulated mutations in PA enhance mRNA transcription through different mechanisms and contribute to the harmful properties of the EA H1N1 virus. Two-thirds of the EA H1N1 viruses reacted poorly with ferret serum antibodies induced by the currently used H1N1 human influenza vaccine, suggesting that existing immunity may not prevent the transmission of the EA H1N1 viruses in humans.