

SUPPLEMENTARY TABLE S1. Poultry environment investigation and testing.

Case No.	Region	Source of poultry	Poultry species	Specify the upstream supplier	Poultry species of upstream supplier	qPCR of poultry environment	qPCR of upstream environment	qPCR of living environment
1	Jinjiang, Chengdu	Market	Chicken, duck	Chenghua district market	Chicken, duck	+	+	+
2	Kaijiang, Dazhou	Domestic	Chicken, duck	Luo's chicken and duck farm	Chicken, duck	+	+	+
3	Xuanhan, Dazhou	Domestic	Chicken, duck	Yunchengzhai chicken farm	Chicken, duck	+	+	+
4	Bazhou, Bazhong	Domestic	Chicken, duck, goose	Not purchased in 2021	Not purchased in 2021	+	ND*	+
5	Nanxi, Yibin	Domestic	Chicken	Private vendors in Wangjia town	Chicken	+	ND	-

\* ND: Not done.

SUPPLEMENTARY TABLE S2. The molecular characteristics of the H5N6 influenza viruses isolated from Sichuan Province.

Genes	AA position	Bazhong/2021	Dazhou/2021	All H5N6*	Poultry species of upstream supplier	qPCR of poultry environment	qPCR of upstream environment	Phenotypic effect
HA	N158D	N	N	<b>N 1803</b>	Chicken, duck	+	+	The substitution at residue 158 leads to a loss of glycan chain modification in the 150-loop, which avoids the potential steric hindrance for binding human receptors.
				S 5				
				D 4				
Q226L	Q	Q	Q	<b>Q 1813</b>	Chicken, duck	+	+	Critical for binding the $\alpha$ -2,6-linked receptor and enabling transmission in mammals
				LRERRRKG 1388				
				<b>LREKRRRKG 305</b>				
Cleavage site	LREKRRRKG	LREKRRRKG	LREKRRRKG	SRERRRKG 47	Chicken, duck	+	+	Virulence increases in chickens
				LKERRRKG 29				
				QRETRG 10				
NA	H274Y	H	H	<b>H 1744</b>	Chicken, duck	+	+	Reduces the susceptibility of neuraminidase inhibitors
				Y 2				
				<b>T 1648</b>				
PB2	T271A	T	T	V 37	Chicken, duck	+	+	Enhances viral replication in mammalian cells in vitro
				M 4				
				I 1				
Q591K	E627K	E	E	<b>Q 1693</b>	Chicken, duck	+	+	Increases pathogenicity in mice
				H 1				
				<b>E 1673</b>				
D701N	P212S	P	P	K 18	Chicken, duck	+	+	Associates with increased virulence of AIVs in mammals
				V 2				
				<b>D 1689</b>				
D92E	L26F, V27A, A30T, S31N, G34E	D	D	N 3	Chicken, duck	+	+	Altered virulence in mice
				<b>P 1681</b>				
				L 367				
M2	L26F, V27A, A30T, S31N, G34E	L-V-A-S-G	L-V-A-S-G	E 1329	Chicken, duck	+	+	Promotes viral replication in mice
				<b>D 355</b>				
				G 1				
L26F, V27A, A30T, S31N, G34E	L-V-A-S-G	L-V-A-S-G	L-V-A-S-G	<b>L-V-A-S-G 1455</b>	Chicken, duck	+	+	Correlated with increased virulence and/or cytokine resistance
				L-V-A-N-G 165				
				L-A-A-S-G 16				
L26F, V27A, A30T, S31N, G34E	L-V-A-S-G	L-V-A-S-G	L-V-A-S-G	L-I-A-S-G 10	Chicken, duck	+	+	Antiviral amantadine resistance
				L-G-A-N-G 6				

Note: Bold is consistent with the results of this study.

\* Only show top 5, if the candidates are more than 5.