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	Q81

2021-23

LPAIV

**Study on monitoring and infection ability evaluation of LPAIV in eastern
migratory bird migration area from 2021 to 2023**

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AIV	Avian influenza viruses		
CAF	The Central Asian Flyway		
cDNA	complementary DNA		
dNTP	2'-deoxynucleoside 5'-triphosphate	2'-	5'-
DMEM	Dulbecco's Modified Eagle Medium		
d	day		
EAAF	The East Asian - Australasian Flyway	-	
EID50	The 50% egg infectious dose		
g	gram		
h	hour		
HA	Hemagglutinin		
LPAIV	Low pathogenic avian influenza viruses		
min	minute		
mRNA	Messenger Ribonucleic Acid		
M1	Matrix 1		1
M2	Matrix 2		2
mL	mililiter		
NP	Nucleoprotein		
NA	Neuraminidase		
NEP/NS2	Nuclear export protein		
NS1	Non-structural protein 1		1
PB2	Polymerase basic protein 2		2
PB1-F2	Polymerase basic protein 1-F2		1-F2
PB1	Polymerase basic protein 1		1
PA	Polymerase acidic protein		
PBS	Phosphate Buffered Saline		
PCR	Polymerase Chain Reaction		
rpm	revolutions per minute		/
RT-PCR	Reverse Transcription-Polymerase Chain Reaction		
RNA	Ribonucleic Acid		
SPF	Specific pathogen free		
s	second		
vRNP	viral ribonucleoprotein		
μL	microliter		

Influenza viruses												
100												
<i>Anseriformes</i>			<i>Charadriiformes</i>			Avian influenza						
viruses	AIV					Low pathogenic avian influenza						
viruses	LPAIV											
				LPAIV								
				LPAIV								
	LPAIV		2021-2023					LPAIV		—		
			LPAIV									
			LPAIV									
1.												
			2021-2023					17	29	134		
	58,803		LPAIV	124		0.21%		11	HA			
7	NA	22										
								H5	H7	H10	H11	
H13	H16		31			H1	H2	H3	H6	H7	H8	H10
H11		93	H13	H16	H1							

LPAIV

AIV

AIV 8

LPAIV

2.	H1N1 H10Nx H13Nx H16N3							
	H1N1 H10Nx							
	H13Nx							
	H16N3		H13Nx					
ZH1902	ZH186	PB2	ZH385	ZH5299	ZH987	DZ137	NA	
DZ137	PB1	DZ137	C701	NP				

3.	H1N1	H13Nx	H10Nx	H16N3
----	------	-------	-------	-------

			H1N1	ZH1902	TJ141	TMJ1994	TMJ679	
			TJ141					6.4
log10EID ₅₀ /mL								
	H10N5		TMJ1098					
		H1N1	H10N5					
			H10N4	TMJ2193	H16N3		CZ1481	
					H13N6	ZH5299	H13N8	
	CZ949	H16N3	CZ1491					
			H13Nx	H16N3				
						LPAIV		

Abstract

Influenza viruses are one of the main pathogens of zoonotic diseases, which can spread in many species or groups. At present, influenza viruses have been isolated from more than 100 species of wild birds in the world, among which *Anseriformes* and *Charadriiformes* are considered as natural reservoir of Avian influenza viruses (AIV). Low pathogenic avian influenza viruses (LPAIV) have been continuously prevalent in migratory birds, and the continuous evolution and cross-species spread of the virus continue to threaten human health, the safety of mammals, poultry and wild birds. In recent years, the cross-species infection of low pathogenic avian influenza virus to mammals or humans has occurred frequently, which poses a certain threat to public health and safety. Therefore, it is of great significance to study the prevalence, evolution and spread of low pathogenic avian influenza virus in migratory birds. In this study, AIV was taken as the research object, and the situation of migratory birds carrying AIV in the migratory areas of eastern China the East Asia-Australasia migration flyway and the Western Pacific migration flyway pass through China's region from 2021 to 2023 was monitored periodically, which explained the prevalence, diversity and host specificity of low pathogenic avian influenza among migratory birds in multi-host ecosystem. The genetic evolution and biological characteristics of different subtypes of LPAIV were analyzed by molecular biology methods. At the same time, using the method of virus molecular epidemiology, combined with the comprehensive analysis of the migration route of the migratory birds of *Charadriiformes*, the possible route of the migratory birds carrying the virus across the border into China was revealed.

1. Epidemiological investigation of avian influenza in migratory birds in eastern China

Under the background of global migratory birds migration, according to the migratory characteristics of migratory birds and the distribution law of bird species, Tumuji National Nature Reserve, Zhuanghe tidal flat wetland in the eastern coastal area, Bohai Bay tidal flat wetland and other important resting places, breeding places and wintering places were selected to set up monitoring posts, and active early warning monitoring was carried out for three years from 2021 to 2023. A total of 58,803 samples of 134 species of migratory birds belonging to 17 orders and 29 families were collected, and 124 strains of LPAIV were isolated, with a separation rate of 0.21%, including 11 subtypes of HA and 7 subtypes of NA. A series of studies proved the diversity of avian influenza virus subtypes in migratory birds. Statistical analysis showed that the hosts of the isolated strains were both *Anseriformes* and *Charadriiformes*, among which 31 strains were H5, H7, H10, H11, H13 and H16 subtypes in *Charadriiformes*, and 90 strains were H1, H2, H3, H6, H7, H8, H10 and H11 subtypes in *Anseriformes*. However, the serological survey of migratory

birds showed that the positive rates of H13, H16 and H1 specific antibodies were 2.28%, 1.65% and 0.34% respectively. Combined with the virus isolation, the antibody positive rate is much higher than the pathogen isolation rate, so it can be seen that the infection rate of LPAIV in migratory birds can not be fully reflected by the pathogen isolation rate, and the distribution of LPAIV and its subtypes in migratory birds should be analyzed by combining the antibody positive rate. The dynamic movement of migratory birds provides conditions for the spread of avian influenza virus. In this study, based on the long-distance migration characteristics of ostriches, AIV susceptible hosts and other factors, Black-bellied Plover, Bar—tailed Godwit, Asian Dowitcher, Black—headed Gull, Gull-billed Tern, Black—winged Stilt migratory birds were selected and tracked by real-time positioning and tracking methods. They span eight countries(China, Russia, Mongolia, the United States, Australia, New Zealand, South Korea and North Korea.) affected by AIV and Southeast Asia. It has important reference value for revealing the possible route of migratory birds carrying virus to cross-border migration and introduce into China. Gray spotted plovers set out from Yalu River in China and flew to the Russian Far East for breeding for one month, then flew back to the mouth of Yalu River to breed, and flew to the eastern coastal areas of Jiangsu Province in winter to overwinter. Some gray spotted plovers flew to the Philippines in Southeast Asia for wintering, and moved north next spring, stopping at Chongming Dongtan in Shanghai, and continuing to move north to start a new round of migration. Red-billed gull started from the breeding ground of tidal flat wetland around Bohai Bay and moved south to the lakes in Anhui and Hubei provinces for wintering. In spring, it moved north to the Central Siberian Plateau to breed, and returned to the breeding ground of tidal flat wetland around Bohai Bay in summer to start the next round of migration. The black-winged long-legged sandpiper moved southward from the breeding ground of the tidal flat wetland around Bohai Bay, flew to Southeast Asia such as Thailand and Myanmar for the winter, and flew back to the tidal flat wetland around Bohai Bay in the spring of next year. Half-webbed snipe started from the beach wetland stop in Bohai Bay and moved south to the eastern coastal area of Fujian Province for the winter. Gull-billed noise gull started from the breeding ground of tidal flat wetland in Bohai Bay and moved southward, stopping in the eastern coastal area of Yancheng City, and moved southward to the Philippines for the winter.

2. Genetic evolution of avian influenza viruses H1N1, H10Nx, H13Nx and H16N3 from migratory birds

Phylogenetic analysis showed that the H1N1 and H10Nx subtype avian influenza strains from migratory birds were multi-subtype recombinant strains with complex internal gene sources and genetic diversity. The H13Nx subtype isolate from migratory birds has not been recombined with

other subtypes. The internal fragment of H16N3 isolate is closely related to H13Nx subtype strain, and there is frequent gene exchange between them. PB2 gene of ZH1902 and ZH186 strains, NA gene of ZH385, ZH5299, ZH987 and DZ137 strains, PB1 gene of DZ137 strains, NP gene fragments of DZ137 and C701 strains are closely related to the American lineage strains, and the introduction of independent fragments of American lineage strains may have occurred. From the evolution of virus strains, the virus strains in China are closely related to those in Mongolia, South Korea, Russia, Asia, Bangladesh and other regions, which are highly similar to the migratory bird migration areas marked by us, suggesting that a relatively stable local transmission circle including China has been formed by migratory bird migration networks.

3. Evaluation of mammalian infectivity of H1N1, H13Nx, H10Nx and H16N3 strains from migratory birds

The experimental results of mice infectivity showed that H1N1 isolates ZH1902, TJ141, TMJ1994 and TMJ679 from migratory birds could infect the lung tissue of mice, among which TJ141 had the highest virus titer in the lung tissue of mice on the fifth day of infection, reaching $6.4 \log_{10} \text{EID}_{50}/\text{ml}$. Besides brain tissue and intestine, low-level virus replication was also found in heart, liver, kidney, spleen and turbinate. H10N5 isolate TMJ1098 can directly infect mice without adaptation and replicate in multiple tissues of mice. The results showed that H1N1 and H10N5 virus isolates from migratory birds could directly infect mice without adaptation, showing good mammalian adaptability. H10N4 isolate TMJ2193 and H16N3 isolate CZ1481 can replicate at a low level in mouse lungs, which has a certain risk of infecting mammals. H13N6 isolate ZH5299, H13N8 isolate CZ949 and H16N3 isolate CZ1491 have not detected virus replication in heart, liver, kidney, spleen, turbinate, brain and intestine, which indicates that H13Nx and some

1

1.1 A

A		(IAV)		8		RNA		PB2		PB1	PA	HA
NP	NA	M	NS	RNA		[1]						
hemagglutinin		HA		neuroaminidase		NA		[2]		18		
HA		11		NA						A		
		16		HA	9	NA	A					
								[3, 4]				
100						[5]				<i>Anseriformes</i>		
<i>Charadriiformes</i>										[2, 6]		
										A		
8		RNA										
RNA				PB2	PB1	PA	3					
				HA								
								NP				
						NA						
M1		vRNP										
M2												
		NS1(				)		NEP/NS2				
				PB1-F2								
						PA-X						
mRNA				N40		PA-N155	PA-182	M42				
NS3				[7, 8]		HA						
						NA		HA				
								[9, 10]				

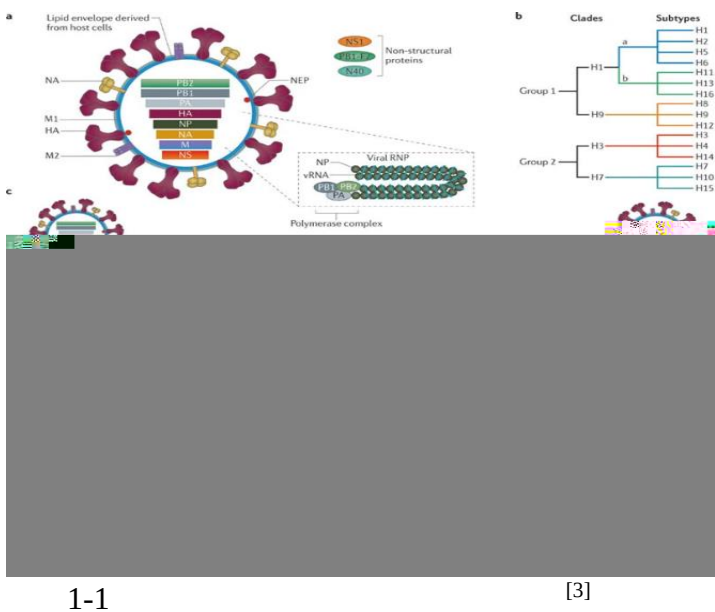


Fig. 1-1 Replication and antigenic classification of influenza A viruses^[3].

1.2

		[11]	26	105	
LPAIV ^[6]		H1N1			
		H1N1			
		H1N1	GSIAID		
H1Nx		H1N1	69.3%	H1N2	H1N3
8%			86%	<i>Anas platyrhynchos</i>	
		46%		H1	
		50%	32%	H1Nx	
		H10	1949		
H10N7	[12, 13]	1953		H10Nx	[14]
H10		1965			
	[15-17]	1984	Mustela vison		
H10N4					

H3 H4 H6 H7 H9 H1

LPAIV

1.3

1.3.1

LPAIV

AIV [30] 9

4 —

(CAF) — (EAAF) —

[31]

IAV

[32, 33]

[2, 33, 34]

2005 4 6 H5N1

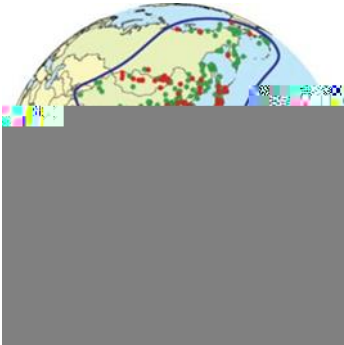
6000 Anser indicus Larus

brunnicephalus Laridae 50% [35, 36]

H5N1
9
4
7
HPAIVH5N1
H5N1
[35, 37] 2014 1
H5N8
2.3.4.4 2014-2015
HPAIVH5N8
2014
2014
[38]
[39]
[33]

A

B



C



1.4.1

[34, 44]		AIVs									
A											
IAV					[2]						
H7N7	H4N5	H4N6	H3N8	H3N3	Phoca vitulina					[45]	
2014	4				2014	6	16	8	13		
					H10N7				HA		
A/mallard/Sweden/133546/2011				H10N4	98.4%	A/mallard/Denmark/16109-					
4/2011	H10N6	98.3%				H10N7				[46]	
2008			H10N5					HA	NA		
[20]											
							2013	3			
H7N9					LPAI H7N9						
LPAI H7N9											
		H9N2	[47]		2019	2					

208R NP I09T RNA

AIV [71-73] NS1 S42P 42

S H5N1 , NS1 RNA

INF-k B IRF-3 P42S L98F I101M

[74, 75]

8 vRNA

[8]

-RNP

[4] 1918

H5N1 H7N9 H5N6

[49, 52, 82]

2

2.1

2.1.1

9-11 SPF

2.1.2

2-1

Tab. 2-1 Main experimental reagents

PBS	Solarbio
DMEM	gibco
RDE	
Agarose	
6×Super GelRed Prestain Loading Buffer	US RVERBRIGHT
50×TAE	Solarbio
DL2000/500 Marker	Takara
TransStart FastPfu DNA Polymerase	
Hiscrypt One Step RT-PCR Kit	Vazyme
QIAamp®Viral RNA Mini Kit(250)	QIAGEN GmbH

2.1.3

2-2

Tab. 2-2 Main experimental instruments

Eppendorf
Thermo
Baker
Retsch
SANTN
SANYO
NanoPhotometer-NP80
BAYGENE
Tanon

2.1.4

2021-2023

—

16

-80

-20

2.1.5

30 s

4

8000 rpm

10 min

1 g

2 mL

/

1 mLDMEM

30 Hz/s

3 min

4

8000 rpm

10 min

200 μL

9-

11

72 h

-80

2.1.6

RNA

QIAamp®Viral RNA Mini Kit(250)

RNA

1

560 μL AVL Buffer

1.5 mL

RNA

5.6 μLCarrier

RNA(

RNA

)

140 μL

		140 μ L	AVL		15 s
	AVL Buffer				
2		10 min	10 min		
3	560 μ L			15 s	
	140 μ L				
4		630 μ L		6000 g\8000 rpm	1 min
		2 mL			
5				500 μ L AW1 Buffer,	
	6000 g\8000 rpm	1 min,	2 mL		
		140 μ L	AW1 Buffer		
6	500 μ L AW2 Buffer	14000 rpm	20000 g	3 min;	2 mL
		1 min(	Buffer	RNA	
		)			
7		1.5 mL		60 μ L	
	AVE	1 min	6000 g	8000 rpm	
8			RNA	-80	

2.1.7

A	RT-PCR	R
	QIAamp®Viral RNA mini Handbook	RNA
One Step PrimeScript™ RT-PCR Kit	Takara	
cDNA		PCR
	2-3	

2-3 RT-PCR		
Tab. 2-3 RT-PCR preparation system		
Component	Volume	Procedure
2× One Step RT-PCR Buffer	12.5 μ l	Stage 1
TaKaRa Ex Taq HS 5 U/ μ l	0.5 μ l	Hold
PrimeScript RT Enzyme Mix	0.5 μ l	42 5 min

RNA

Illumina

2-4

Tab.2-4 Reverse transcription preparation system and program conditions

Component	Volume	Procedure
AMV Reverse Transcriptase 5× Reaction Buffer	10 µl	
10mM dNTP mix	5 µl	
RNasin®Ribonuclease Inhibitor	2 µl	
U12	1 µ	
		42 1h

2013-2023 1729

21

1

Eppendorf 4 RDE 37 16 18 h 56

30 min 4 V PBS 1 2

25 μ L 4

30 min 50 μ L 1%

15-20 min

2.1.10

4-6 g

,

2019 2023

2.2

2.2.1

2021 2023

17 29 134

58,803

2-1 (

)

2-6

Tab.2-6 Collection sample host source

<i>Anser cygnoid</i>	Swan Goose
<i>Anser fabalis</i>	Bean Goose
<i>Anser anser</i>	Graylag Goose
<i>Anser albifrons</i>	Greater White—fronted Goose
<i>Anser indicus</i>	Bar—headed Goose
<i>Cygnus olor</i>	Mute Swan
<i>Cygnus columbianus</i>	Tundra Swan
<i>Cygnus cygnus</i>	Whooper Swan
<i>Cygnus atratus</i>	Black Swan
<i>Tadorna tadorna</i>	Common Shelduck
<i>Tadorna ferruginea</i>	Ruddy Shelduck
<i>Aix galericulata</i>	Mandarin Duck
<i>Mareca penelope</i>	Eurasian Wigeon
<i>Anas platyrhynchos</i>	Mallard
<i>Anas zonorhyncha</i>	Eastern Spot—billed Duck
<i>Anas acuta</i>	Northern Pintail
<i>Anas crecca</i>	Green—winged Teal
<i>Aythya ferina</i>	Common Pochard
<i>Aythya baeri</i>	Baer's Pochard
<i>Aythya nyroca</i>	Ferruginous Duck
<i>Aythya fuligula</i>	Tufted Duck
<i>Anas gracilis</i>	Grey Teal
<i>Anas clypeata</i>	Northern Shoveler
<i>Anas strepera</i>	Gadwall
<i>Anas falcata</i>	Falcated Teal
/	
<i>Mergellus albellus</i>	Smew
<i>Sibirionetta formosa</i>	Baikal Teal
<i>Himantopus himantopus</i>	Black—winged Stilt
<i>Recurvirostra avosetta</i>	Pied Avocet
<i>Pluvialis squatarola</i>	Grey Plover
<i>Charadrius alexandrinus</i>	Kentish Plover
<i>Charadrius dubius</i>	Little Ringed Plover
<i>Charadrius leschenaultii</i>	Greater Sand Plover
<i>Pluvialis squatarola</i>	Black-bellied Plover
<i>Rostratula benghalensis</i>	Greater Painted Snipe
<i>Scolopax rusticola</i>	Eurasian Woodcock
<i>Limosa limosa</i>	Black—tailed Godwit
<i>Limosa lapponica</i>	Bar—tailed Godwit
<i>Numenius madagascariensis</i>	Eastern Curlew
<i>Numenius phaeopus</i>	Whimbrel
<i>Tringa erythropus</i>	Spotted Redshank
<i>Numenius arquata</i>	Eurasian Curlew
<i>Tringa totanus</i>	Common Redshank

Tab.2-6 Collection sample host source (continued)

<i>Tringa stagnatilis</i>	Marsh Sandpiper
<i>Tringa nebularia</i>	Common Greenshank
<i>Actitis hypoleucos</i>	Common Sandpiper
<i>Arenaria interpres</i>	Ruddy Turnstone
<i>Calidris canutus</i>	Red Knot
<i>Calidris ferruginea</i>	Curlew Sandpiper
<i>Calidris alpina</i>	Dunlin
<i>Calidris acuminata</i>	Sharp-tailed Sandpiper
<i>Calidris ruficollis</i>	Red-necked Stint
<i>Limnodromus semipalmatus</i>	Asian Dowitcher
<i>Crocethia alba</i>	Sanderling
<i>Tringa glareola</i>	Wood Sandpiper
<i>Calidris subminuta</i>	Long-toed Stint
<i>Limicola falcinellus</i>	Broad-billed Sandpiper
<i>Chroicocephalus brunnicephalus</i>	Brown-headed Gull
<i>Chroicocephalus ridibundus</i>	Black-headed Gull
<i>Ichthyaelus relictus</i>	Relict Gull
<i>Ichthyaelus ichthyaelus</i>	Pallas's Gull
<i>Larus smithsonianus</i>	Siberian Gull
<i>Sternula albifrons</i>	Little Tern
<i>Sterna hirundo</i>	Common Tern
<i>Chlidonias hybrida</i>	Whiskered Tern
<i>Chlidonias leucopterus</i>	White-winged Tern
<i>Chlidonias hybrida</i>	Whiskered Tern
<i>Larus crassirostris</i>	Black-tailed Gull
<i>Gelochelidon nilotica</i>	Gull-billed Tern
<i>Pavo cristatus</i>	Common Peafowl
<i>Arborophila gingica</i>	White-browed Hill Partridge
<i>Chrysolophus pictus</i>	Golden Pheasant
<i>Alectoris chukar</i>	Chuckar
<i>Lophura nycthemera</i>	silver pheasant
<i>Podiceps cristatus</i>	Great Crested Grebe
<i>Podiceps nigricollis</i>	Black-necked Grebe
<i>Tachybaptus ruficollis</i>	Little Grebe
<i>Streptopelia decaocto</i>	Eurasian Collared Dove
<i>Streptopelia orientalis</i>	Oriental Turtle Dove
<i>Spilopelia chinensis</i>	Spotted Dove
<i>Fulica atra</i>	Common Coot
<i>Gallinula chloropus</i>	Common Moorhen
<i>Rallus indicus</i>	Brown-cheeked Rail
<i>Grus leucogeranus</i>	Siberian Crane
<i>Grus vipio</i>	White-naped Crane
<i>Grus japonensis</i>	Red-crowned Crane
<i>Grus grus</i>	Common Crane
<i>Otis tarda</i>	Great Bustard

2-6

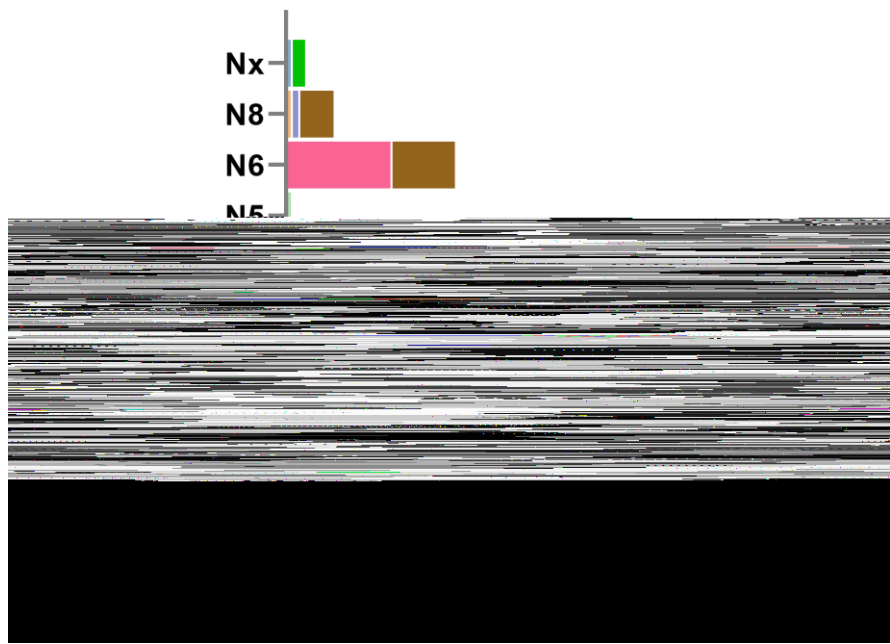
Tab.2-6 Collection sample host source (continued)

<i>Ciconia boyciana</i>	Oriental Stork
<i>Ciconia ciconia</i>	White Stork

	H5N1	AIV	6		2.76%			343	
	AIV					1196		AIV	
						3090		AIV	
48		1.32%	H1N1	21	H6N1	21	H6N2	4	
H7N3	1	H11Nx	1					155	
	AIV					188		AIV	
			466	AIV	10		2.15%		
	H5N6	1	H5N1	6	H3N1	1	H6N1	1	
	H5N1	1		280	2	H1N1		0.71%	
			164	AIV			590		
AIV				50		AIV			
	3610		AIV	29		0.80%		15	H8N6
	AIV		3	H5N3	AIV	5	H13N8	AIV	1
1	H7Nx	AIV	1	H11N1	AIV	2	H5N1	AIV	1
	9348		AIV	1	H5N1		0.01%		

2-7 2021-2023 AIV
Tab. 2-7 Statistics of separation rate of AIV in 2021-2023

	AIV	%	%
	3038	0	0
2021	3722	13	0.34
	10158	18	0.18
	6135	20	0.32
	3283	10	0.30
2022	3212	1	0.03
	10447	2	0.02
	307	6	1.95
	2872	48	1.67
2023	3610	29	0.80
	9348	1	0.01
	2671	18	0.67



2-2 2021-2023 LPAIV
 Fig. 2-2 Subtype statistics of LPAIV isolates in eastern China from 2021 to 2023

2-8 2021-2023 LPAIVs
 Tab.2-8 Host distribution of LPAIVs isolates in eastern China from 2021 to 2023

H5N3	3	H7Nx	1	H7N2(10)
H10N5(1)	H11Nx(1)	H13N6(9)	H13N8(5)	H16N3(1)
H1N1(30)	H1N2(5)	H2N2(1)	H3N1(1)	H3N2(1)
H3N8(1)	H6N1(23)	H6N2(4)	H6N8(1)	H7N2(9)
H7N3(1)	H8N6(15)	H11Nx(1)		

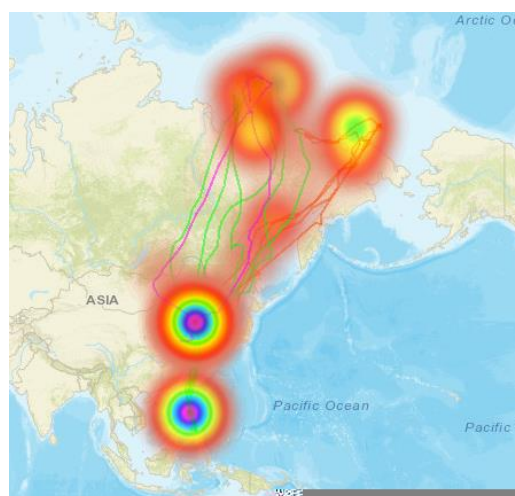
2.2.3

2013-2023					
		H13		2.28%	H16
1.65%	H1		0.34%	H13	H16
	H1				2021-2023
H1	H13	H16	0.141%	0.025%	0.005%

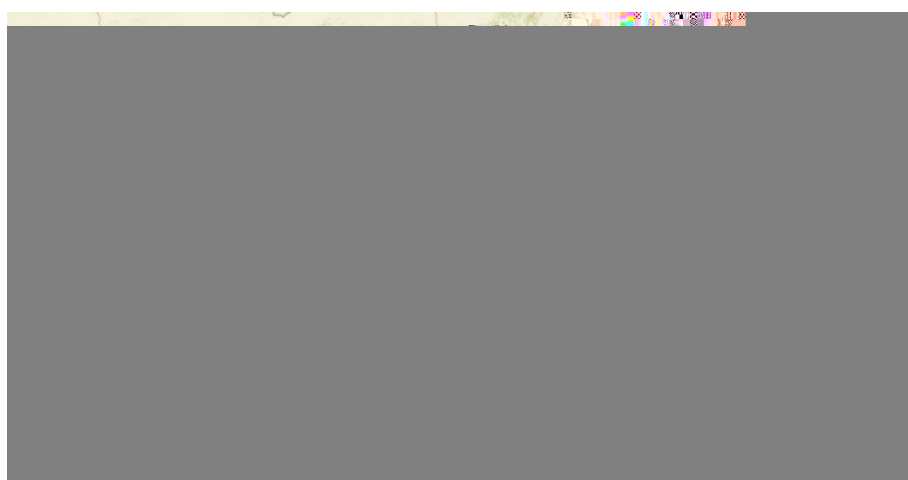
H1

20	HQP3480		2021	4	2										
			5												
			7												
							11	26							
	2022		4	8			5								
				8	12					9					
										2023		1			
7	HQP3490		2021	4	2					5	26				
	6		1			1		7		28					
	30						8		4						
			5						10		7				
5	HQP3599		2021	5	5										
					1	5									
							8								
									1	8					
							2022		4						
								2023		4					
												1			
5															
HQP3599															
HQP1126		2020	8	30											
9		17													
2021	10	24													

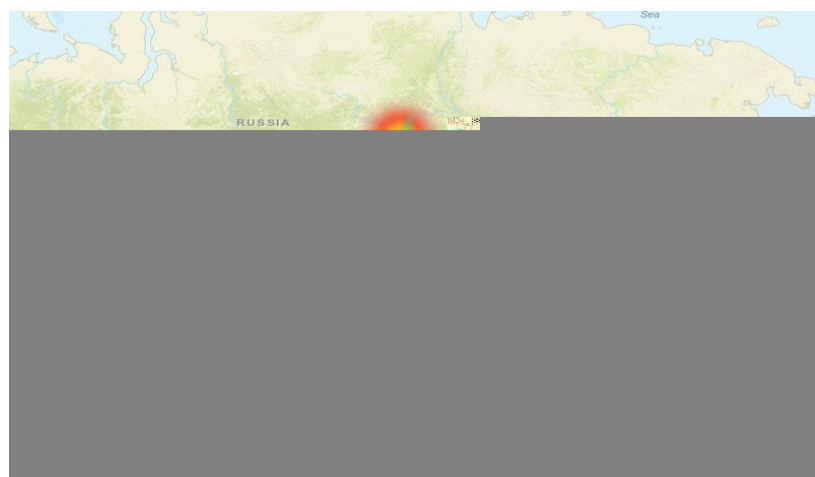
2-3 HQP3638
 Fig. 2-3 Migration Route of *Limosa lapponica* HQP3638



2-4 HQP3480 HQP3490 HQP3599
 Fig. 2-4 Migration Route of *Pluvialis squatarola* HQP3480, HQP3490 and HQP3599



2-5 HQP1126
 Fig. 2-5 Migration Route of *Limnodromus semipalmatus* HQP1126



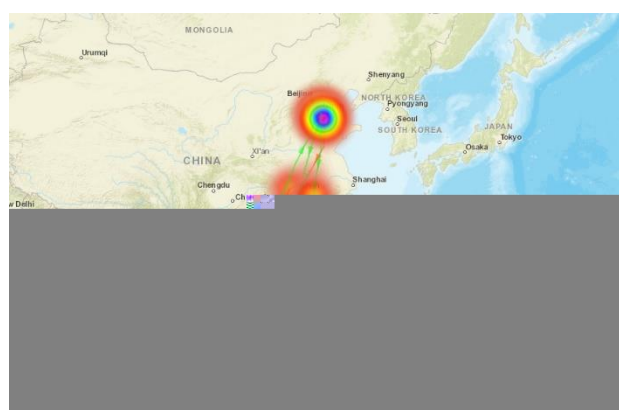
2-6 HQP1127

Fig. 2-6 Migration Route of *Chroicocephalus ridibundus* HQP1127



2-7 HQP1125

Fig. 2-7 Migration Route of *Gelochelidon nilotica* HQP1125



2-8 HQP1121 HQP1123

Fig. 2-8 Migration Route of *Gelochelidon nilotica* HQP1121 and HQP1123

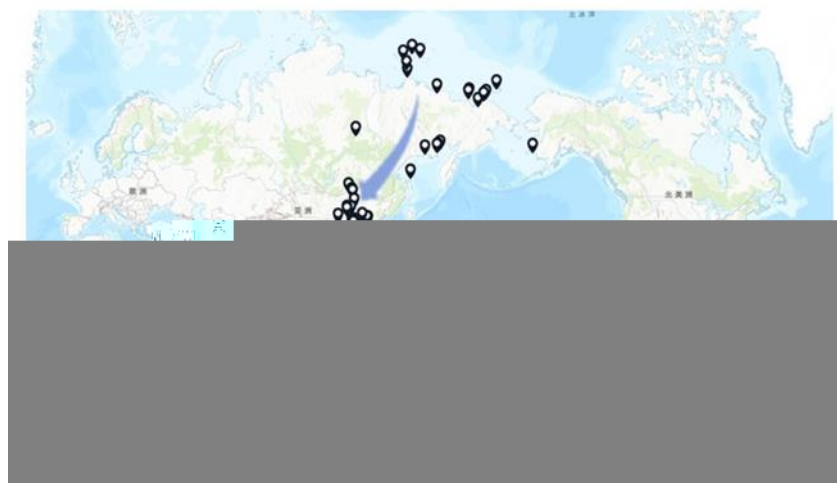
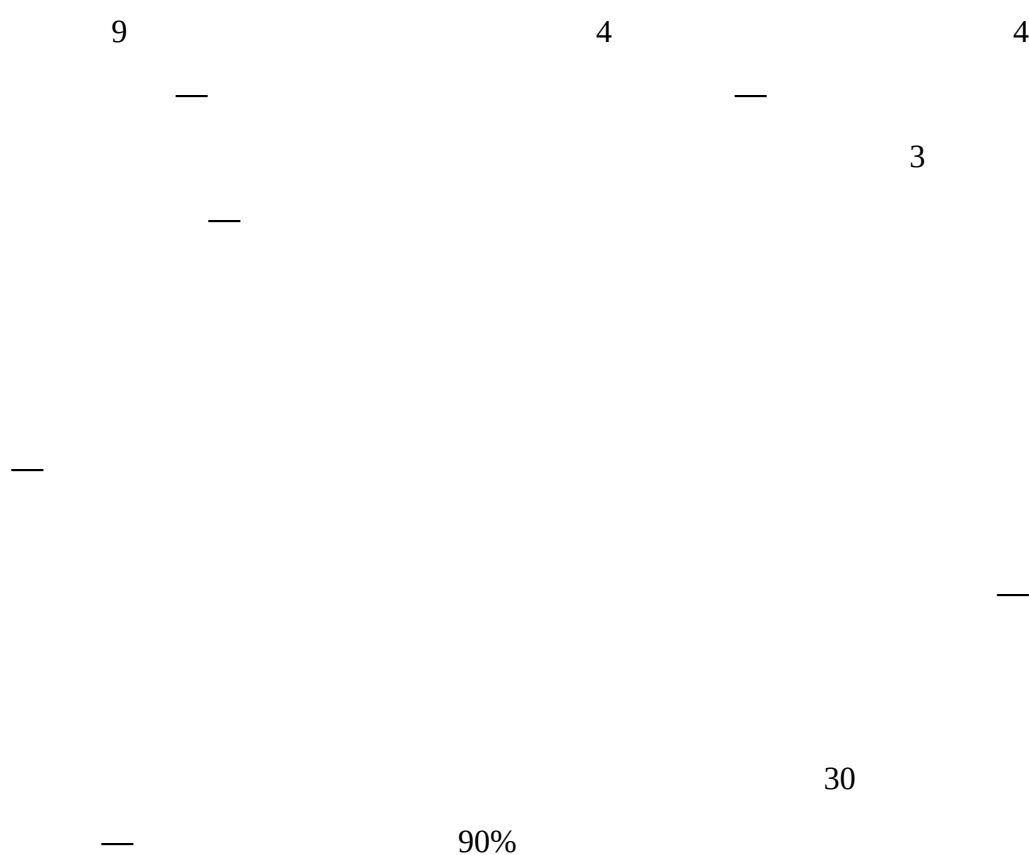


Fig. 2-9 Track the main direction map of migratory birds moving into China. The black icons show the breeding grounds, wintering grounds and stopping places of migratory birds. Purple is the migration direction of Black—winged Stilt, blue is the migration direction of Black—headed Gull and Black-bellied Plover, and green is the migration direction of Bar—tailed Godwit and Black-bellied Plover.

2.3



20

EAAF

(

)

3

	4	5		9	10	
			-			-

	2021	4	
H13N6	2021	4	
	H11N9		

2.4

	1. 2021		2023												16
17	29	134	58,803		LPAIV				124	LPAIV				0.21%	
2021	2022		2023		0.22%		0.11%		0.52%						
					0.65%		0.39%		0.07%		LPAIV				11
HA	7		NA	22							H1	H6	H7	H13	LPAIV
		H3	H8												
					H5		H7	H10	H11	H13	H16				31
			H1	H2	H3	H6	H7	H8	H10	H11					93
LPAIV															LPAIV

2. H13 H16 H1

2.28% 1.65% 0.34% H1 H13 H16

H13 H16 H1 0.025% 0.005% 0.141%

LPAIV

H13 H16 H13 H16 H1

1 40-1 160

3.

8

3 LPAIV

3.1

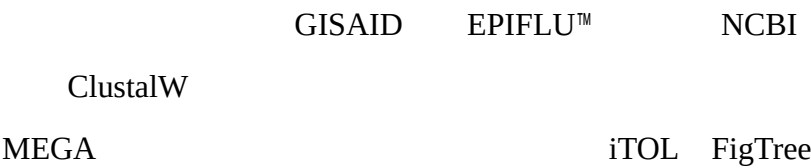
3.1.1

3-1

Tab. 3-1 Experimental strain information

Viruses Name	Suptype	Abbreviation	Acquisition time	Collection location
A/environment/Dalian/ZH1902/2020/H1N1	H1N1	ZH1902	2020.04.08	
A/environment/Tumuji/TMJ2168/2022/H1N1	H1N1	TMJ2168	2022.1	
A/environment/Tumuji/TMJ1994/2022/H1N1	H1N1	TMJ1994	2022.1	
A/environment/Tumuji/TMJ50/2023/H1N1	H1N1	TMJ50	2023.04	
A/environment/Tumuji/TMJ93/2023/H1N1	H1N1	TMJ93	2023.04	
A/environment/Tumuji/TMJ493/2023/H1N1	H1N1	TMJ493	2023.04	
A/environment/Tumuji/TMJ679/2023/H1N1	H1N1	TMJ679	2023.04	
A/environment/Tianjin/TJ141/2023/H1N1	H1N1	TJ141	2023.03.25	
A/environment/Inner Mongolia/TMJ1098/2018/H10N5	H10N5	TMJ1098	2018.6.12	
A/Mallard/Inner Mongolia/TMJ28/2019/H10N4	H10N4	TMJ28	2019.07.22	
A/Anatidae/Inner Mongolia/TMJ2193/2020/H10N4	H10N4	TMJ2193	2020.10.14	
A/environment/Qihai/QH82/2022/H10N7	H10N7	QH82	2022.07.24	
A/environment/Hebei/CZ1753/2023/H10N5	H10N5	CZ1753	2023.11.04	
A/mallard/Dalian/DZ-137/2013/H13N6	H13N6	DZ-137	2013.10.01	
A/Eurasian curlew/Liaoning/ZH-385/2014/H13N8	H13N8	ZH-385	2014.10.18	
A/Eurasian curlew/Liaoning/ZH-186/2014/H13N6	H13N6	ZH-186	2014.10.18	
A/environment/Dalian/ZH2943/2020/H13N8	H13N8	ZH2943	2020.05.16	
A/environment/Dalian/ZH987/2021/H13N6	H13N6	ZH987	2021.04	
A/environment/Dalian/ZH5299/2022/H13N6	H13N6	ZH5299	2022.10.17	
A/environment/Cangzhou/CZ949/2023/H13N8	H13N8	CZ949	2023.09.05	
A/environment/Hebei/CZ1503/2023/H13N8	H13N8	CZ1503	2023.09.17	
A/environment/Fujian/FJ124/2024/H13N6	H13N6	FJ124	2024.01	
A/Black-headed gull/Hebei/c701/2017/H13N6	H13N6	C701Y	2017.08.01	
A/environment/Hebei/CZ1451/2020/H16N3	H16N3	CZ1451	2020.10.06	
A/environment/Hebei/CZ1481/2020/H16N3	H16N3	CZ1481	2020.10.06	
A/environment/Hebei/CZ1491/2023/H16N3	H16N3	CZ1491	2020.10.06	

3.1.2



	EID ₅₀		PBS	10
10 ⁻⁶ -10 ⁻¹⁰	9-10	SPF	3	37
48 h			Reed-Muench	EID ₅₀
BALB/c				H1N1
H13			4-6	BALB/c
n=11/			50 µL	10 ⁶ EID ₅₀ /50µL
	n=5/		PBS	
	14 d		3 d	5 d
n=3/				
9-11				

3.2.1 H1N1

83.1-99.9% 9

TMJ50 TMJ93 TMJ493 TMJ679 TMJ1994
 TMJ2168 TJ141 TMJ50 TMJ1994 A/Common
 Teal/Buryatia/89i/2019/H1N1 A/mallard/Russia Primorje/166T/2020/H1N1
 TMJ2168 TJ141 A/Goose/Korea/H277/2022/H5N3
 A/Em/Korea/22WF191-23P/2022/H5N1 TMJ93 TMJ493 TMJ679
 H10N5 ZH1902
 A/gull/South Korea/GNU54/2021/H13N6
 ZH1902 H13
 H16
 H1N1 PB1 A/teal/Russia
 Primorje/390/2016/H1N1 A/Anser albifrons/South Korea/163-5/2022/H10N7 A/Wild
 duck/South Korea/KNU2020-29/2020/H1N1 A/common teal/ShanghaiJDS110215/2020/
 H9N2 A/Bean Goose(*Anser fabalis*)/South Korea/KNU2021-42/2021/H1N1 A/chicken/Laos/
 M-FA01243/2022/H5N1
 PB1
 PA
 TMJ93 TMJ493 TMJ679 TJ141 A/Spot-billed
 duck(*Anas poecilohyncha*)/South Korea/KNU2021-51/2021/H8N4 TMJ50
 TMJ1994 A/mallard/Yakutia/47/2020/H7N7 A/duck/Okayama/22D2T/2022/H5N1
 A/duck/Mongolia/345/2018/H4N6 TMJ2168
 A/duck/Moscow/5743/2019/H1N1 A/*Anas platyrhynchos*/Belgium/00358 0006/2023/H1N1
 ZH1902 A/Mallard(*Anas platyrhynchos*)/South Korea/KNU2019-
 54/2019/H5N3 NP NS
 TMJ93 TMJ493 TMJ679 NP
 H6N1 H1N1 2023 H10N5
 TMJ50 TMJ1994 TMJ2168 TJ141 ZH1902 NP
 A/Northern Pintail/Russia Primorje/298/2019/H3N8

A/chicken/Korea/H124/2022/H5N1 A/Bean goose/Korea/22WC059/2022/H5N1

TMJ50 TMJ1994 NS A/chicken/Kagawa/22A2T/2022/H5N1

A/whooper swan/Shanxi/SX126/2020/H9N2 A/common teal/Shanghai/JDS110203/2019/H12N8 A/duck/Mongolia/419/2019/H5N3 A/mallard/Yakutia/47/2020/H7N7

NS H1N1 H6N1

M TMJ50 ZH1902 TJ141 TMJ93

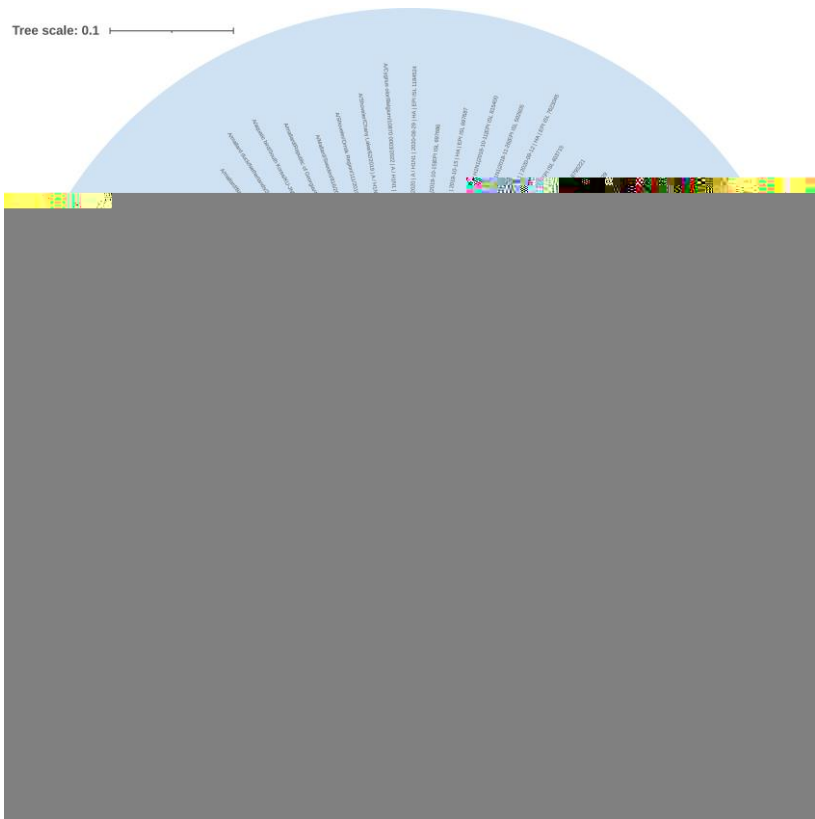
TMJ493 TMJ679 TMJ1994 TMJ2168 A/duck/Mongolia/314/2018/H3N6

A/kentish plover/Liaoning/DD646/2020/H10N9 A/wild duck/Korea/H296/2020/H7N9

A/common teal/Shanghai/NH110923/2019/H1N1 A/Mallard (Anas platyrhynchos)/South Korea/KNU2019-65/2019/H4N6 H1N1 PB2

ZH1902 PB2

H1N1



3-1 HA

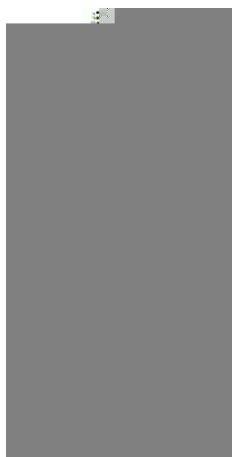
Fig.3-1 Phylogenetic tree of HA gene of H1 influenza virus. The green branch is American strain, the purple branch is isolated strain, and the blue branch is Eurasian strain.



3-2 NA

Fig.3-2 Phylogenetic tree of NA gene of N1 influenza virus. The green branch is American strain, the purple branch is isolated strain, and the blue branch is Eurasian strain

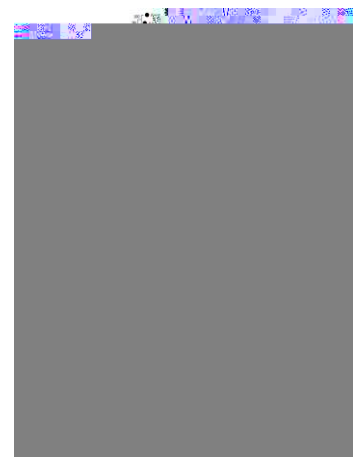
PB2

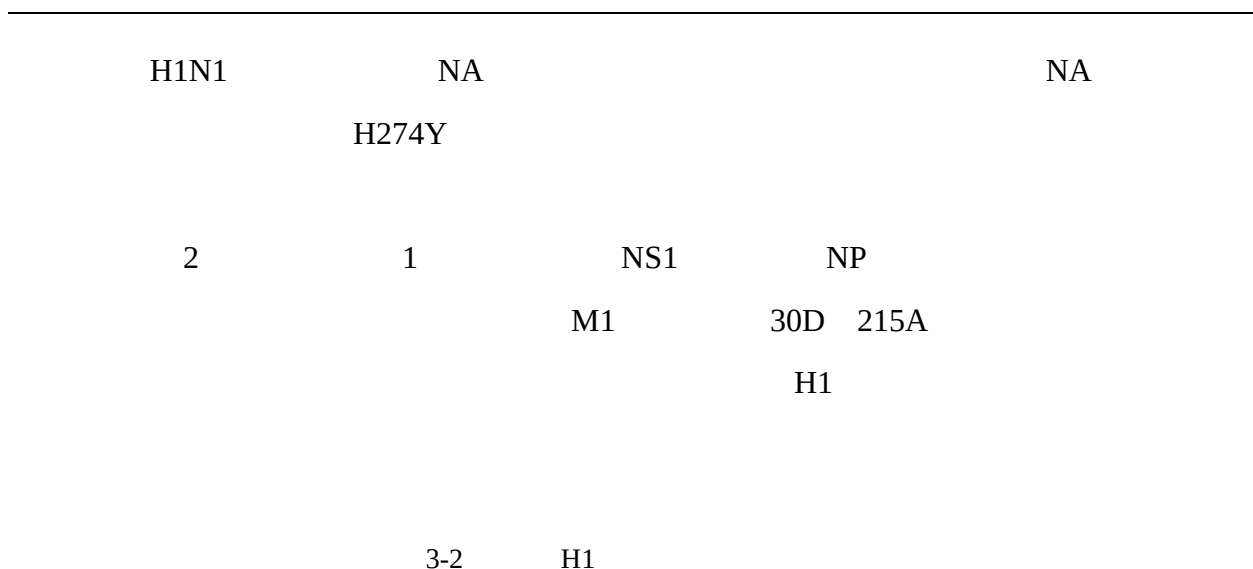


PB1



PA





Tab.3-2 Molecular characteristics of H1 subtype avian influenza from migratory bird

		TJ141	ZH1902	TMJ679	TMJ1994		
		/					
	HA	³³⁹ PSIQ SR↓GL F ³⁴⁷	³³⁹ PSIQ SK↓GL FG ³⁴⁷	³³⁹ PSIQ SR↓GL F ³⁴⁷	³³⁹ PSIQS R↓GLF ³⁴⁷		
HA	Q226L	Q	Q	Q	Q		
	G228S	G	G	G	G		
NA	Deletion in the stalk	/	/	/	/		
	H274Y	Y	Y	Y	Y		
PB2	E158G	E	E	E	E	RNA	,
	T271A	T	T	T	T		
	E627K	E	E	E	E		
	D701N	D	D	D	D		
PB1	S622G	G	G	G	G		
	V709I	V	V	V	V		
PA	T157A	T	T	T	T		
	H510A	H	H	H	H	mRNA	

NP	I ¹⁰⁹ T	I	I	I	I	RNA	,	AIV
M1	N ³⁰ D	D	D	D	D			
	T ²¹⁵ A	A	A	A	A			
NS1	S ⁴² P	S	S	S	A			
	L ⁹⁸ F	M	M	M	I			
	I ¹⁰¹ M	D	D	D	E			
/ - ;HA Subtype Numbering Conversion (https://www.bv-brc.org)								

3.2.1.3

H1N1

BALB/c

H1N1

2020-2023

H1N1

ZH1902

TJ141

TMJ1994

TMJ679

14

ZH1902

4

4.6

5.4

4.75

5.4log10EID₅₀/ml,

4

6.4

6

5.8log10EID₅₀/ml,

TMJ1994

3.75log10EID₅₀/ml

ZH1902

TMJ1994

TMJ679

TJ141

TMJ1994

4

H1N1

TJ141

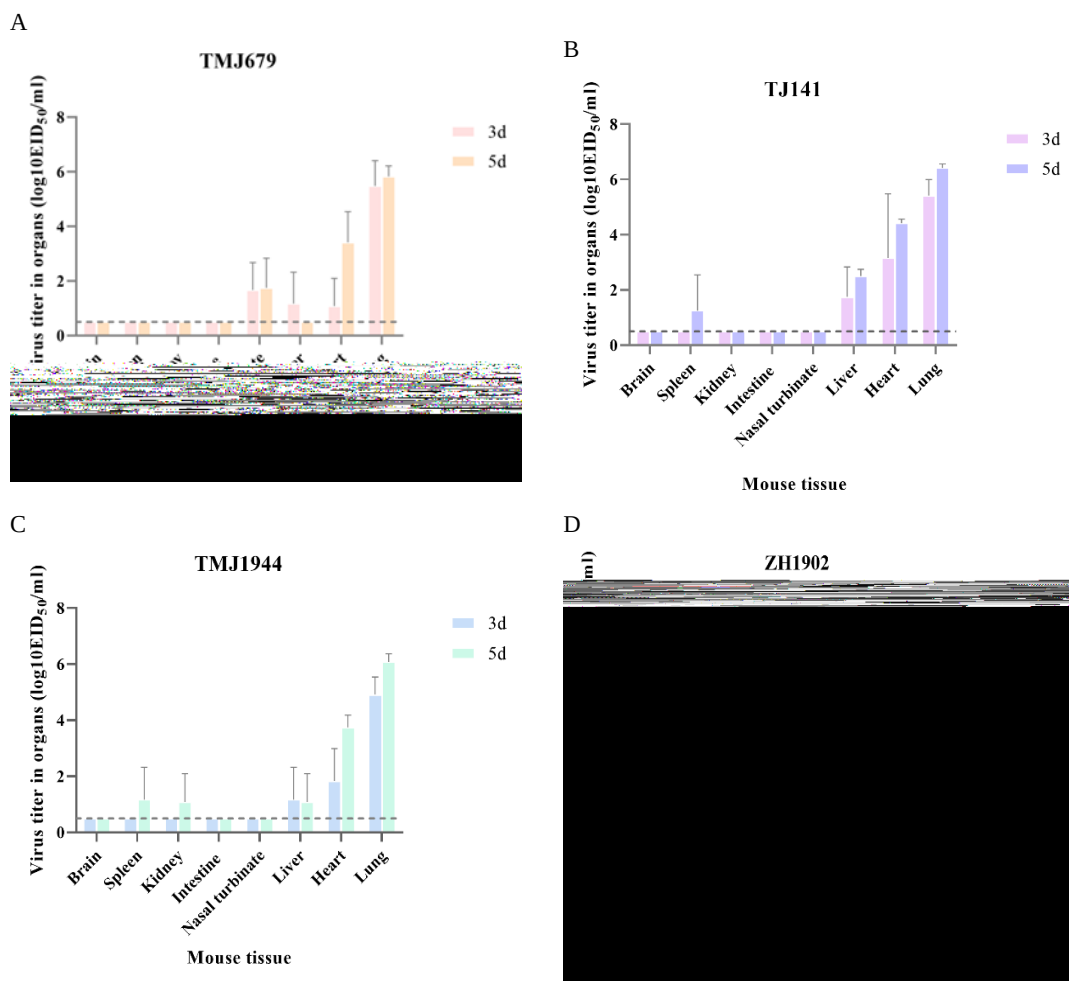
TMJ1994

TMJ679

ZH1902

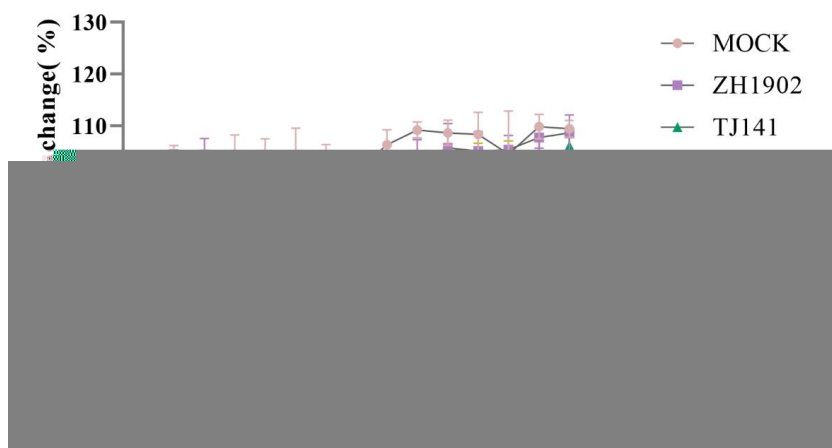
4

H1N1



3-4

Fig.3-4 Replication of tissue virus in mice infected with avian influenza virus



3-5

Fig.3-5 Weight changes of mice post inoculation

3.2.2 H13Nx

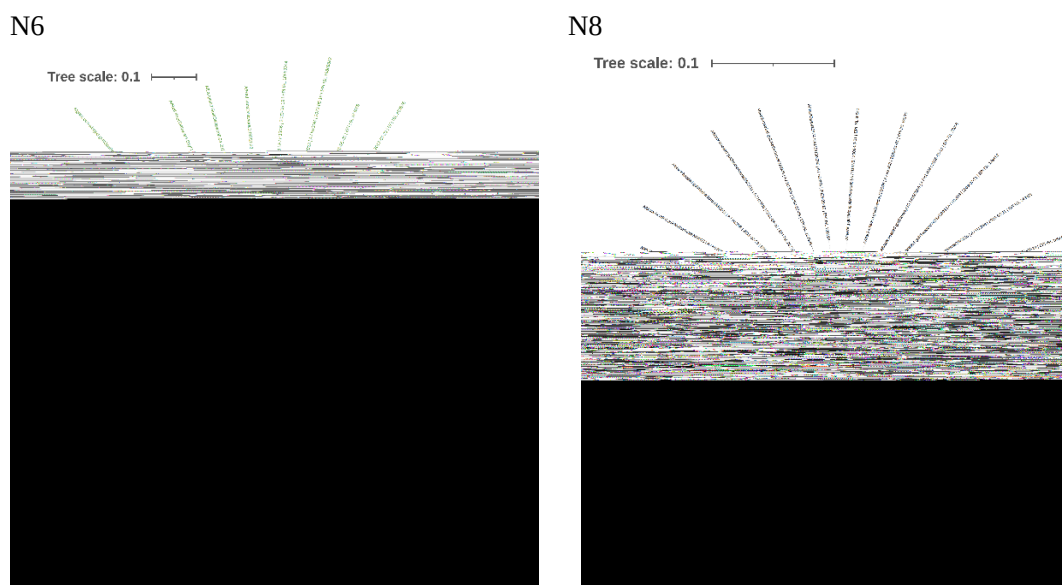
3.2.2.1 H13

H13

	H13N6	H13N8					H13N6
			H13	HA		3	
			ZH385				
						H13	
	NA			N6	N8		N6
N8		ZH385	ZH5299	ZH987	DZ137		
	PB2			DZ137	ZH385		
DZ137		H13		ZH385			
H13		H13					
ZH186	A/glaucous-winged gull/Southcentral Alaska/15MB01429/2015/H13N6						
				PB1			
DZ137PB1		CZ949	ZH987				
				DZ137	ZH385		
	PA						
			NP		DZ137	C701	
A/glaucous-winged gull/Alaska/545/2013/H13N2				A/Glaucous-Winged Gull/Alaska/20MB02			
182/2020/H13N6				C701 CZ949			
ZH5299	CZ1503	M1	A/Glaucous-Winged Gull/Alaska/20MB02182/2020/				
H13N6	H13		NS		H1	H10	H16
	H13						

3-6 HA

Fig.3-6 Phylogenetic tree of HA gene of H13 influenza virus. The green branch is American strain, the red branch is isolated strain, and the black branch is Eurasian strain.



3-7 NA

Fig.3-7 Phylogenetic tree of NA gene of N6 N8 influenza virus. The green branch is American

strain, the red branch is isolated strain, and the black branch is Eurasian strain.

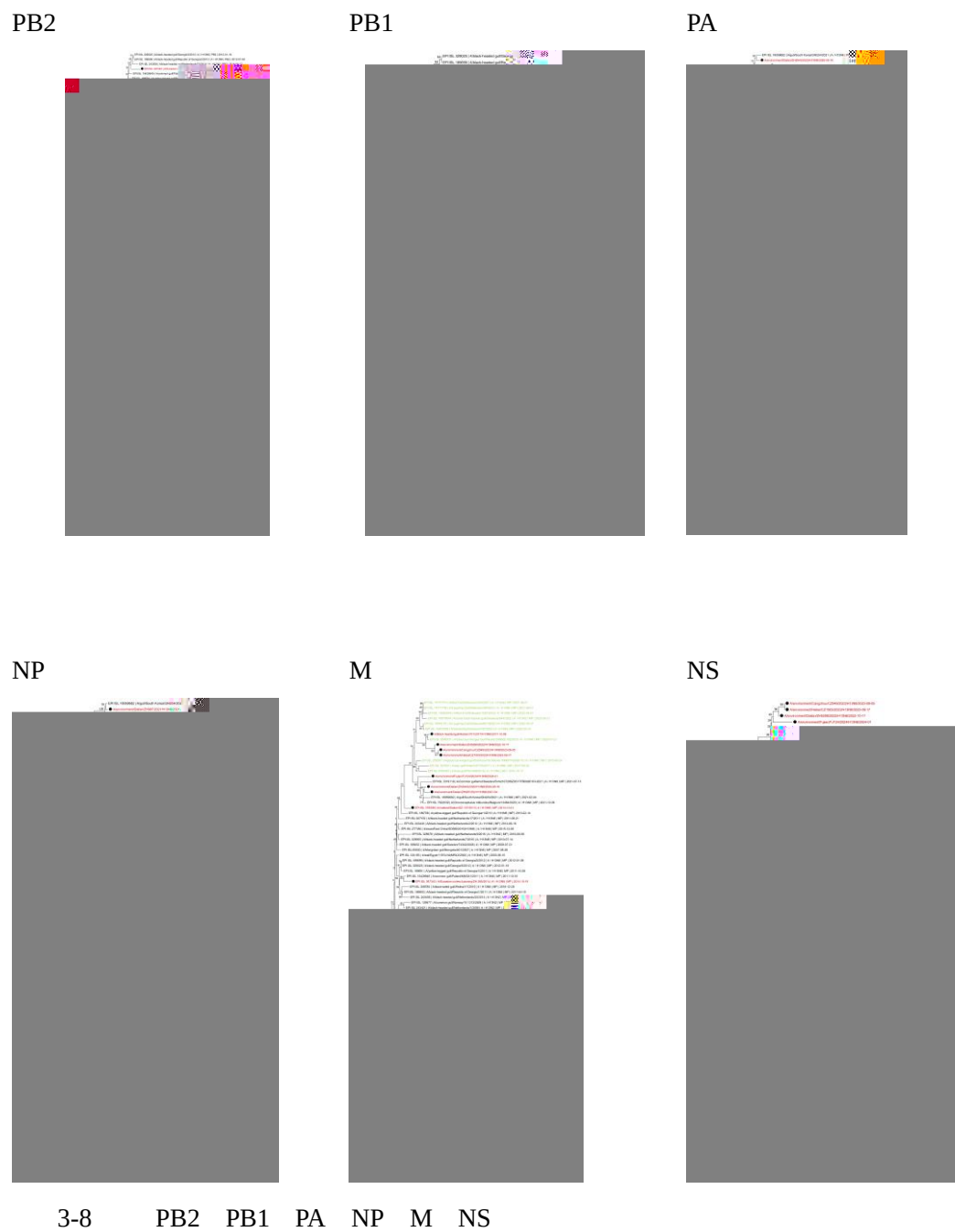


Fig.3-8 Phylogenetic tree of NA gene of PB2 PB1 PA NP M NS influenza virus. The green branch is American strain, the red branch is isolated strain, and the black branch is Eurasian strain.

3.2.2.2 H13

H13 HA

PAISNR ↓ GLF			LPAIV		
			HA	226Q	
228S(H3 Numbering	226			228	
	NA		H13	NA	
		NA			
		2			
1	M1		622G	30D	
	NS1	NP			
		H13 AIV		HA	PB1 M1

3-3 H13

Tab.3-3 Molecular characteristics of H13 subtype avian influenza from migratory bird

		ZH987	ZH2943	ZH385	DZ137	ZH5299	ZH186	CZ949	2017C701	FJ124
/										
HA		³²⁴ PAISNR ↓	³²⁴ PAISNR ↓	³²⁴ PAISNR ↓ GL	³²⁴ PAISNR ↓ GL	³²⁴ PAISNR ↓ GL	³²⁴ PAISNR ↓ GL	³²⁴ PAISNR ↓ GL	³²⁴ PAISNR ↓ G	³²⁴ PAISNR ↓ G
		GLF ³³²	GLF ³³²	F ³³²	F ³³²	F ³³²	F ³³²	F ³³²	LF ³³²	LF ³³²
HA	Q ²²⁶ L	Q	Q	Q	Q	Q	Q	Q	Q	Q
	G ²²⁸ S	S	S	S	S	S	S	S	S	S
	Deletion									
NA	in the stalk	/	/	/	/	/	/	/	/	/
	H274Y	Q	G	G	Q	R	Q	G	N	Q
	E ¹⁵⁸ G	E	E	E	E	E	E	E	E	E
	T ²⁷¹ A	T	T	T	T	T	T	T	T	T
PB2	E ⁶²⁷ K	E	E	E	E	E	E	E	E	E
	D ⁷⁰¹ N	D	D	D	D	D	D	D	D	D
	S ⁶²² G	G	G	G	G	G	G	G	G	G
PB1	V ⁷⁰⁹ I	V	V	V	V	V	V	V	V	V
PA	T ¹⁵⁷ A	T	T	T	T	T	T	T	T	T

	H ⁵¹⁰ A	H	H	H	H	H	H	H	H	H
NP	I ¹⁰⁹ T	V	V	V	V	V	V	V	V	V
M1	N ³⁰ D	D	D	D	D	D	D	D	D	D
	T ²¹⁵ A	A	A	A	A	A	A	A	A	A
	S ⁴² P	S	S	S	S	S	S	S	S	S
NS1	L ⁹⁸ F	M	M	M	M	M	M	M	M	M
	I ¹⁰¹ M	D	D	D	D	D	E	D	D	D

/
-
HA Subtype Numbering Conversion (<https://www.bv-brc.org>)
3-2

3.2.2.3	H13				
	BALB/c			H13	
		3-9	ZH5299	CZ949	
			8		14
ZH5299	CZ949				
	H13				
A			B		

H16N3

228

NA

NA

627 E 158 701

CZ1481 271 A RNA ,

CZ1491 M1 30D 215A

PA PB1 NS1 NP

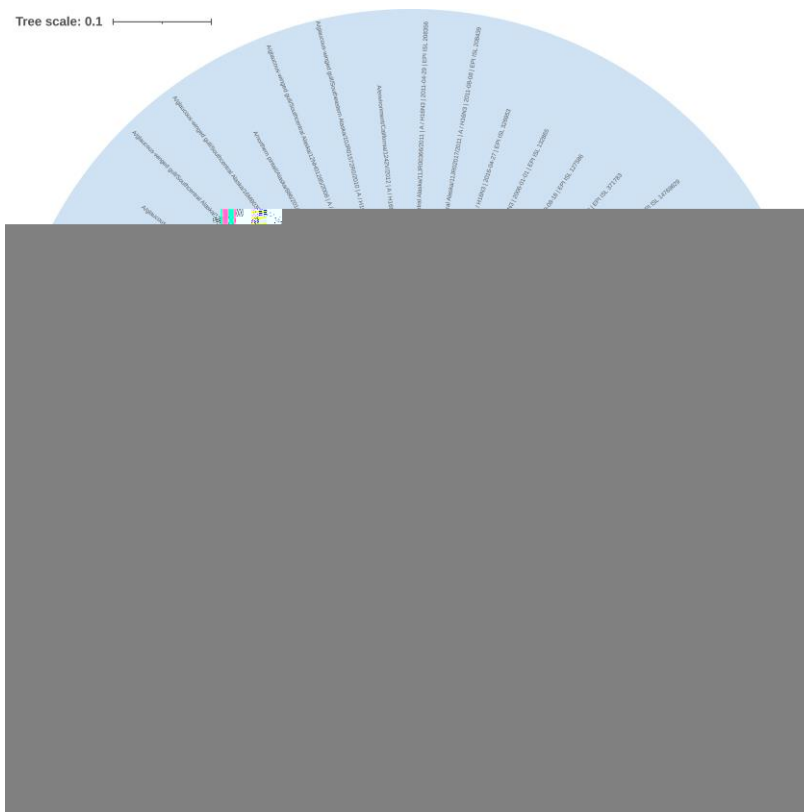
H16 AIV

PB2 M1



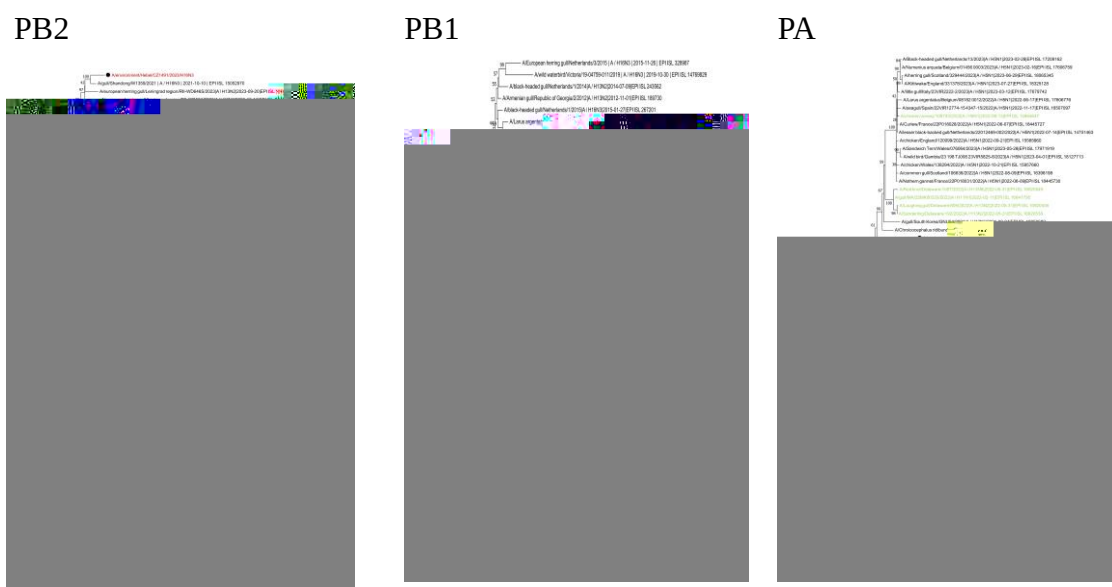
3-11 HA

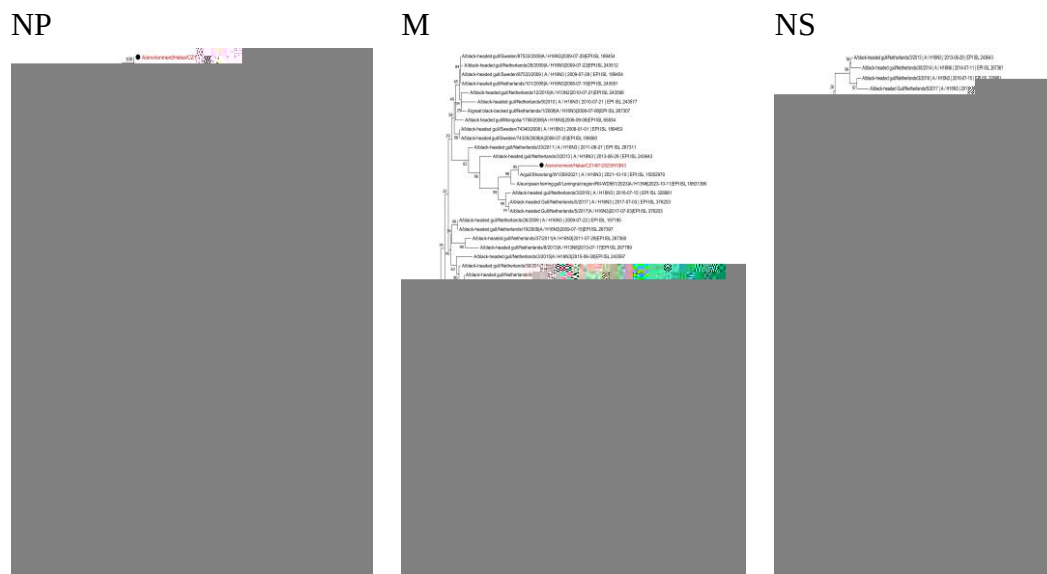
Fig.3-11 Phylogenetic tree of HA gene of H16 influenza virus. Blue is American strain, green is Eurasian strain, and purple is isolated strain.



3-12 NA

Fig.3-12 Phylogenetic tree of NA gene of N3 influenza virus. Blue is American strain, orange is Eurasian strain, and purple is isolated strain.





3-13 PB2 PB1 PA NP M NS

Fig.3-13 Phylogenetic tree of NA gene of PB2 PB1 PA NP M NS influenza. The green branch is American strain, the red branch is isolated strain, and the black branch is Eurasian strain.

3.2.3.2 H16N3

BALB/c

H16N3

3-14

CZ1481

7

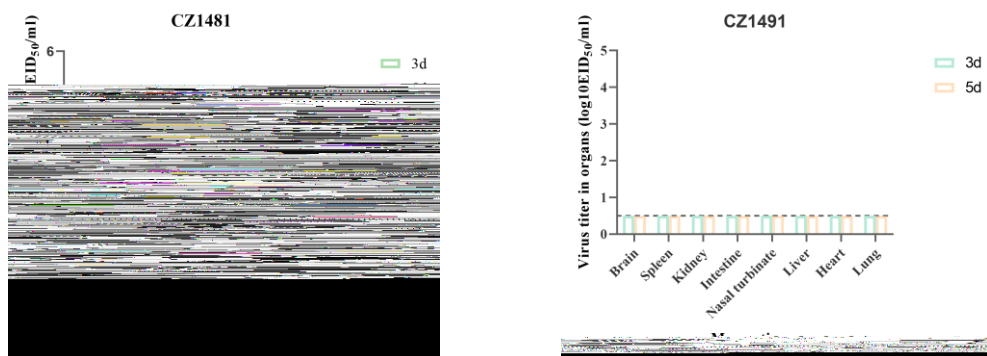
CZ1491

14

CZ481

CZ1491

H16N3



3-14

Fig.3-14 Replication of tissue virus in mice infected with avian influenza virus



3-15

Fig.3-15 Weight changes of mice post inoculation

3.2.4 H10Nx

3.2.4.1 H10Nx

2024	1	H3N2	H10N5	
H10			H10N3	H10N8
			H10Nx	
			H10Nx	HA
	TMJ2193	TMJ28	CZ1753	QH82
	TMJ1098		TMJ2193	H10N5
A/Zhejiang/ZJU01/2023/H10N5				
	H10		TMJ1098	A/mallard/Korea/H276-
1/2018/H10N1			H10	
			N4	N5 N7

TMJ2193 T

MG02/2021/H3N8

A/mallard/Korea/H276-1/2018/H10N1

A/Eurasian

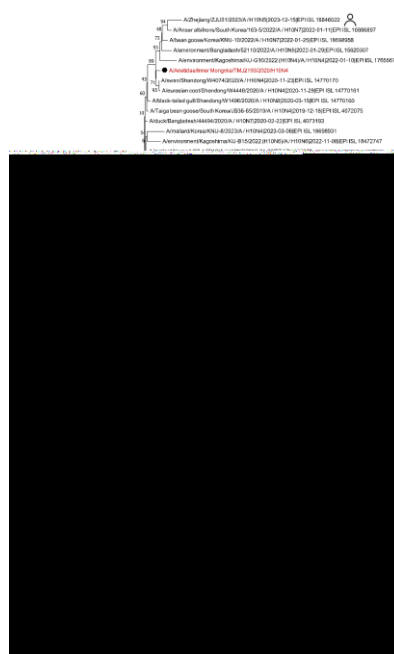
wigeon/Kagoshima/KU-D45/2018/H6N2

A/environment/Kagoshima/KU-

E1/2018/H4N2

NS

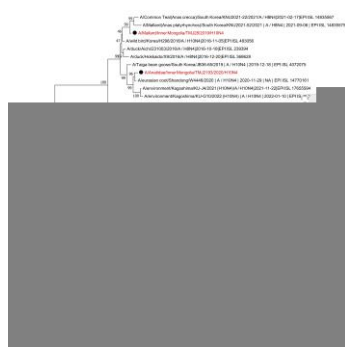
H10



3-16 HA

Fig.3-16 Phylogenetic tree of HA gene of H10 influenza virus. The green branch is American strain, the red branch is isolated strain, and the black branch is Eurasian strain.

N4



N5



N7

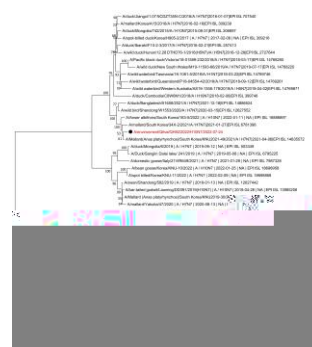


Fig.3-15 Phylogenetic tree of NA gene of N4 N5 N7 influenza virus. The green branch is American strain, the red branch is isolated strain, and the black branch is Eurasian strain.

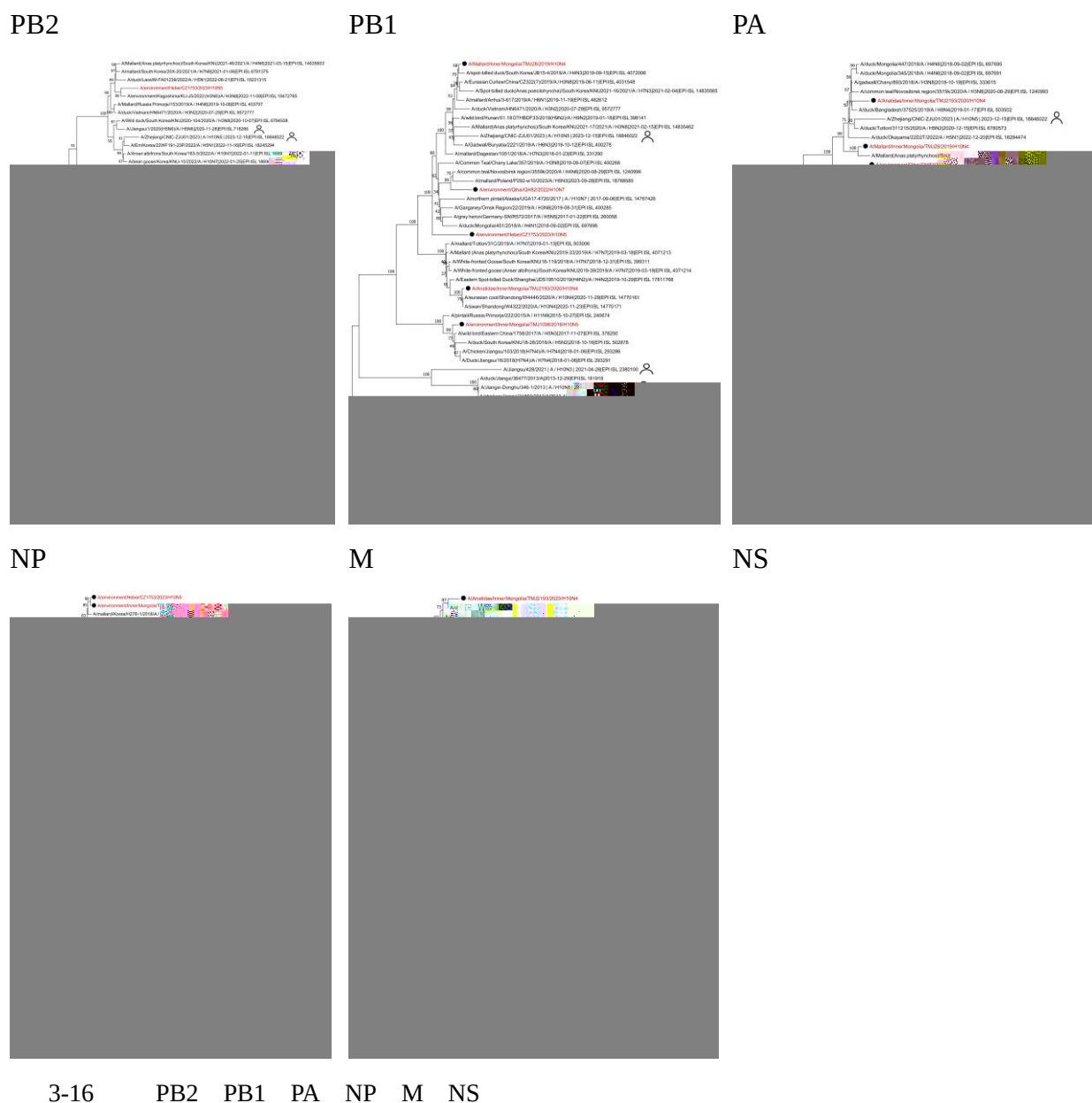


Fig 3-16 Phylogenetic tree of NA gene of PB2 PB1 PA NP M NS influenza virus. The green branch is American strain, the red branch is isolated strain, and the black branch is Eurasian strain.

H10 TMJ28 TMJ2193 QH82
CZ1753 HA PEVVQGR ↓ GLF TMJ1098HA

	D ⁷⁰¹ N	D	D	D	D	D	
PB1	S ⁶²² G	G	G	G	G	G	
	V ⁷⁰⁹ I	V	V	V	V	V	,
	T ¹⁵⁷ A	T	T	T	T	T	
PA	H ⁵¹⁰ A	H	H	H	H	H	mRNA
							RNA ,
NP	I ¹⁰⁹ T	I	I	I	I	I	AIV
M1	N ³⁰ D	D	D	D	D	D	
	T ²¹⁵ A	A	A	A	A	A	
	S ⁴² P	S	S	S	S	S	
NS1	L ⁹⁸ F	M	M	M	M	M	
	I ¹⁰¹ M	D	D	D	D	D	

/ - ;HA Subtype Numbering Conversion (<https://www.bv-brc.org>)

3.2.4.2 H10Nx

BALB/c H0N4(TMJ2193)

H10N5(TMJ1098) 3-19

TMJ2193

7 TMJ1098

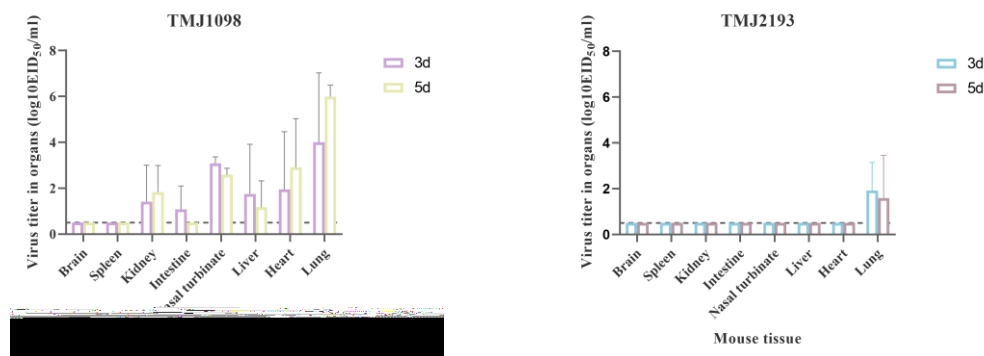
6

14

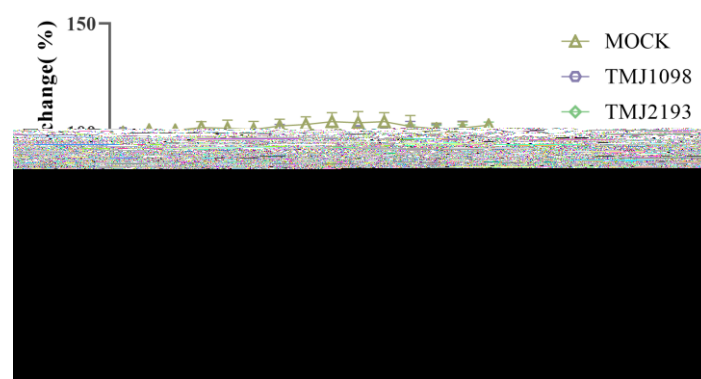
TMJ2193 TMJ1098

H10N5

H10N4



3-19
Fig.3-19 Replication of tissue virus in mice infected with avian influenza virus



3-20
Fig.3-20 Weight changes of mice post inoculation

3.3

H1

ZH1902 PB2

H13

H13N6

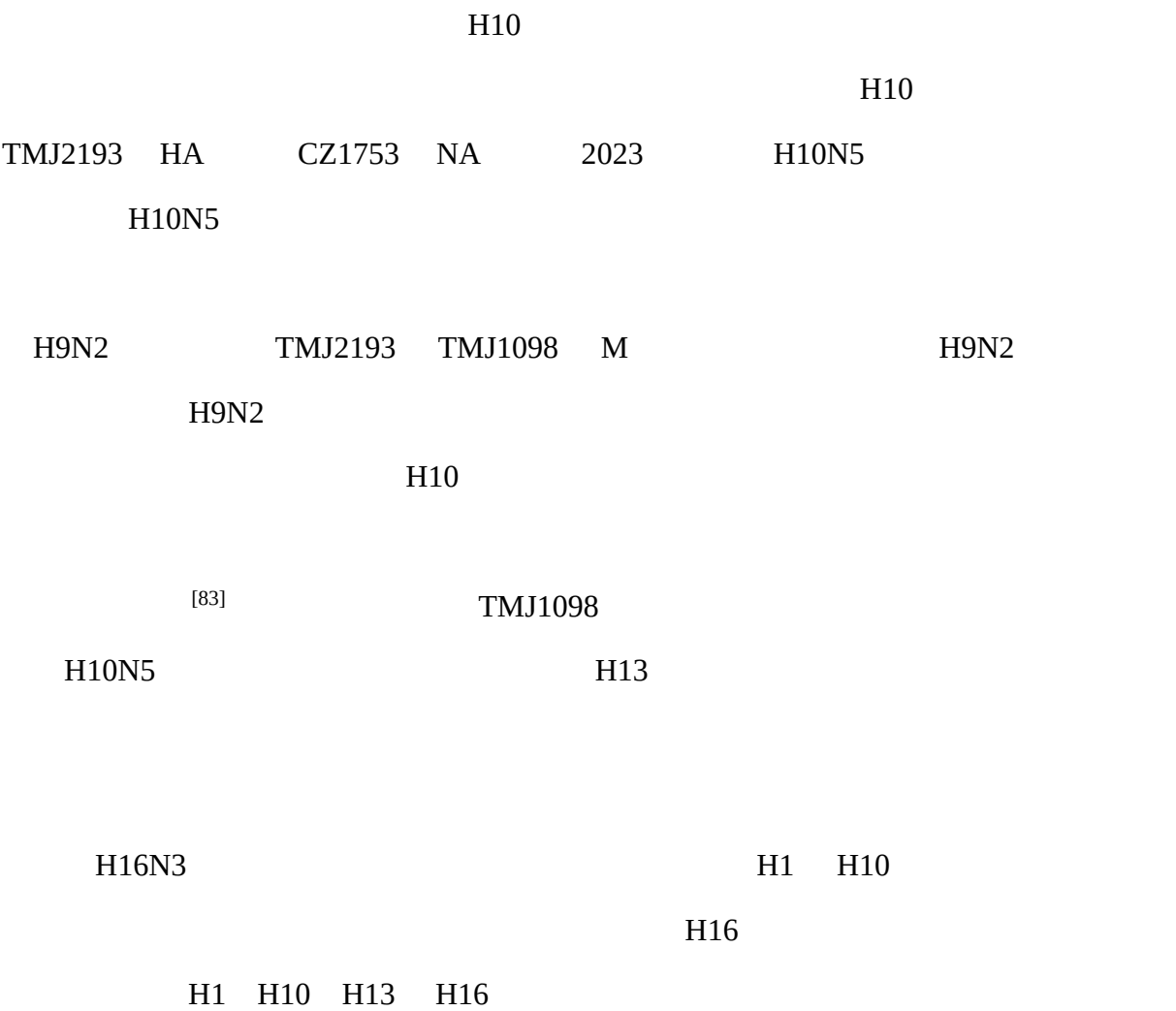
M1

H1

H1N1

H1N1

H1N1



3.4

1. H1N1

ZH1902 PB2

H1N1

2. H13Nx

ZH385 ZH5299 ZH987 DZ137 NA ZH186 PB2

DZ137 PB1 DZ137 C701 NP

H16N3 H13

H13	ZH5299	CZ949	H16N3
CZ1481			
3.	H10N _x		
LPAIV			
H10N4	H10N5		H10N5
5.			

4

4.1

1. 2021-2023	3		16	17	29	134
58,803	124	22	LPAIV		0.21%	LPAIV
					LPAIV	
			AIV			
	AIV				AIVs	
						LPAIV
2.	H1N1	H10N5				
	H1N1		H10N4	H16N3		
			H1N1	H10N5		
	H1N1	H10N5			H10N4	H16N3
3.		H13N6	H13N8	H16N3		
				H13Nx	H16N3	
			H1N1	H10Nx	LPAIV	
			LPAIV			
4.						
						LPAIV
				LPAIV		
						LPAIV

4.2

LPAIV

AIV

AIV

ZR2021MC119 H13

31970502

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