



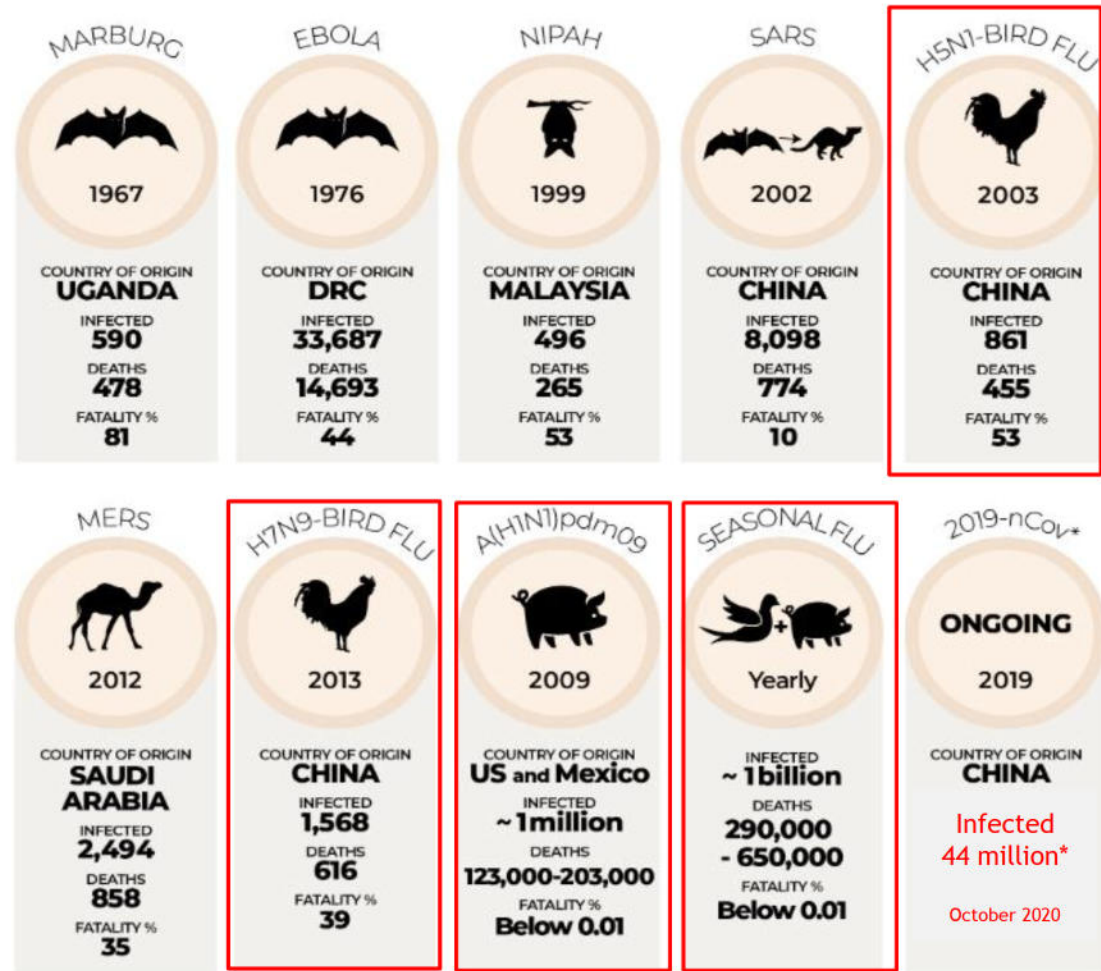
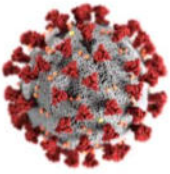
LPAIV PREVALENCE, SUBTYPE DIVERSITY AND MIGRATION CONNECTIVITY OF DABBLING DUCK THE AZOV-BLACK SEA REGION IN UKRAINE

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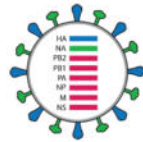
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Influenza viruses: a zoonotic threat

Global crisis



Influenza virus



- *Pandemic potential*
- *Natural infection of wild birds*
- *Spillover to domestic poultry*
- *Spillover to mammals, including humans and swine*
- *Economic losses to commercial and backyard poultry*
- *Reassortment, genetic drift and shift*

Deadly viral outbreaks that originated from animals

Influenza remains a serious infectious disease for humans and animals

People



Human pandemic influenza:

H1N1 (“Spanish flu” 1918-1920)

H2N2 (“Asian flu” 1957-1958)

H3N2 (“Hong Kong flu” 1968-1969)

H1N1 (Pandemic 2009)

Sporadic: H5N1 (HPAIV 1996-present)

H7N9 (HPAIV 2013-present)

Seasonal flu: yearly, ~ 1 billion infected, ~ 290 000 - 650 000 deaths

Swine



H1N1

H2N3

H3N8

H5N1

Economic losses to swine industry. “Mixing vessel” influenza viruses

Poultry



H5N2

H5N3

H5N1 (2004-2017)

H7N1, H7N7, H7N9

H9

H10

H5N8 (2014-2020)

New recent outbreak in Russia, Kazakhstan

New hosts and new viruses:

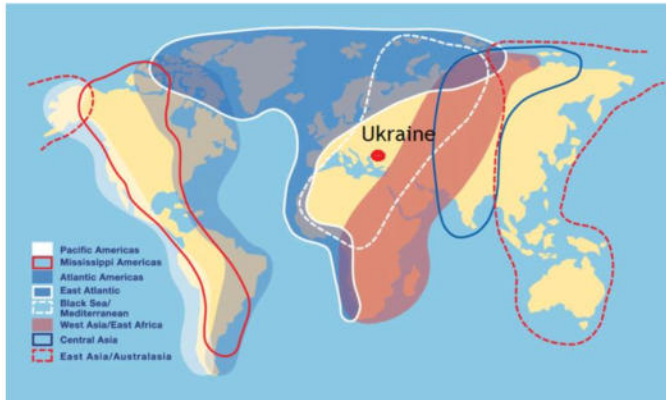


**Bat: Influenza A
H17N10, H18N11**



**Cattle:
Influenza D**

Wild birds and Avian influenza Virus



Position of Ukraine in the global wild bird's flyways

Anseriformes (duck, geese)



From duck and geese the most often isolated AIV of subtypes H3, H4, H6, H8, H9 H11, LPAIV H5, H7

Charadriiformes Gulls, waders



Gulls and waders are hosts of AIV rare subtypes (H13, H14, H15 H16)

Wild birds are the primary reservoir of AIV. AIV were isolated from >100 wild bird species (12 orders). *Anseriformes* and *Charadriiformes* are primary natural hosts of **ALL** subtypes of AIV.

Ukraine: unique geographic location in Europe

- Intersection of transcontinental migratory routes
 - North Asia, North Europe, Western Siberia
 - Black Sea-Azov & Caucasus
 - Mediterranean Sea, Southwest Asia, Levant
 - East Africa, North Africa
- Azov-Black Sea region in southern Ukraine important for wild bird migration, nesting, wintering.
- Rich ornithological fauna: 416 species (21 orders).
- All these points make Ukraine an important center for international AIV surveillance.

The LPAIV and HPAIV situation in Ukraine (2005-2020)

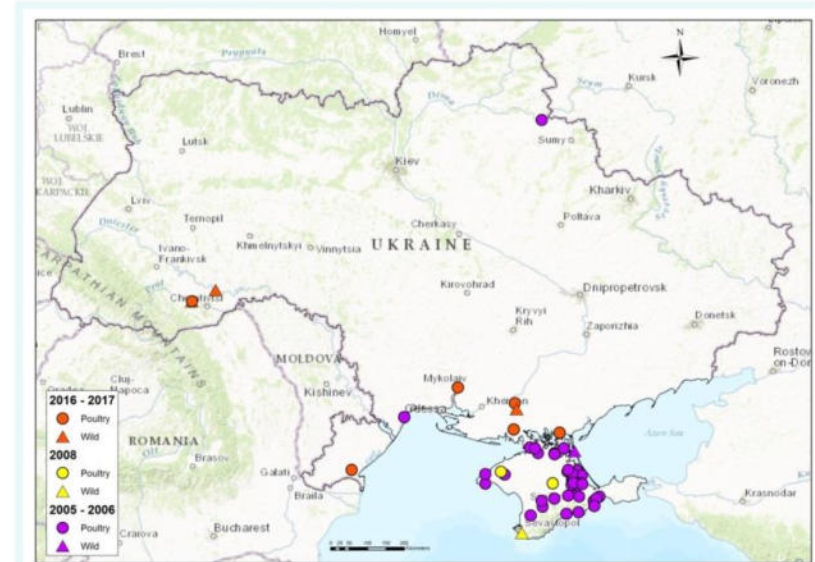
- Poultry farming: industrial and backyard (235-250 million birds). Ukraine is a major exporter of poultry products.
- LPAIV was not reported in poultry (2001-2020).
- HPAIV H7 subtype has never been reported in Ukraine.
- HPAIV H5N1 and H5N8: Ukraine had four waves of HPAIV H5.**

2005-2006 (H5N1) - 42 outbreaks (AR Crimea, Kherson, Odessa, Sumy)

2008 (H5N1) - 3 outbreaks (AR Crimea)

2016-2017 (H5N8) - 9 outbreaks (Kherson, Mykolaiv, Odesa, Ternopil, Chernivtsy)

2020 (H5N8) - 1 outbreak (Vinnytyca Oblast)



HPAIV H5N1 and H5N8 outbreaks in Ukraine in 2005-2006, 2008, 2016-2017

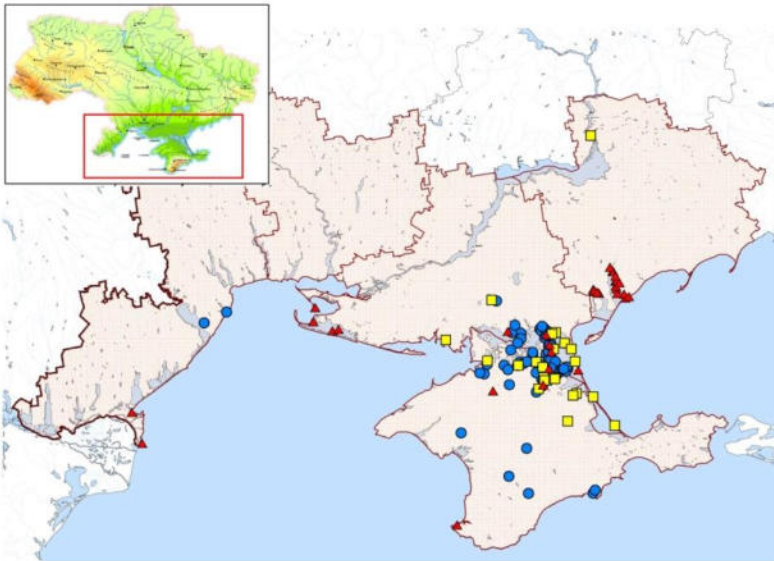
Affected species:

Poultry: hen, duck, geese, turkey

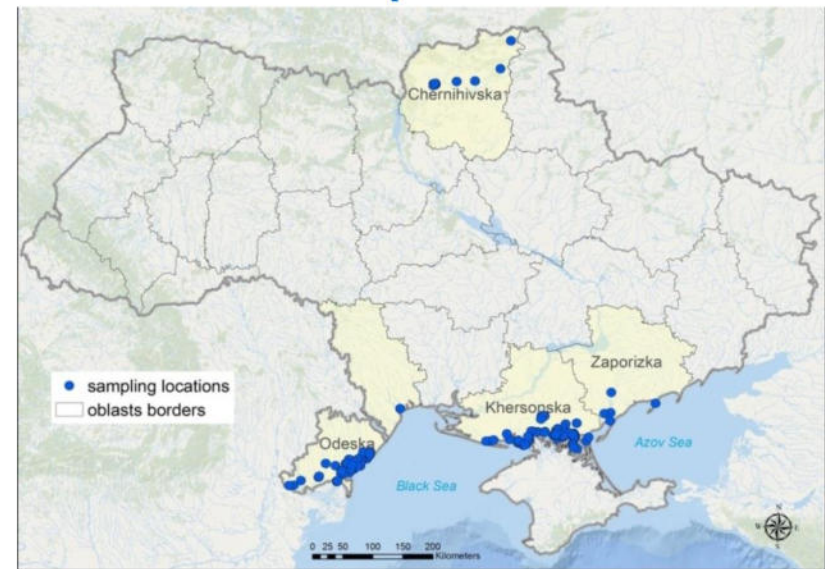
Wild Birds: Mute Swan (*Cygnus olor*), Cormorant (*Phalacrocorax carbo*), Great Crested Grebe (*Podiceps cristatus*)



Surveillance in wild birds in an AIV hotspot



Sampling sites in 2006-2016



Sampling sites in 2016-2020

- **Locations:** Azov-Black Sea region (Odesa, Kherson, Zaporizhzhia Oblast) and North Region (Chernihiv Oblast)
- **Biosampling:** cloacal, tracheal swabs, environmental/fecal samples
- **Species (2006-2016):** 21,511 wild birds from 105 species (11 orders: *Pelecaniformes*, *Ciconiiformes*, *Anseriformes*, *Galliformes*, *Gruiformes*, *Charadriiformes*, *Coraciiformes*, *Passeriformes*, *Falconiiformes*, *Columbiformes*, *Podicipediformes*).
- **Species (2017-2019):** >8000 environmental samples (ongoing) from 40 species (4 order: *Pelecaniformes*, *Anseriformes*, *Gruiformes*, *Charadriiformes*)
- **AIV diagnostics:** virology testing, RT-PCR (MP), serological subtyping by hemagglutination inhibition (HI) tests and virus genome sequencing (by Sanger, Illumina or MinION)

High AIV subtype diversity in AIV isolates from wild birds (virology study)

Subtype	Neuraminidase subtype										Total	
	N1	N2	N3	N4	N5	N6	N7	N8	N9	N?		
H1	22	1									1	24
H2			2								1	3
H3								5				5
H4		1				5					2	8
H5	8	2							6		3	19
H6	7	2				1					1	11
H7			3			2	3				2	10
H8		1		3								4
H9		3									3	6
H10	1						4				2	7
H11		2				1		1			1	5
H12								1			1	2
H13		1						1			1	3
H14												0
H15							1					1
H16			1									1
Total	38	13	6	3	0	9	8	14			18	109

From these samples, **95 LPAIV's** and **14 HPAIV's** belonging to 15 of the 16 known HA subtypes and 7 of 9 known NA subtypes were isolated. No H14, N5 or N9 subtypes were identified. There were **28** HA and NA antigenic combinations. Additional we had 18 AIV H1N1 from sentinel ducks.

Infection Rate of AIV of wild birds in 2010-2016 (virology study)

Bird species	Sampling Periods			Positive/Total samples (infection rate, %)
	Autumn migration	Wintering	Spring migration, nesting, after-nesting movements	
ANSERIFORMES				
Mallard <i>Anas platyrhynchos</i>	16/700 (2.28%)	15/1313 (1.14%)	0/266	31/2279 (1,36) ^c
White-fronted Goose <i>Anser albifrons</i>	1/891 (0.11%)	14/3267 (0.42%)	2/1682 (0.12%)	17/5840 (0,29)
Ruddy Shelduck <i>Tadorna ferruginea</i>	3/614 (0.49%)	6/857 (0.70%)	0/335	9/1806 (0,49)
Shelduck <i>Tadorna tadorna</i>	3/246	10/378	0/427	13/1051 (1,23)
Wild duck ^D	0/111	2/45	50	2/206 (0.97)
Teal <i>Anas crecca</i>	2/194	0/85	0/112	2/391 (0.51)
Garganey <i>Anas querquedula</i>	4/56	-	0/51	4/107 (3.73)
Shoveler <i>Anas clypeata</i>	1/56	-	-	1/56 (1.78)
Total ANSERIFORMES	30/3399 (0.88%)	47/7853 (0.60%)	2/3261 (0.06%)	79/14513(0.54%)
CHARADRIIFORMES				
Yellow-legged Gull <i>Larus cachinnans</i>	0/99	2/342	2/598	4/1039 (0.38)
Black-headed Gull <i>Larus ridibundus</i>	0/182	0/185	1/418	1/785 (0.13)
Slender-billed Gull <i>Larus genei</i>	0/1	-	2/229	2/230 (0.87)
Wood Sandpiper <i>Tringa glareola</i>	-	-	1/45	1/45 (2.56)
Total CHARADRIIFORMES	0/681	2/604 (0.33%)	6/3386 (0.18%)	8/4671 (0.17%)
PELECANIFORMES				
Cormorant <i>Phalacrocorax carbo</i>	0/10	-	5/204 (HPAI)	5/214 (2.33)
PODICIPEDIFORMES				
Great Crested Grebe <i>Podiceps cristatus</i>	-	-	3/6 (HPAI)	3/6 (50.00)
Total ALL species	30/4244 (0.70)	49/9635 (0.51)	16/7632 (0.21)	95/21511 (0.45)

AIV prevalence, subtype diversity of dabbling duck in 2010-2016 (virology study)

Bird species	Sampling Periods			Positive/Total samples (%)
	Autumn migration	Wintering	Spring migration, nesting, after-nesting movements	
ANSERIFORMES				
Mallard <i>Anas platyrhynchos</i>	16/700 (H1N1, H2N3, H3N8 [5], H5N2 [2], H7N7 [2], H8N4, H10N7, H11N8, H4N?[2])	15/1313 (H1N1 [2], H5N8 ^B , H6N2, H7N7, H7N3 [3], H7N6 [2], H10N7 [3], H12N8, H15N7)	266	31/2279 (1,36) ^C
Teal <i>Anas crecca</i>	2/194 (H5N2, H6N1)	0/85	0/112	2/391 (0.51)
Garganey <i>Anas querquedula</i>	4/56 (H4N6 [4])	-	0/51	4/107 (3.73)
Shoveler <i>Anas clypeata</i>	1/56 (H8N4)	-	-	1/56 (1.78)

All viruses of dabbling duck were isolated during autumn migration and wintering.

Infection Rate of AIV of wild birds in 2017-2018 (PCR)

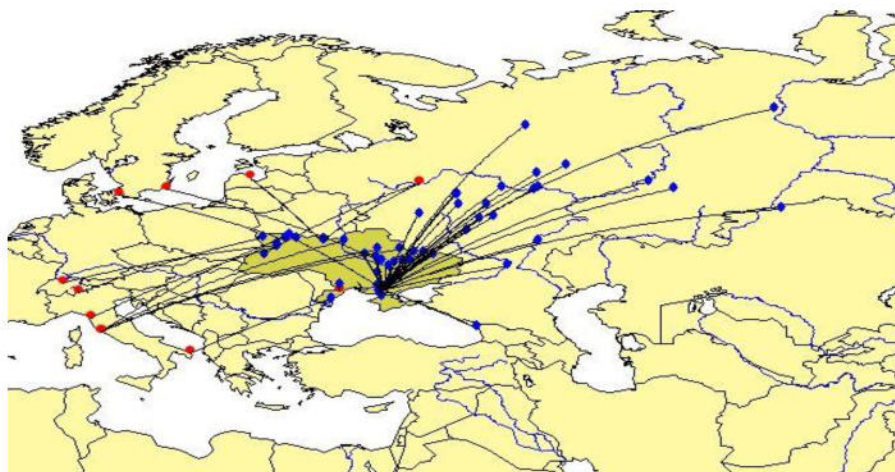
Species	Positive samples AIV A/Total samples (% prevalence)	Positive samples for AIV H5 (% prevalence)
South Region (Kherson, Odesa Oblast)		
Mallard	25/1116 (2.24%)	1 (0.08%)
Garganey	11/84 (13.0%)	-
Shelduck	14/578 (2.42)	-
Red-breasted Goose	1/304 (0.32%)	-
White-fronted Goose	13/900 (1.44%)	-
Greylag Goose	3/176 (1.7%)	-
Yellow-legged Gull	1/194 (0.51)	-
Wigeon	1/161 (0.62%)	1 (0.62%)
Great Black- headed Gull	1/16 (6.25%)	-
Whooper Swan	2/387 (0.51%)	-
Cormorant	1/174 (0.57%)	-
Total of South Region	73/5608 (1.3%)	2 (0.03%)
North Region (Chernihiv Oblast)		
Mallard	11/183 (6.03)	-
White-fronted Goose	2/215 (0.93%)	-
Total of North Region	13/399 (3.25)	-

Infection Rate of AIV of wild birds in 2019-2020 (PCR)

Species	Positive samples AIV A/Total samples (% prevalence)	Positive samples for AIV H5 (% prevalence)	Positive samples for AIV H7 (%prevalence)
South Region (Kherson, Odesa, Mykolaiv Oblast)			
Mallard	26/824 (3.1%)	2 (0.24%)	2 (0.24%)
Ruddy shelduck	21/90 (23.3%)	-	-
Shelduck	5/435 (1.14%)	-	-
Black-headed gull	1/190 (0.52%)	-	-
Teal	2/47 (4.25%)	-	-
White-fronted goose	6/800 (0.75%)	-	-
Total of South Region	61/3674 (1.66%)	2 (0.05%)	2 (0.24%)
North Region (Chernihiv Oblast)			
Mallard	4/158 (2.53%)	-	-
Total od North Region	4/329 (1.21%)	-	-

All AI viruses of dabbling duck were detected during autumn migration and wintering.

Ringing results



Directions of migration of wild ducks from the South Ukraine by the results of ringing
(Center for Bird Ringing, Poluda A.M.)

According to the ringing results in the Southern Ukraine, the geography of the ring findings is very wide. The predominant direction of mallards from Askania-Nova during spring migration is Eastern and Northern and much less - to the West and South. The maximum duration of return of ring is up to 10.5 years, and the largest migratory distance is 3206 km.

Species	Label number	Date	Place	Date of band return	Location of band return	Distance, km	Time after the labeling, days
Mallard	DB-410759	17.01.2018	Kherson region 46.28 N/33.50 E	05.05.2018-14.05.2018	Vovchansk, Sverdlov Region, Russia	2284	108
Mallard	DB-410791	29.01.2018	Kherson region 46.28 N/33.50 E	23.09.2018	Dniprovskia Oblast, Ukraine	273	237
Mallard	DB-410916	13.02.2018	Kherson region 46.28 N/33.50 E	12.05.2018	Tumen Region, Russia	3206	88

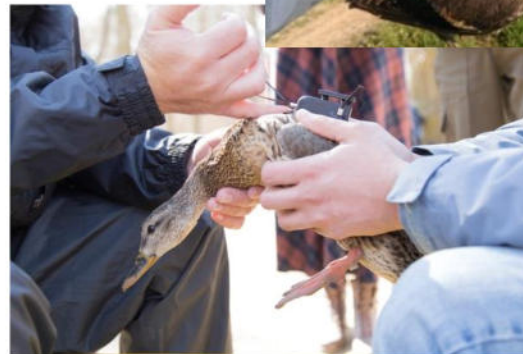
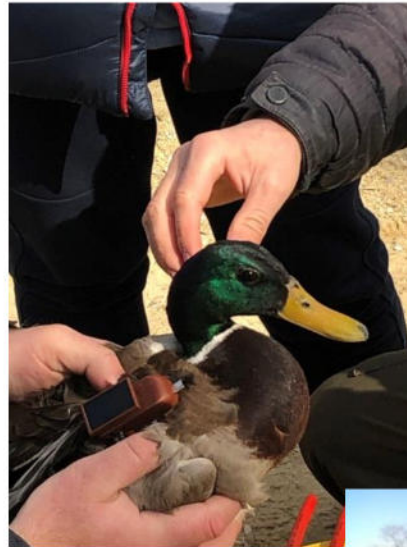
GPS/GSM loggers



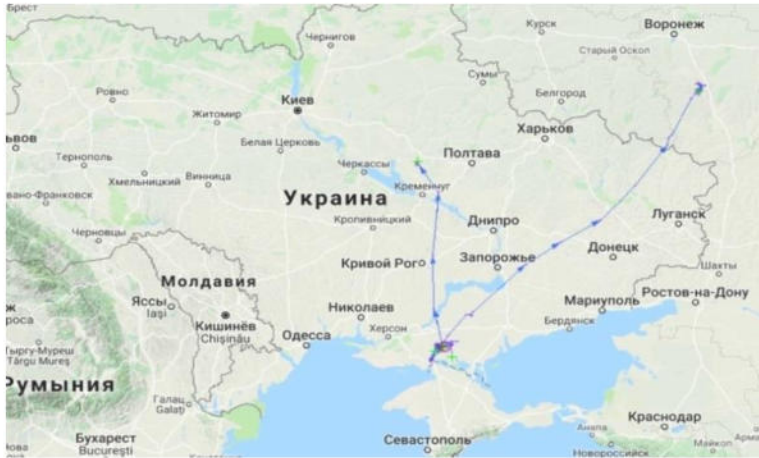
We used the Ornitela
GPS/GSM loggers (10g, 25g).

The loggers were provided by Linnaeus
University (Kalmar, Sweden).

Linnaeus University 



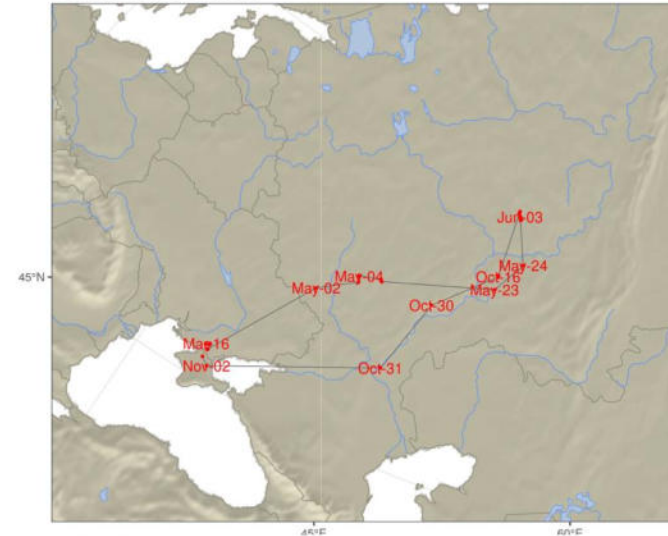
GPS tracking of duck in Ukraine



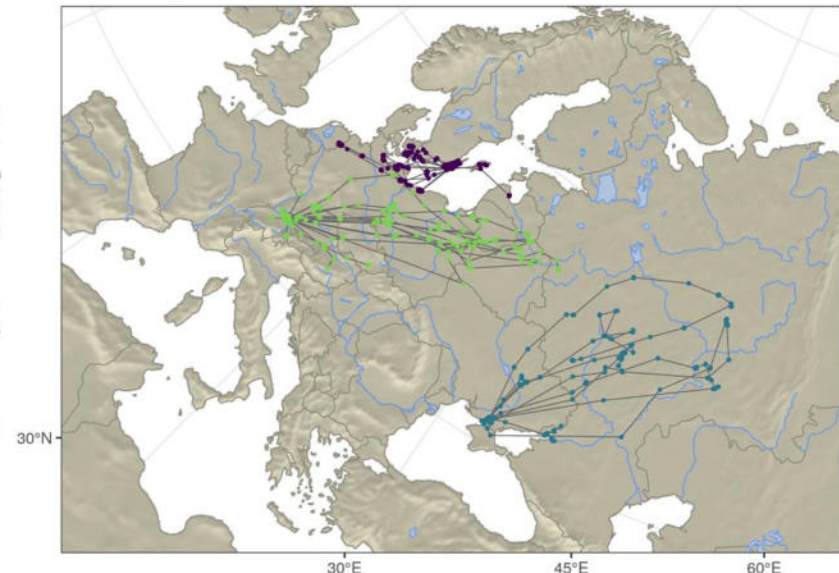
Directions of migration of wild ducks from the South Ukraine by the results of GPS tracking (March 2019)

- During spring migration, all birds migrated to the North-East also. The biggest distance was over 2000 km and some birds covered this distance in 3-4 days.
- The birds stayed for breeding in the central Russia.
- With the beginning of autumn migration, birds returned in Kherson region (Ukraine).

Mallards 'T003017' (2019)

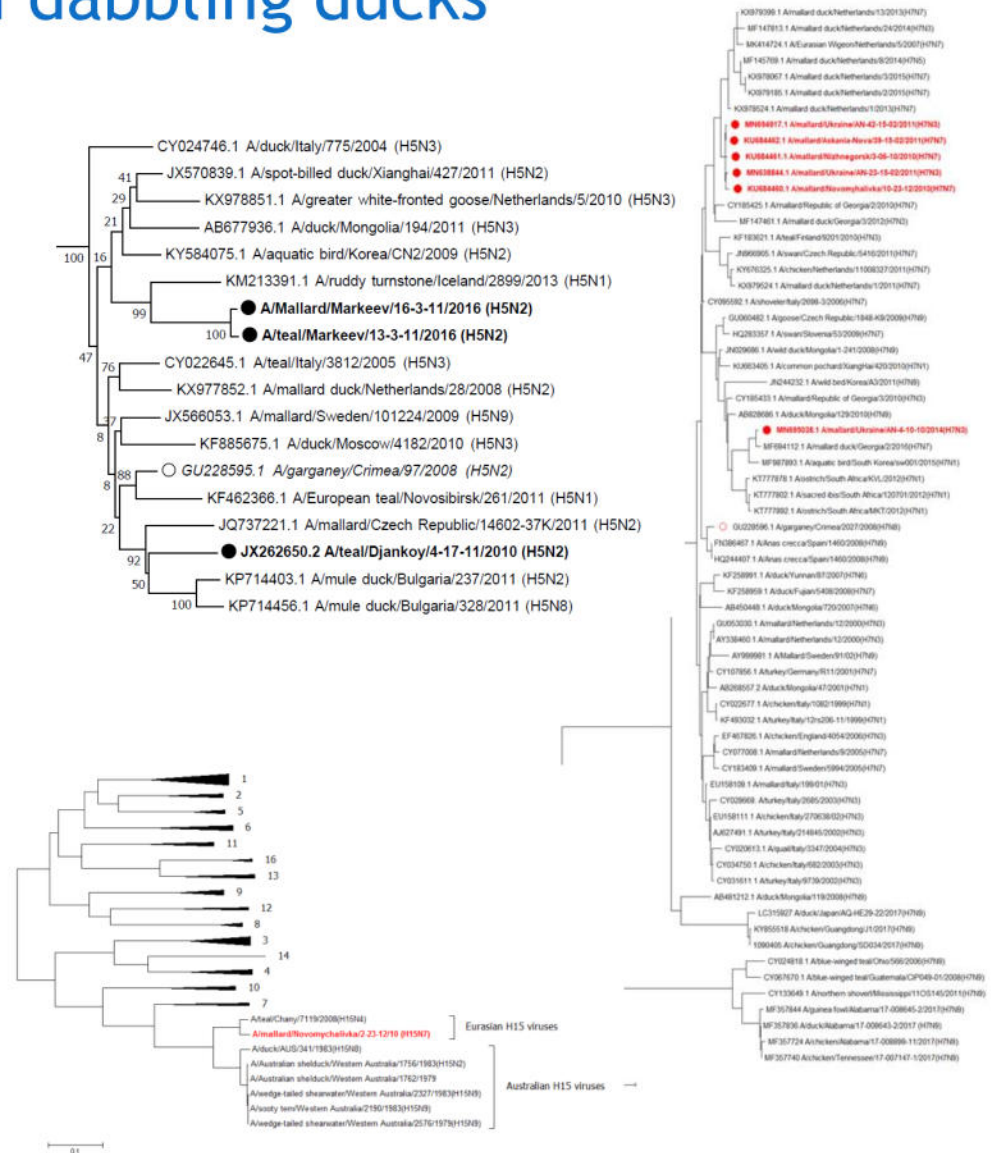


Mallard migrations



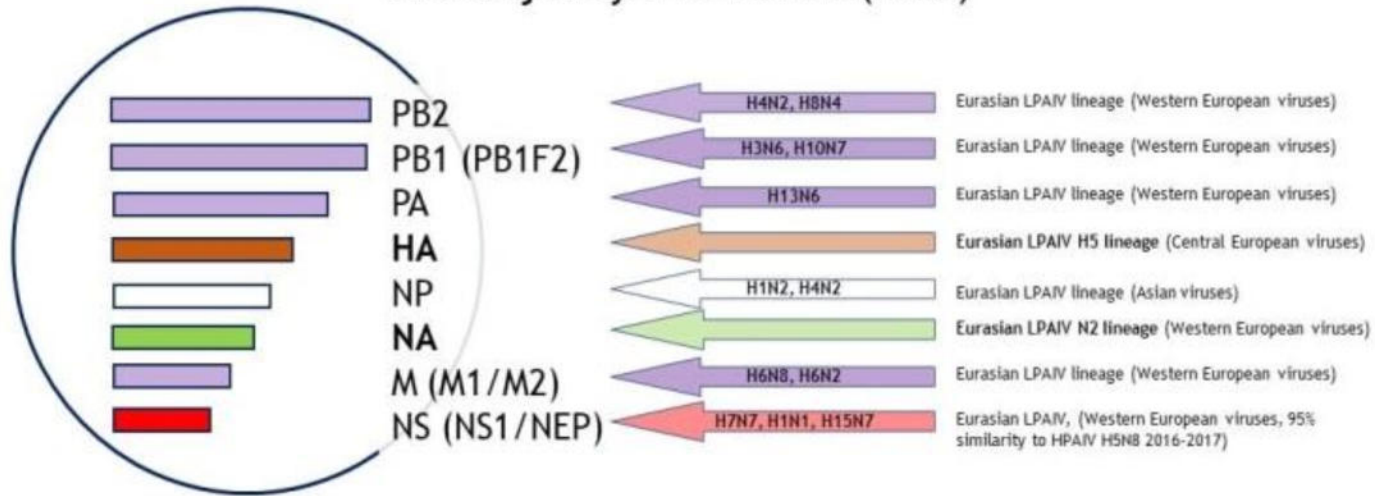
Phylogeny of LPAIV from dabbling ducks

- Genetic analysis revealed a large genetic diversity and their association with pathogens from Europe, Siberia, and Asia.
- LPAIV's **H5N2** were most closely related to wild bird LPAIVs found in wild birds in Europe.
- The LPAIV's **H7N3**, **H7N7** are related to the AIV same subtype isolated in Georgia.
- The LPAIV's **H1N1** viruses related to AIV same subtypes circulating in Mongolia and Georgia.
- The LPAIV's **H13** viruses showed that all these viruses are related to viruses from Georgia and Russia.

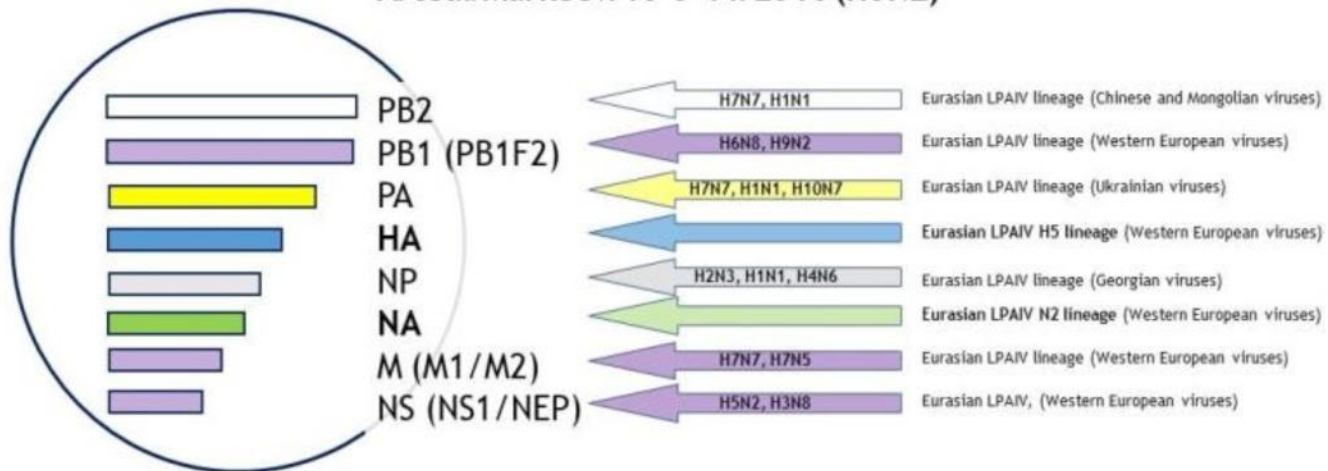


Genetic analysis

A/teal/Djankoy/4-17-11/2010 (H5N2)



A/teal/Markeev/13-3-11/2016 (H5N2)



Conclusions

- Detection of multiple AIV subtypes in diverse wild bird species suggests a natural ecological reservoir for continual circulation of AIV in Ukraine.
- Detection of wide diversity of LPAIV subtypes in dabbling duck confirms the importance of Ukraine as a potential hotspot for the European influenza surveillance system.
- The role of wild migratory birds in the introduction of novel H5 HPAIV viruses is not clear and more deep study.
- Detailed study of migration contacts of virus major natural carriers will allow to study the ecology of influenza viruses, potential directions, stopping locations, virus transmission rate more deeply and will develop a strategy for monitoring, preventing and eradication of the pathogen. Also, it will help to fill in some gaps in the ecology of zoonotic pathogens.
- We are going to continue the surveillance of LPAIV and HPAIV in Ukraine and will study susceptibility of different wild birds to HPAIV.



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Our team



Pictures by Denys Muzyka



Thank you!

