

Results of WGS of Serbian AIV isolates in period 2022/2023



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Some facts about Republic of Serbia

- **Area 88.499 km²**
- **Cattle 725408**
- **Sheep 1.716.000**
- **Pigs 2.140.000**
- **Poultry 14.278.000**



Veterinary Specialized Institute Kraljevo

National Reference Laboratory for:

- **Avian influenza (AI)**
- **Newcastle disease (NDV)**
- **West Nile fever (WNV)**
- **Chlamydiosis**
- **Mycoplasmosis**
- **Gumboro disease (IBD)**
- **Infectious Bronchitis (IBV)**
- **Capripox viruses**

Employees

- 45 employees
- 12 DVM (6 PhD, 5 MSc, 8 vet. specialists)
- 2 chemist (1 MSc)
- 20 vet. technicians
- 8 Administrative and helping stuff



National monitoring program for avian influenza in Serbia

Active monitoring

- Backyard poultry in risk areas: The following types of domestic poultry are included in the supervision: ducks, geese, chickens (all age and production categories), quail (Japanese), all other ornamental poultry and farmed birds (hobby birds and poultry).
- Domestic poultry, farmed and ornamental poultry in markets, fairs and exhibitions
- Migratory wild waterfowl, especially those whose natural habitat is related to water bodies (ponds, swamps, lakes), which Serbia abounds in.
- Poultry from intensive farm production

Passive monitoring

- All dead or sick wild or domestic birds
- Wild carnivores

National monitoring program for avian influenza in Serbia

Прилог 1. - Попис „циљаних врста“ дивљих птица које се приоритетно узоркују у оквиру програма мониторинга авијарне инфлуенце доњљих птица у Републици Србији

Број	Латински назив	Име по српској номенклатури
1.	Accipiter gentilis	Јастреб
2.	Alopochen aegyptiacus	Египатска утва
3.	Anas acuta	Шилјан
4.	Anas crecca	Крца
5.	Anas platyrhynchos	Глувара
6.	Anser albifrons	Лисаста гуска
7.	Anser anser	Дивља гуска
8.	Anser brachyrhynchus	Краткокулна гуска
9.	Anser erythropus	Мала лисаста гуска
10.	Anser fabalis	Гуска глоговњача
11.	Ardea cinerea	Сива чапља
12.	Aythya ferina	Риђоглава патка
13.	Aythya fuligula	Ћубаста патка
14.	Aythya marila	Морска црнка
15.	Botaurus stellaris	Водени бик
16.	Branta bernicla	Гриваста гуска
17.	Branta canadensis	Канадска гуска
18.	Bubo bubo	Буљина
19.	Bucephala clangula	Патка дупљашница
20.	Buteo buteo	Мишар
21.	Buteo lagopus	Гаџасти мишар
22.	Larus ridibundus	Обични галеб
23.	Ciconia ciconia	Бела рода
24.	Cygnus cygnus	Велики лабуд
25.	Cygnus olor	Лабуд грбац
26.	Casmerodius albus	Велика бела чапља
27.	Egretta garzetta	Мала бела чапља
28.	Falco peregrinus	Сиви соко
29.	Haliaeetus albicilla	Белорепан
30.	Larus argentatus	Сребрнасти галеб
31.	Larus canus	Сиви галеб
32.	Limosa limosa	Муљача
33.	Anas penelope	Звиздара
34.	Anas strepera	Чегртуша
35.	Mergelus albellus	Мали ронац
36.	Mergus meganser	Велики ронац
37.	Netta rufina	Превез
38.	Pelecanus crispus	Кудрани несит
39.	Pelecanus onocrotalus	Ружичасти несит
40.	Phalacrocorax carbo	Велики враџац
41.	Pica pica	Сврака
42.	Podiceps cristatus	Ћубасти гњурац
43.	Podiceps nigricollis	Црногрлати гњурац
44.	Porphyrio porphyrio	Султанка
45.	Somateria mollissima	Гавка
46.	Tadorna tadorna	Шарена утва
47.	Tachybaptus ruficollis	Мали гњурац
48.	Tringa ochropus	Спрудник нијукавац
49.	Turdus pilaris	Дрозд борњак

National monitoring program for avian influenza in Serbia

Active monitoring September, October, November, March, April, May
Passive monitoring – whole year

AI MONITORING FOR SEASON 2021/22

CATEGORY	PLANNED
Individual farms	2556
commercial farms	0
Wild birds	852

AI MONITORING FOR SEASON 2022/23

CATEGORY	PLANNED
Individual farms	3231
commercial farms	405
Wild birds	852

AI MONITORING FOR SEASON 2023/24

CATEGORY	PLANNED
Individual farms	3906
commercial farms	810
Wild birds	852



2021

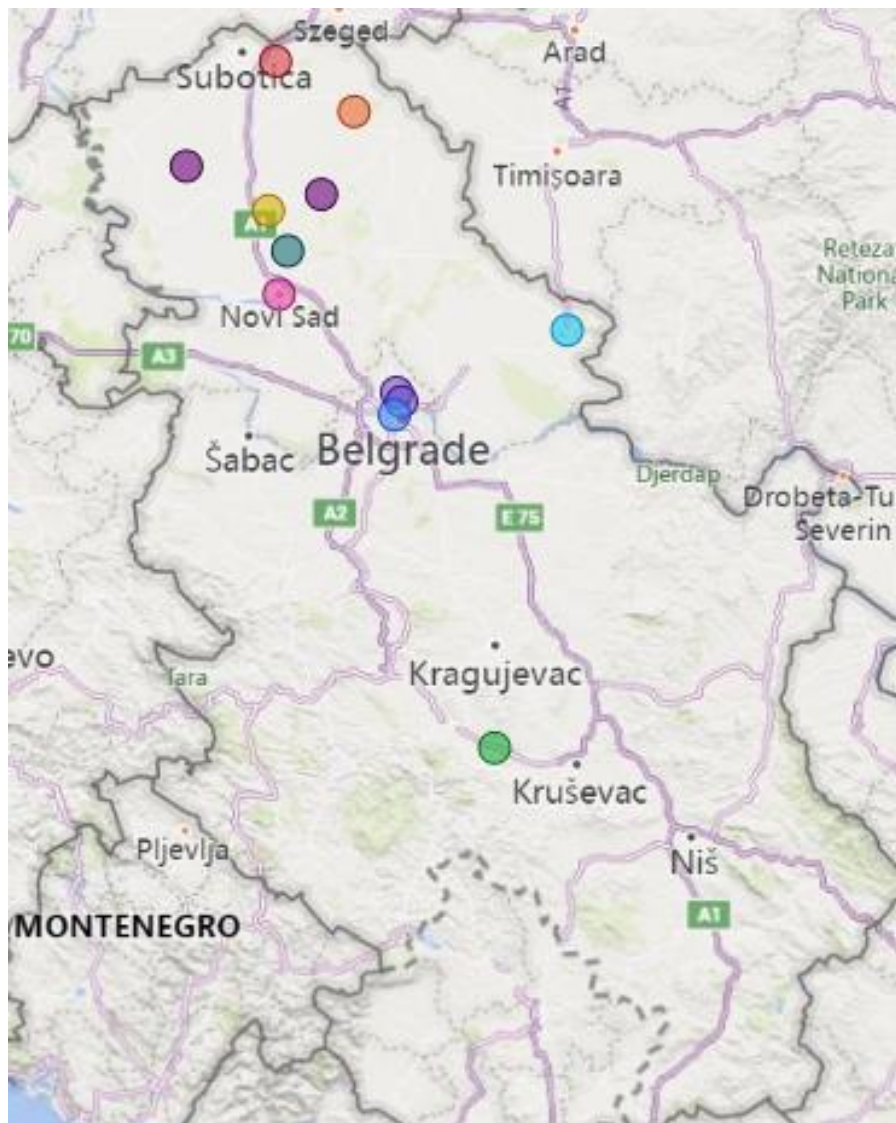
Epizootiological situation on 2021

1	Veliki Bački Kanal u naseljenom mestu Kula, ul.Lajoš Košut br.41	Kula	Zapadno-Bački	H5N8
2	Veliki Bački Kanal, naseljeno mesto Sivac	Kula	Zapadno-Bački	H5N8
3	Kej oslobođenja, Zemun	Zemun	Grad Beograd	H5N8
4	Borča, ul.Borčanskih žrtava 1914, (most kod kanala prema Crvenki)	Palilula	Grad Beograd	H5N8
5	Beljarica,potez Udbina rampa, Kovilovo	Palilula	Grad Beograd	H5N8
6	Zoovrt "Metaloplastika Srečković" d.o.o.,Vrnjci, ul.Moravska dolina br.1/1	Vrnjačka Banja	Raški	H5N1
7	ribnjak Zlatica, DTD "Ribarstvo" d.o.o. Jazovo	Čoka	Severno-Banatski	H5N1
8	Luka Novi Sad, kanal DTD, na teritoriji Grada Novog Sada	Novi Sad	Južno-Bački	H5N1
9	Mini Zoo Vrt Temerin, ul.Rakoci Ferenca br.209/4	Temerin	Južno-Bački	H5N1
10	Vršačko jezero, ul.Miloša Obilića br.88, na teritoriji grada Vršca	Vršac	Južno-Banatski	H5N1
11	Specijalni rezervat prirode "Ludoško jezero" u naseljenom mestu Šupljak	Subotica	Severno-Bački	H5N1
12	Lipar, ul.Bratstva i jedinstva br.19	Kula	Zapadno-Bački	H5N1
13	Srbobran, ul.Petra Bigina br.16	Srbobran	Južno-Bački	H5N1



Epizootiological situation on 2021

2021



Species of wild birds 2021

Wild - Mute Swan	H5N1 x 2, H5N8 x 4
Wild - Mallard	H5N8 x 1
Wild - Black swan	H5N1 x 1
Wild – Elliot	H5N1 x 1
Wild - Swan goose	H5N1 x 2
Wild - Greater white-fronted goose	H5N1 x 1
Wild - Greylag goose	H5N1 x 1



Epizootiological situation on 2022

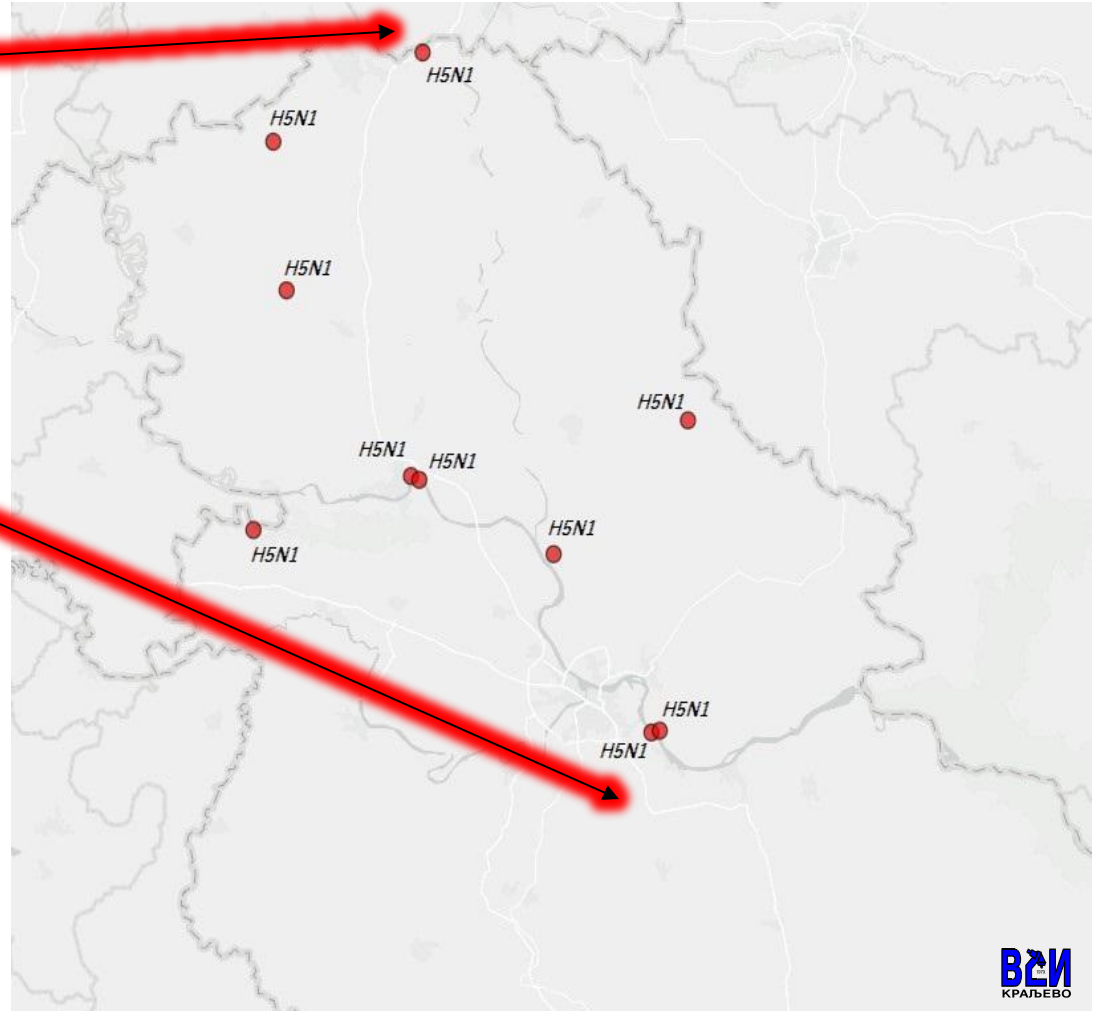
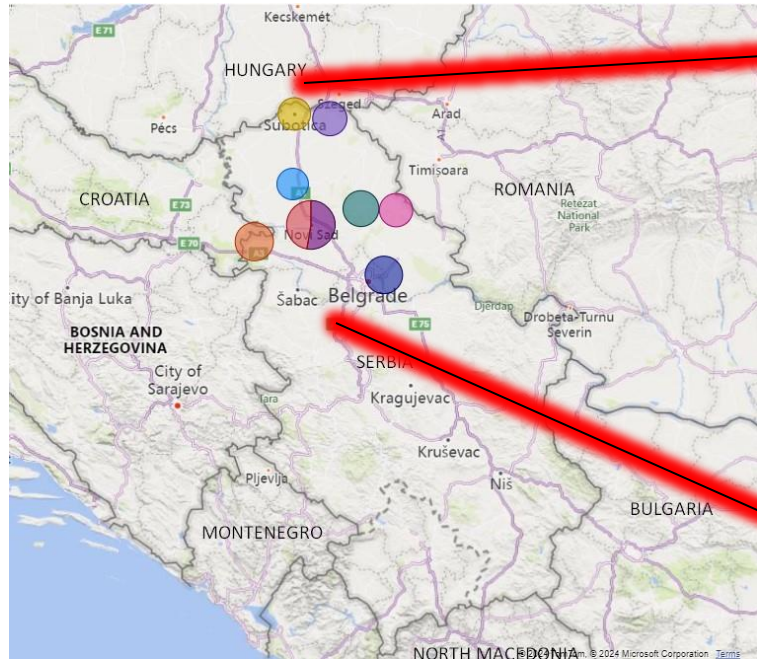
2022

AVIAN INFLUENZA IN SERBIA IN 2022				
6p.	Location	Municipality	District	Virus
1.	Veliki Bački Kanal u naseljenom mestu Crvenka, ul.Mošé Pijade	Kula	Zapadno-Bački	H5N1
2.	Subotica, ul.9.Nova br.134	Subotica	Severno-Bački	H5N1
3.	Priroda dela toka Tamiš u neposrednoj blizini ribnjaka Sutjeska	Sečanj	Srednje-Banatski	H5N1
4.	Specijalni rezervat prirode "Selevinjske pustare" u naselju Horgoš	Kanjiža	Severno-Banatski	H5N1
5.	Ušće kanala DTD , na teritoriji Grada Novog Sada	Novi Sad	Južno-Bački	H5N1
6.	Čenta, ul.Pionirska bb	Zrenjanin	Srednje-Banatski	H5N1
7.	Obala Dunava na Beogradskom keju, teritorija Grada Novog Sada	Novi Sad	Južno-Bački	H5N1
8.	Ivanovo i Omoljica u opštini Pančevo i Ritopek u opštini Grocka	Pančevo/Grocka	Južno-Banatski/	H5N1
9.	Jezero Sot, naseljeno mesto Sot	Šid	Grad Beograd	H5N1

Species of wild birds 2022

Wild - Common buzzard	H5N1 x 1
Wild - Black headed gull	H5N1 x 1
Wild - Mute Swan	H5N1 x 5
Wild – Great egret	H5N1 x 1
Wild - Mallard	H5N1 x 1

Map of AI outbreaks in Serbia 2022



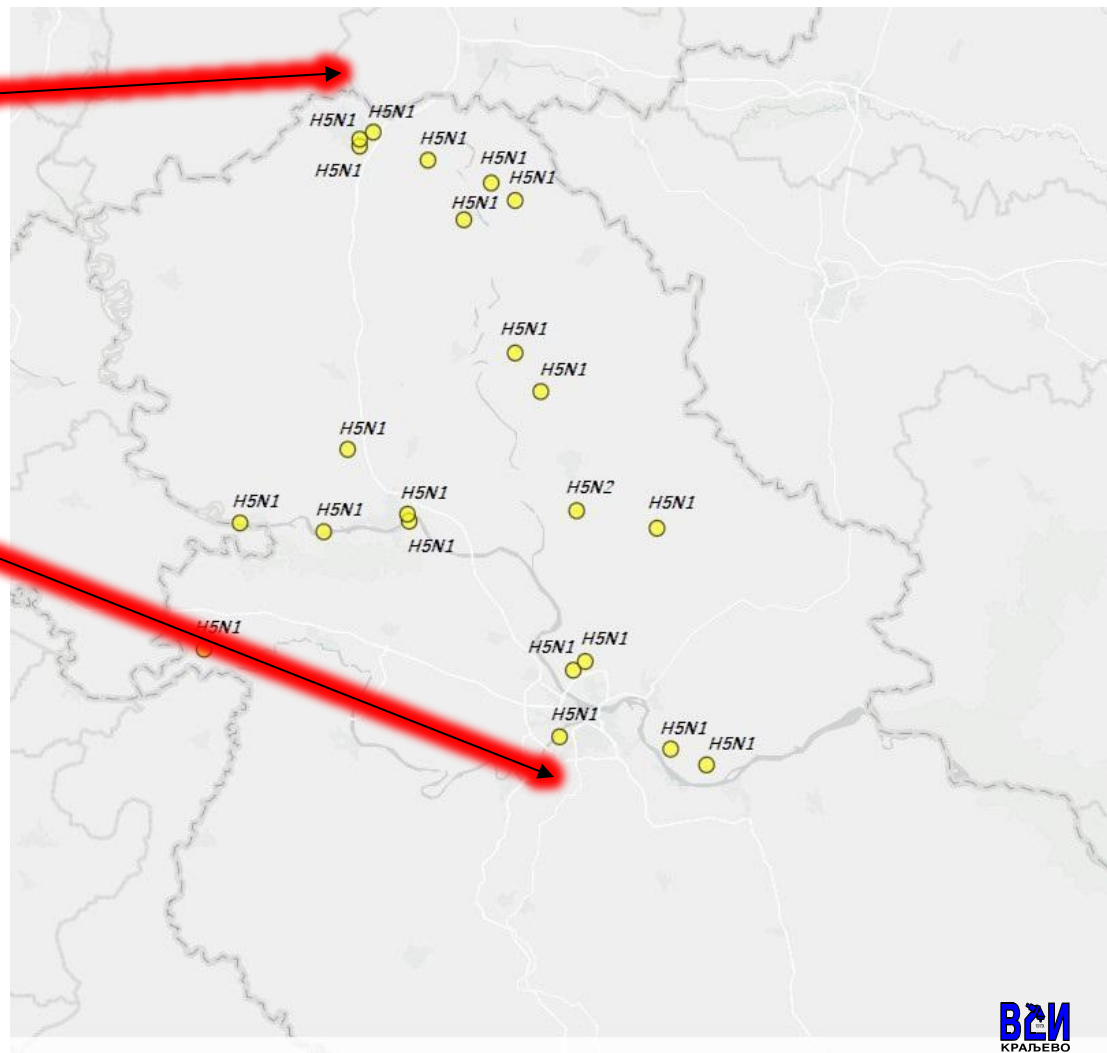
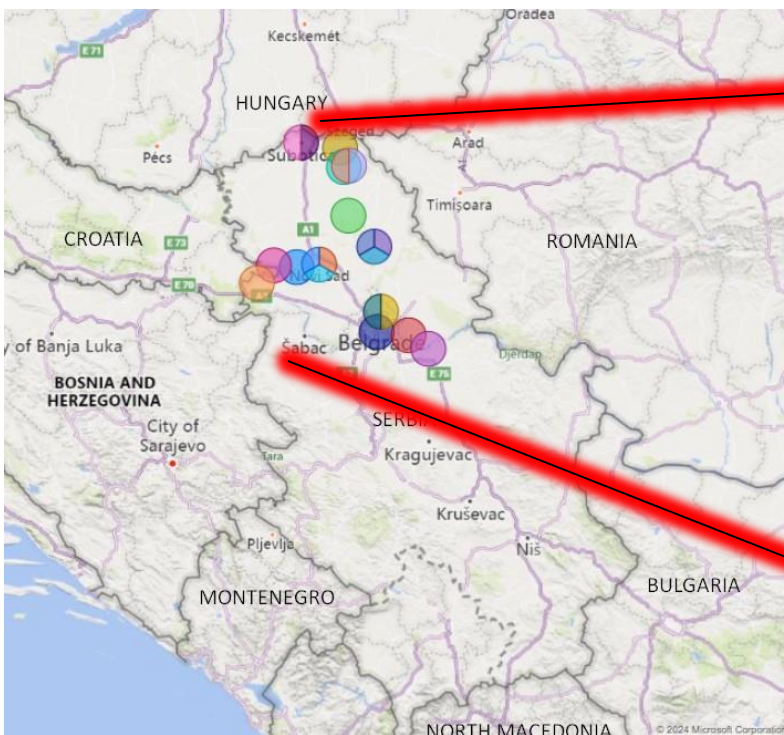
2023

Epizootiological situation on 2023



Бр.	Lokacija	Opština	Okrug	Virus
1	Palić-Ludoš	Subotica	Severno-Bački	H5N1
2	Mali Bački kanal-prevodnica DTD kanala	Novi Sad	Južno-Bački	H5N1
3	deo Novog Sada, Petrovaradin i Sremska Kamenica	Novi Sad	Južno-Bački	H5N1
4	jezero Palić	Subotica	Severno-Bački	H5N1
5	jezero Palić	Subotica	Severno-Bački	H5N1
6	Kanal Vizelj, Borča	Palilula	Grad Beograd	H5N1
7	Veliki Makiš	Čukarica	Grad Beograd	H5N1
8	Kovilovo	Palilula	Grad Beograd	H5N1
9	Jezero Tikvara(park prirode)	Bačka Palanka	Južno-Bački	H5N1
10	Senta	Senta	Severno-Banatski	H5N1
11	ribnjak Kapetanski rit,Velebit	Kanjiža	Severno-Banatski	H5N1
12	Morović	Šid	Sremski	H5N1
13	jezero u Parku prirode banje Rusanda	Zrenjanin	Zrenjanin	H5N1
14	Specijalni rezervat prirode "Slano Kopovo "	Novi Bečej	Srednje-Banatski	H5N1
15	Tomaševac	Zrenjanin	Srednje-Banatski	H5N1
16	Prirodni rezervat Čokansko kopovo Slatina	Čoka	Severno-Banatski	H5N1
17	ribnjak Ribobel, Prirodni rezervat Slatina	Čoka	Severno-Banatski	H5N1
18	naselje Jugovo u Smederevu	Smederevo	Podunavski	H5N1
19	kanal Nadel	Omoljica	Južno-Banatski	H5N1
20	Begečka jama	Begeč	Južno-Bački	H5N1
21	Luka Novi Sad i Rečno ostrvo	Novi Sad	Južno-Bački	H5N1

Map of AI outbreaks in Serbia 2023



Species of wild birds 2023

Wild - Greylag goose	H5N1 x 2
Wild - Black headed gull	H5N1 x 4
Wild - Mute Swan	H5N1 x 11
Wild - Common crane	H5N1 x 4
Wild - Rook	H5N1 x 1

AIV diagnostic methods in VSI Kraljevo

- RNA extraction on Kingfisher mL and Kingfisher Flex
- RT-qPCR on AriaMx devices
- Influenza A -Wise and Heine protocols
- H5 - Slomka et al., 2007
- H5 RT-qPCR Pathotyping (FLI protocol)
- H7 - Hassan et al., 2022
- H9 - Monne et al. 2008
- N1 - Payungporn et al., 2006
- Other subtypes - SOP VIR 1004 IZSV

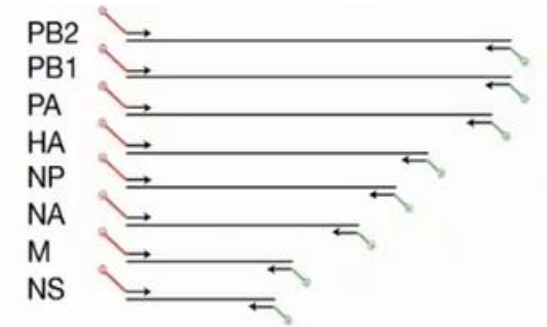
AIV diagnostic methods in VSI Kraljevo

- Isolation of the virus on embryonated chicken eggs
- Typing by conventional (using reference sera) or molecular methods (RT-PCR, NGS)
- Detection of antibodies
 - ELISA
 - HI Test

AIV WGS on Minion device

Primers

- MTBuni-12 5'-GCGTGATCAGCAAAGCAGG -3'
- MTBuni-12.4 5'-GCGTGATCAGCGAAAGCAGG -3'
- MBTUni-13 5'-ACGCGTGATCAGTAGAAACAAGG -3'



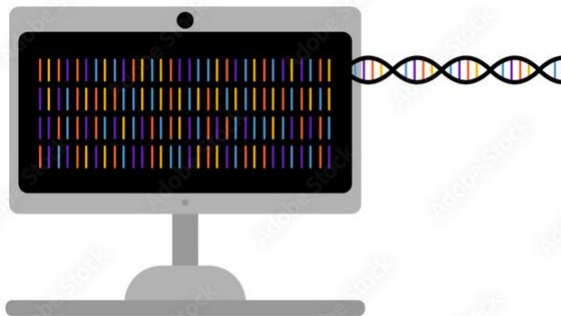
- SuperScript™ III One-Step RT-PCR System with Platinum™ Taq DNA Polymerase
- Ligation Sequencing Kit V14
- Native Barcoding Kit 24
- MinION Mk1B



AIV WGS on Minion device

Bioinformatic

- Basecalling - Guppy
- Assembly - Geneious prime
- Assembly - IZSV (Gianpiero Zamperin and bioinformatic team)



AIV WGS on Minion device

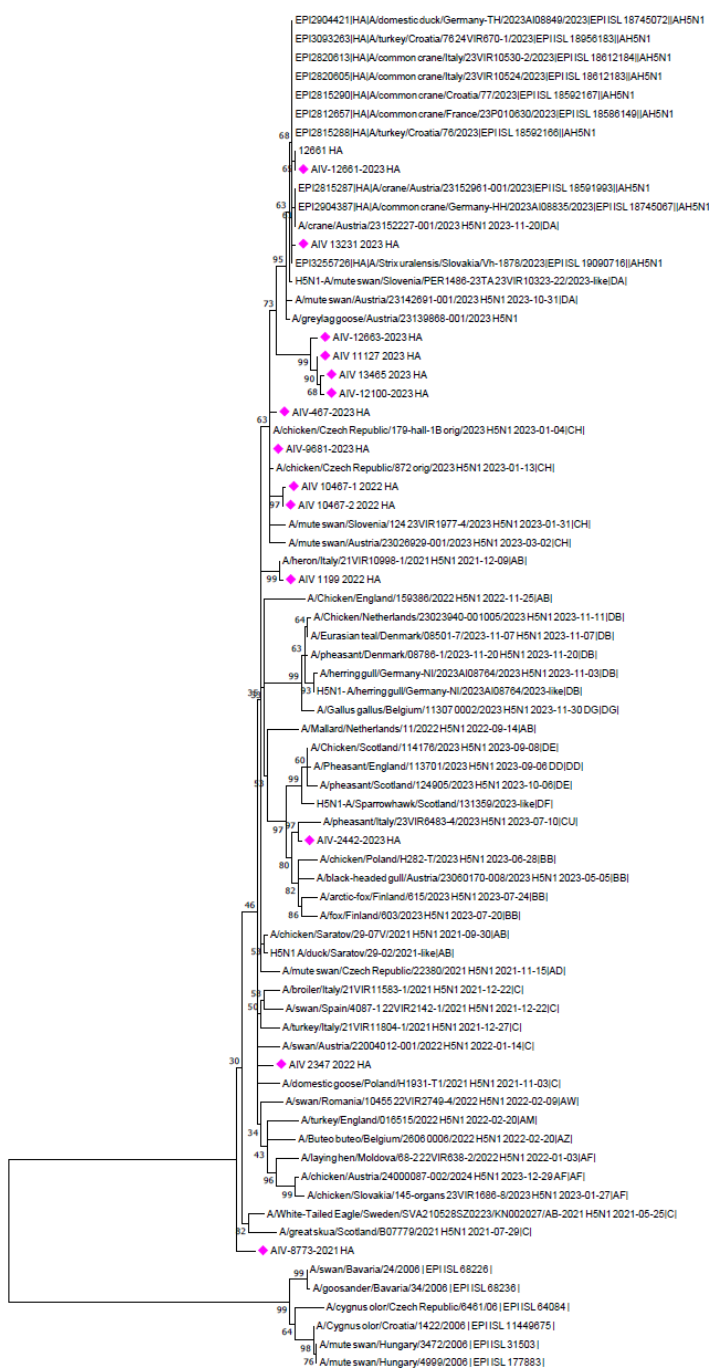
Pathotyping

- HPAI PLREKRRKR/GLF
- HPAI PLRGKRRKR/GLF

Genotyping

- Genotypes within clade 2.3.3.4b
- DA x 6, CH x 4, AB x 1, CU x 1, C x 2,
- 2022: CH, AB, CU and C
- 2023: DA and CH





Phylogenetic tree of 14 Serbian isolates of AIV H5N1 compared with standard panel of reference sequences (IZSV)

Pathotypes, genotypes and CS motifs

Sample ID	Pathotype	CS motif	Genotype	Species
A/mute swan/Serbia/8773/2021 H5N8	HPAI	PLREKRRKRGLF	C	Mute swan
A/white heron/Serbia/1199/2022 H5N1	HPAI	PLREKRRKRGLF	AB	White heron
A/black-headed gull/Serbia/2347/2022 H5N1	HPAI	PLREKRRKRGLF	C	Black-headed gull
A/mute swan/Serbia/10467-1/2022 H5N1	HPAI	PLREKRRKRGLF	CH	Mute swan
A/mute swan/Serbia/10467-2/2022 H5N1	HPAI	PLREKRRKRGLF	CH	Mute swan
A/mute swan/Serbia/11127/2023 H5N1	HPAI	PLREKRRKRGLF	DA	Mute swan
A/mute swan/Serbia/13231/2023 H5N1	HPAI	PLRGKRRKRGLF	DA	Mute swan
A/mute swan/Serbia/13465/2023 H5N1	HPAI	PLREKRRKRGLF	DA	Mute swan
A/greylag goose/Serbia/467/2023 H5N1	HPAI	PLREKRRKRGLF	CH	Greylag goose
A/black-headed gull/Serbia/2442/2023 H5N1	HPAI	PLREKRRKRGLF	CU	Black-headed gull
A/mute swan/Serbia/9681/2023 H5N1	HPAI	PLREKRRKRGLF	CH	Mute swan
A/mute swan/Serbia/12100/2023 H5N1	HPAI	PLREKRRKRGLF	DA	Mute swan
A/common crane/Serbia/12661/2023 H5N1	HPAI	PLRGKRRKRGLF	DA	Common crane
A/mute swan/Serbia/12663/2023 H5N1	HPAI	PLREKRRKRGLF	DA	Mute swan

Mutation analysis using FLUMUT tool

AIV_1199_2022	Marker	Mutations in your sample	Effect	Subtype
	HA1-5:K218Q,HA1-5:S223R	HA1-5:K218Q,HA1-5:S223R	Increased virus binding to α 2-6	H5N1
	HA1-5:S154N	HA1-5:S154N	Increased virus binding to α 2-6	H5N1
	HA1-5:T156A	HA1-5:T156A	Increased virus binding to α 2-6	H5N1
	PA:N383D	PA:N383D	Increased polymerase activity in mammalian cells	H5N1
	PB1:D3V	PB1:D3V	Increased polymerase activity in mammalian cells	H5N1
	PB1:D622G	PB1:D622G	Increased polymerase activity in mammalian cells	H5N1
	PB2:L89V,PB2:G309D	PB2:L89V,PB2:G309D	Increased polymerase activity in mammalian cells	H5N1
	PB2:L89V,PB2:G309D,PB2:T:PB2:L89V,PB2:G309D,PB2:T339K,PB2:R477G,PB2:I495V,PB2:K627E,PB2:A676T	PB2:L89V,PB2:G309D,PB2:T339K,PB2:R477G,PB2:I495V,PB2:K627E,PB2:A676T	Increased polymerase activity in mammalian cells	H5N1

UPCOMING ACTIVITIES

- Monitoring program will continue in 2024-2025
- WGS sequencing of isolates from 2021 and 2024 is ongoing
- Training of staff in bioinformatic

THANK YOU FOR YOUR ATTENTION

