IAVSur - BME

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Outcome of surveillance activities and summary of the national assessment across animal and public health and the environment on One Health approach (Year 1)

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1 Summary

The *Influenza A Virus Surveillance in Birds, Mammals, and the Environment* (IAVSur - BME) project is a three-year initiative under the EU4Health programme, aimed at strengthening the understanding of the ecology and zoonotic potential of influenza A viruses (IAVs) through One Health approach. In its first year, the project successfully established a national surveillance framework that integrates monitoring of wild mammals, environmental water bodies, and domestic pig populations.

Active and passive surveillance of wild mammals led to the detection of IAV RNA in one red fox (HPAI H5N1) and one wild boar (swIAV H1N1), with additional serological evidence of past infections in several species, including red foxes, martens, badgers, and wild boars. These findings support the hypothesis that a broad range of wild mammal species play a role in the IAV epidemiological cycle.

Environmental monitoring of two migratory wild bird habitats (Medvedce and Zbilje lakes) revealed the presence of IAV RNA, including subtype H5, particularly during autumn migration period. These results underscore the value of environmental matrices as early indicators of virus circulation. Identified PCR inhibitors suggest the need for methodological optimisation in future sampling rounds.

In the domestic pig component, comprehensive sampling and biosecurity protocols were developed, alongside veterinary network strengthening. Training and awareness activities for veterinarians specialising in pig health were carried out to enhance surveillance capacity. Although virological data from pig farms is still limited, baseline systems for data collection and sampling have been established.

2 Background

Emerging infectious diseases (EIDs), particularly those of zoonotic origin, pose a significant threat to public health, animal health and environmental sustainability. Approximately 75% of all EIDs and 60% of all human pathogens are of animal origin. The increasing frequency of interactions between wildlife, domestic animals and humans — driven by globalisation, climate change, land use change and biodiversity loss — has facilitated the emergence and spread of novel pathogens. Among these, influenza A viruses (IAVs) are of particular concern due to their high mutation rate, potential for genetic reassortment and broad host range.

Wild birds serve as a natural reservoir for IAVs and are often asymptomatic carriers. Spillover events from wild birds to domestic poultry and pigs can lead to serious economic consequences and in some cases to human infections with pandemic potential. Pigs are considered "mixed vessels" as they are susceptible to both avian and human influenza strains, which opens the possibility of reassortment and the emergence of new strains with zoonotic potential.

In response to these risks, the European Commission has launched the CP-g-22-04.01 initiative under the EU4Health programme, which aims to support Member States in setting up coordinated surveillance systems based on the One Health approach. The IAVSur - BME project was initiated within this framework to improve the surveillance of IAV in wildlife, domestic mammals (especially pigs) and environmental matrices to increase preparedness for zoonotic transmission and possible pandemics.

3 Introduction

The Influenza A Virus Surveillance in Birds, Mammals and the Environment (IAVSur - BME) project is a three-year initiative being implemented in Slovenia to improve the epidemiological understanding of IAV ecology within One Health approach. The project builds on the recognition that comprehensive and harmonised surveillance of IAVs in different reservoirs — including wild mammals, domestic pigs and the environment — is crucial to mitigate public health risks and ensure effective outbreak preparedness.

The main objectives of IAVSur - BME include:

- Active and passive surveillance of IAV in wild mammals to assess zoonotic risk.
- Environmental monitoring of water bodies frequently used by waterfowl to determine the persistence and circulation of the virus.
- Targeted surveillance of swine influenza virus (swIAV) in domestic pigs and wild boar with a focus on genomic characterisation and detection of zoonotic markers.
- Establishment of a real-time data network for the exchange of data between veterinary, environmental and public health services to enable rapid response and risk communication.

Through its multidisciplinary consortium and comprehensive surveillance approach, the IAVSur - BME project addresses critical gaps in current surveillance systems and supports EU-wide efforts to develop early warning mechanisms and cross-border cooperation on zoonotic disease threats.

This 2024 annual report of the IAVSur - BME project provides the outcome of surveillance activities and summary of the national assessment for the first year of the duration of the project. Part of the report is the 2024 surveillance report downloaded from the EFSA One Health dashboard (see Appendix 1).

4 Surveillance results

4.1 Investigation of influenza A viruses in wild mammals (WP2)

Prior to the start of surveillance, we established a protocol for surveillance of influenza A virus (IAV) infections in mammals. The protocol was based on two important references: Guidelines and Minimum Requirements for the Diagnosis of H5Nx HPAI Infections in Mammals (EURL for AI/NCD IZSVe, 2023) and Practical Guide for Authorized Field Responders to HPAI Outbreaks in Marine Mammals (WOAH, 2024).

For IAV virological surveillance, we collected samples from mammals found dead or road-killed, including:

- red foxes (planned number: 200),
- wild boar (planned number: 150),
- other carnivorous and omnivorous mammals (planned number: 80), such as brown bear (*Ursus arctos*), grey wolf (*Canis lupus*), golden jackal (*Canis aureus*), Eurasian lynx (*Lynx lynx*), wild cat (*Felis silvestris*), otter (*Lutra lutra*), beech marten (*Martes foina*), pine marten (*Martes martes*), European polecat (*Mustela putorius*), pygmy weasel (*Mustela nivalis*), stoat (*Mustela erminea*) and European badger (*Meles meles*).

Virological samples were taken from each individual animal, consisting of:

- swabs from the upper respiratory tract (nasal mucosa, oropharynx and trachea),
- swabs from the lower respiratory tract (several lung sections),
- brain swabs.

For serological monitoring, we took samples of the same animal species. In the case of wild boar, blood sera were obtained from shot animals. We collected:

- blood clots and body cavity fluids,
- several tissue samples (lung, heart or diaphragm) to obtain meat juice.

Swabs from each individual animal were pooled prior to virological testing; the original samples were archived for possible further testing or virus isolation. Total RNA was extracted using the QIAamp Viral RNA Kit (Qiagen, Germany). RT-qPCR was performed according to EURL recommended protocols for the detection of IAV subtypes in birds (Spackman et al., 2002) and WOAH recommended methods for the detection of swIAV.

Samples that tested positive for IAV were further subtyped using RT-qPCR assays (Hassan et al., 2022; Slomka et al., 2007b (HA2)).

For the detection of antibodies against IAV in sera and meat juice, we used the commercial ELISA kits ID Screen® Influenza A Antibody Competition Multi-species and ID Screen® Influenza H5 Antibody Competition 3.0 Multi-species.

ELISA-positive samples were then tested with the haemagglutination inhibition test (HIT) to determine antibodies against subtypes H5, H7, H1 and H3.

4.1.1 Surveillance results

In 2024, we sampled and analysed the following animals: 123 red foxes, 135 wild boars, 21 golden jackals, 13 martens, 46 badgers, 3 bears, 3 otters and 2 polecats (Figure 1).

Viral RNA was detected in one red fox and one wild boar. Subtyping was confirmed: HPAI H5N1 clade 2.3.4.4, genotype EA-2024-DI in the fox and swIAV H1N1 in the wild boar.

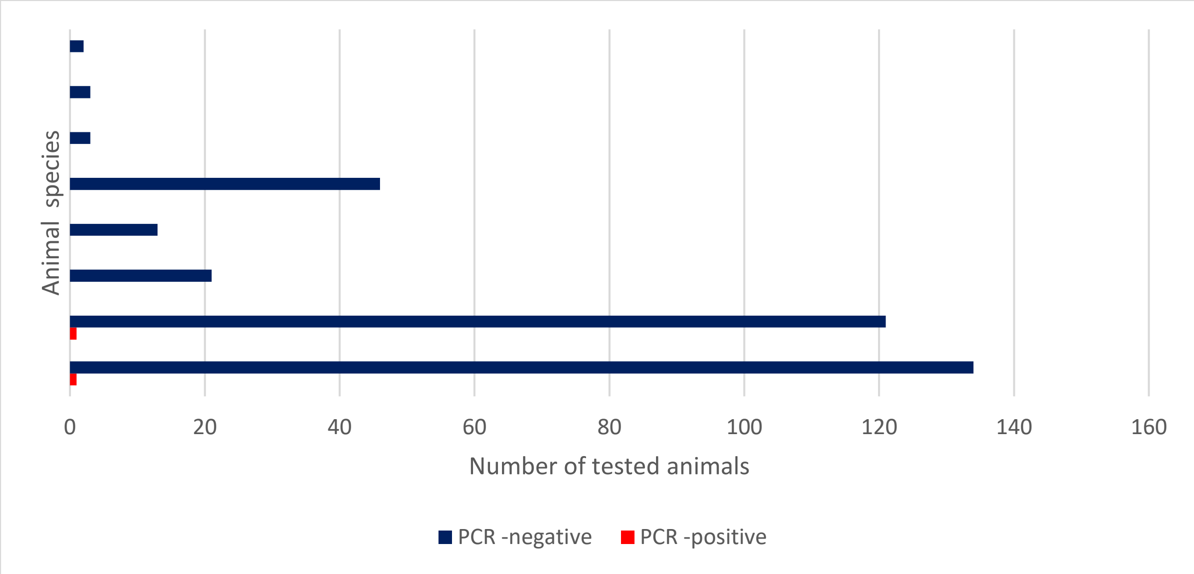


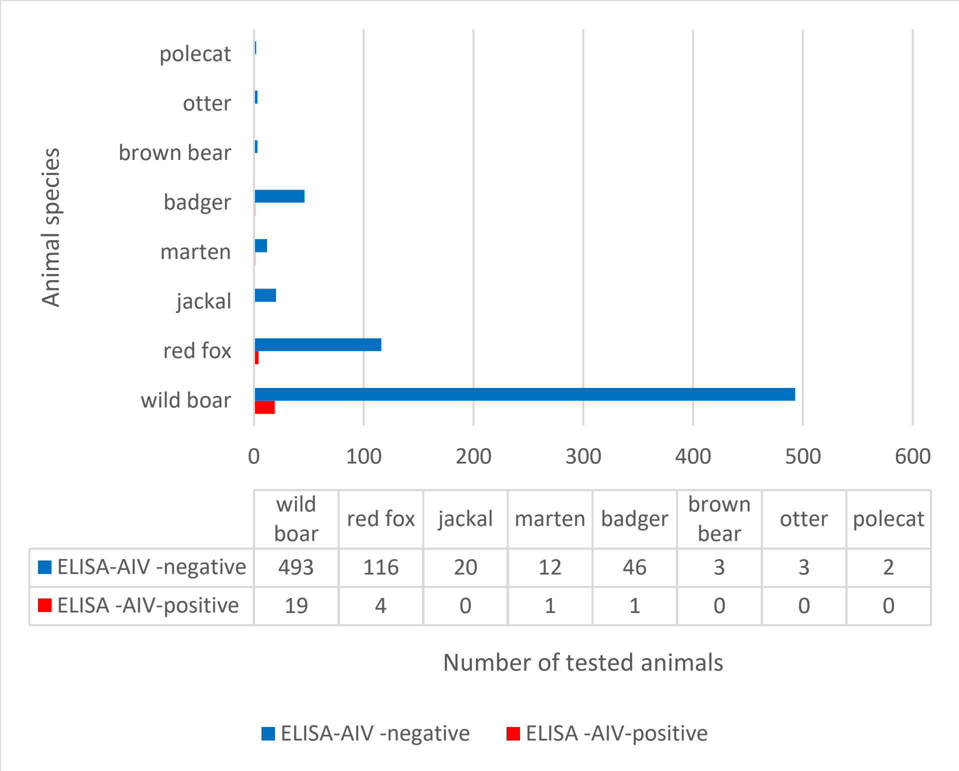
Figure 1: Influenza A virus surveillance in wild mammals: Virological findings from field surveillance in 2024.

HPAI H5N1 was successfully isolated using embryonated chicken eggs; the isolate was sent to the EURL for genotyping and whole genome sequencing. The sequence (A/red-fox/Slovenia/PER2154PA-2024_25VIR818-11/2024) was submitted to the GISAID database. The isolation of H1N1 from wild boar was not successful and genome sequencing is still ongoing. All other samples tested were IAV negative.

Serological tests were performed on serum or meat juice samples or both from 122 red foxes, 21 jackals, 13 martens, 46 badgers, 3 bears, 3 otters, 2 polecats and 25 wild boars found dead or shot with clinical signs (Figure 2).

In addition, 471 sera from shot healthy wild boars were tested.

Using ELISA screening, IAV antibodies were detected in four red foxes, one marten, one badger, and 19 wild boars. Further subtype-specific testing with the H5 ELISA revealed H5 antibodies in one red fox, one marten, and one wild boar. Antibodies against the H1 subtype were detected in one wild boar using the hemagglutination inhibition test (HIT). Subtyping of the remaining 22 samples was not successful. When comparing sample types (blood vs. meat juice), both sample types were positive in 62% of cases (5/8 positive animals). Meat juice samples were positive in 75% (6/8), while blood samples were positive in 87.5% (7/8 positive animals).



	wild boar	red fox	jackal	marten	badger	brown bear	otter	polecat
ELISA-AIV -negative	493	116	20	12	46	3	3	2
ELISA -AIV-positive	19	4	0	1	1	0	0	0

Figure 2: Influenza A virus surveillance in wild mammals: Serological findings from the 2024 field surveillance

4.1.2 Conclusion

Based on the results of the first year of surveillance, we conclude that our initial hypothesis regarding the relevance of testing a wider range of mammalian species for the IAV virus is well founded. Influenza A virus was detected in 0.6% of all virologically tested animals, including one red fox that tested positive for HPAI H5N1. As expected, the proportion of seropositive animals was higher at 3,5% of the ELISA-positive samples, 12% were found to carry antibodies against the H5 subtype.

4.2 Environmental monitoring (WP3)

Pilot environmental monitoring began in July 2024 and will continue until 2026 in two Slovenian reservoirs with a high diversity of migratory wild birds: Zbilje lake, an artificial reservoir on the Sava River, which is mainly visited by swans and ducks and where human-wild bird contact is frequent, and the Medvedce reservoir, the third largest water body in the Pannonian part of Slovenia. There is no large human presence here, but a greater diversity of migratory wild birds. After comparing different methods for sampling, virus concentration and detection using water samples spiked with an inactivated IAV suspension, we decided on the following procedure: on-site sampling and concentration of 50-70 litres of water using large volume concentrator ultrafiltration units (Innovaprep) > additional concentration in the laboratory using concentration pippets (Innovaprep) > RNA/DNA extraction on a magnetic basis using a Maxwell EnviroTNA kit (Promega) > and RT-qPCR detection using two qPCR assays, one for universal detection of Influenza A sequences and another specific for the Eurasian H5 type, both approved by the Italian European Reference Laboratory, using luciferase control RNA as internal amplification control.

4.2.1 Monitoring results

The results of the environmental water monitoring for 2024 (July to December - 24 samples in total) are shown in Table 1. 13 of the 24 samples analysed so far gave positive results for EURL - Uni (Influenza A), and 6 of these 13 samples were also positive for the H5 test. Three sediment samples from the Medvedce site were positive for Influenza A, and one of these three samples was positive for H5. Most of the H5 positives occurred in the months of October to December, when H5N1 findings were also reported during the mandatory surveillance of wild birds. A water sample from August 2024 was positive for H5 (in droplet Digital PCR) even no positive findings were reported during the mandatory wild bird monitoring on these dates.

We observed a clear inhibition in concentrated samples, as indicated by the results of our internal amplification luciferase control. The inhibitors are most likely concentrated together with the viruses in the concentration step. If inhibition is observed in subsequent monitoring, this may lead to a greater number of positive samples.

Table 1: Results of water monitoring for AIV in Medvedce and Zbilje in 2024 (July to December) and sediment monitoring in Medvedce. Results are given as RT-qPCR quantification cycle (Cq), mean of three measurements. For some samples, digital droplet PCR (ddPCR) was performed to confirm the results. "und" means indeterminate (negative results in the RT-qPCR reaction). For the sampling dates/locations shaded in orange, we confirmed the presence of Influenza A, while for the samples labelled in pink, we additionally confirmed the H5 subtype. Red colour indicates positive results.

Date	Location	FraCqion	Sample ID	EURL_UNI	EURL_H5	ddPCR		Sedimemt	
				Cq Mean	Cq Mean	Eurl Uni	Eurl H5	Eurl Uni	Eurl H5
10.07.2024	Zbilje	RAW	W001-R	und	und				
			W001-R 10x	und	und				
		LVC	W001-L	und	und				
			W001-L 10x	und	und				
		CP	W001-E	und	und				
			W001-E 10x	und	und				
11.07.2024	Medvedce	RAW	W002-R	und	und			Neg	Neg
			W002-R 10x	und	und				
		LVC	W002-L	und	und				
			W002-L 10x	und	und				
		CP	W002-E	und	und				
			W002-E 10x	und	und				
31.7.2024	Zbilje	RAW	W004-R	und	und				
			W004-R 10x	und	und				
		LVC	W004-L	und	und				
			W004-L 10x	und	und				
		CP	W004-E	und	und				
			W004-E 10x	und	und				
2.08.2024	Medvedce	RAW	W005-R	und	und			Neg	Neg
			W005-R 10x	und	und				
		LVC	W005-L	39.26	und				
			W005-L 10x	37.34	und	YES	YES		
		CP	W005-E	37.32	und				
			W005-E 10x	37.22	und				
14.08.2024	Zbilje	RAW	W007-R	und	und				
			W007-R 10x	und	und				
		LVC	W007-L	37.82	und				

			W007-L 10x	und	und				
		CP	W007-E	und	und				
			W007-E 10x	38.59	und				
15.08.2024	Medvedce	RAW	W008-R	und	und			Poz	Neg
			W008-R 10x	und	und				
		LVC	W008-L	38.37	und				
			W008-L 10x	und	und				
		CP	W008-E	und	und				
			W008-E 10x	und	und				
29.08.2024	Zbilje	RAW	W010-R	und	und				
			W010-R 10x	und	und				
		LVC	W010-L	37.61	und				
			W010-L 10x	37.94	und				
		CP	W010-E	39.90	und	YES	NO		
			W010-E 10x	38.08	und	YES	NO		
30.08.2024	Medvedce	RAW	W011-R	und	und			Neg	Neg
			W011-R 10x	und	und				
		LVC	W011-L	und	und				
			W011-L 10x	und	und				
		CP	W011-E	und	und				
			W011-E 10x	und	und				
10.09.2024	Zbilje	RAW	W013-R	und	und				
			W013-R 10x	und	und				
		LVC*	W013-C	38.37	und				
			W013-C 10x	und	und				
		CP	W013-E	und	und				
			W013-E 10x	und	und				
11.09.2024	Medvedce	RAW	W014-R	und	und			Neg	Neg
			W014-R 10x	und	und				
		LVC*	W014-C	und	und				
			W014-C 10x	und	und				
		CP	W014-E	und	und				
			W014-E 10x	und	und				
25.09.2024	Zbilje	RAW	'W016-R	und	und				
			W016-R 10x	und	und				
		LVC*	'W016-C	und	und				
			'W016-C 10x	und	und				
		CP	'W016-E	und	und				
			W016-E 10x	und	und				
26.09.2024	Medvedce	RAW	W017-R	und	und			Neg	Neg
			W017-R 10x	und	und				
		LVC*	W017-C	und	und				
			W017-C 10x	und	und				
		CP	W017-E	und	und				
			W017-E 10x	und	und				
7.10.2024	Zbilje	RAW	W019-R	und	und				
			W019-R 10x	und	und				
		LVC*	W019-C	38.21	und				
			W019-C 10x	und	und				
		CP	W019-E	35.80	und				
			W019-E 10x	und	und				
9.10.2024	Medvedce	RAW	W020-R	und	und			Neg	Neg
			W020-R 10x	und	und				
		LVC*	W020-C	und	und				
			W020-C 10x	und	und				
		CP	W020-E	und	und				
			W020-E 10x	und	und				
22.10.2024	Zbilje	RAW	W022-R	und	und				
			W022-R 10x	und	und				
		LVC*	W022-C	35.56	36.75				
			W022-C 10x	36.74	und				
		CP	W022-E	32.26	36.57				
			W022-E 10x	34.87	und				
23.10.2024	Ptujsko jezero	RAW	W023-R	und	und				
			W023-R 10x	und	und				
		LVC*	W023-C	und	und				
			W023-C 10x	und	und				
		CP	W023-E	und	und				
			W023-E 10x	37.70	und				
24.10.2024	Medvedce	RAW	W024-R	und	und			Neg	Neg
			W024-R 10x	und	und				
		LVC*	W024-C	und	und				
			W024-C 10x	und	und				
		CP	W024-E	und	und				
			W024-E 10x	und	und				
6.11.2024	Zbilje	RAW	W026-R	und	und				

			'W026-R 10x	und	und				
		LVC	'W026-L	37.75	und				
			W026-L 10x	und	und				
		LVC*	W026-C	36.88	37.12				
			W026-C 10x	und	und				
		CP	W026-E	und	und				
			W026-E 10x	und	und				
8.11.2024	Medvedce	RAW	W027-R	und	und			Neg	Neg
			W027-R 10x	und	und				
		LVC	W027-L	und	und				
			W027-L 10x	und	und				
		LVC*	W027-C	und	und				
			W027-C 10x	und	und				
		CP	W027-E	und	und				
			W027-E 10x	und	und				
22.11.2024	Zbilje	RAW	W031-R	und	und				
			W031-R 10x	und	und				
		LVC	W031-L	34.18	36.20				
			W031-L 10x	39.30	37.00				
		LVC*	W031-C	39.73	36.79				
			W031-C 10x	und	und				
		CP	W031-E	37.06	35.98				
			W031-E 10x	und	35.98				
21.11.2024	Medvedce	RAW	'W029-R	und	und			Neg	Neg
			'W029-R 10x	und	und				
		LVC	'W029-L	und	und				
			W029-L 10x	und	37.34				
		LVC*	W029-C	38.85	und				
			W029-C 10x	und	und				
		CP	W029-E	und	und				
			W029-E 10x	und	und				
6.12.2024	Zbilje	RAW	W034-R	und	und				
			W034-R 10x	und	und				
		LVC	W034-L	und	und				
			W034-L 10x	und	und				
		LVC*	W034-C	und	und				
			W034-C 10x	und	und				
		CP	W034-E	39.54	und				
			W034-E 10x	und	und				
5.12.2024	Medvedce	RAW	W032-R	und	und			Poz	Poz
			W032-R 10x	und	und				
		LVC	W032-L	37.26	und				
			W032-L 10x	und	und				
		LVC*	W032-C	und	und				
			W032-C 10x	und	und				
		CP	W032-E	und	und				
			W032-E 10x	und	und				
23.12.2024	Zbilje	RAW	W035-R	und	und				
			W035-R 10x	und	und				
		LVC	W035-L	und	und				
			W035-L 10x	und	und				
		LVC*	W035-C	und	und				
			W035-C 10x	und	und				
		CP	W035-E	und	und				
			W035-E 10x	und	und				
24.12.2024	Medvedce	RAW	W036-R	und	und			Poz	Neg
			W036-R 10x	und	und				
		LVC	W036-L	und	und				
			W036-L 10x	und	und				
		LVC*	W036-C	und	und				
			W036-C 10x	und	und				
		CP	W036-E	und	und				
			W036-E 10x	und	und				

On the same dates and at the same locations as water sampling, the wild birds were counted and the structure of the wild bird population recorded (Figure 4). The absolute number of wild birds in Medvedce was higher and the species diversity was also greater, so it is probably more representative. As AIV monitoring by RT-qPCR is a qualitative method, with most positive results close to the limit of detection and affected by PCR inhibitors, it is not possible to establish a correlation at this stage. In Medvedce, there was an increase in the number of migratory wild birds in November (Figure 4), mainly due to the arrival of swans and greylag geese (data not shown), and we can only speculate that these migratory wild bird events may have had an impact on the positive results detected in environmental monitoring (and also in mandatory wild bird monitoring) during these months by favouring the spread of HP-AIV.

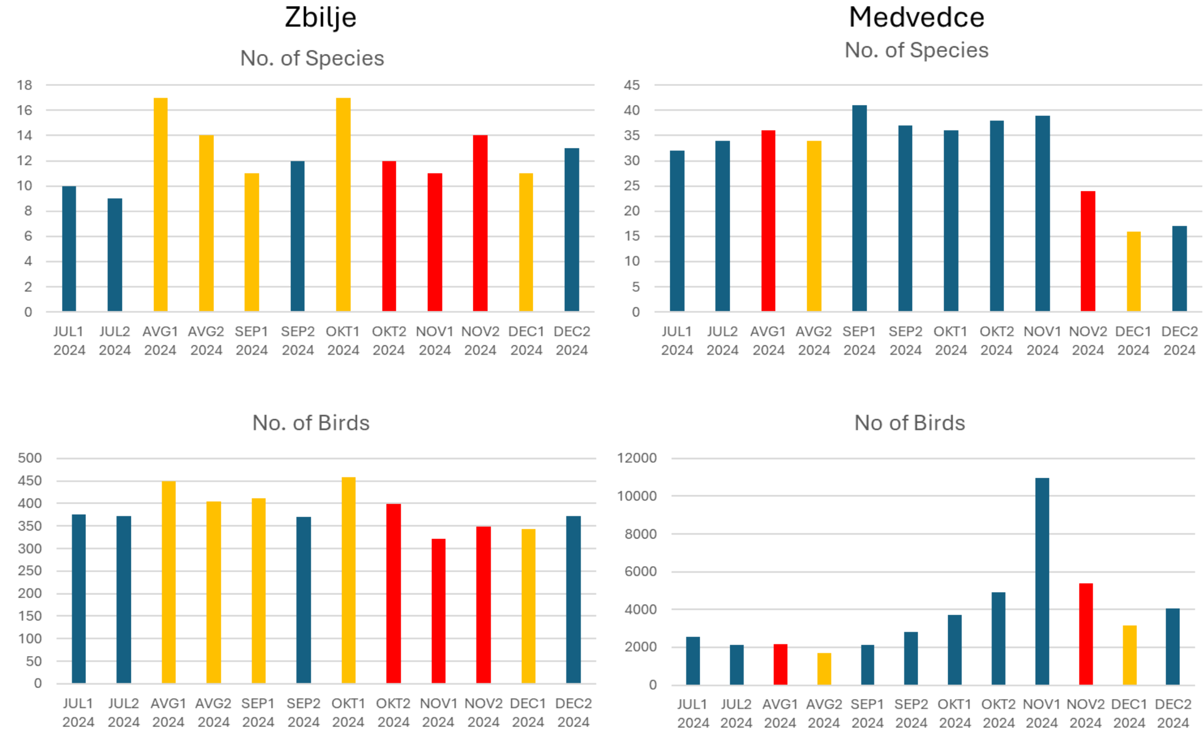


Figure 4: Number of migratory wild bird species and absolute number of wild birds counted at the monitoring sites on the same sampling days of the AIV environmental survey. The samples that gave a positive FluA result in the water monitoring are marked in orange and the samples in which H5 was also confirmed are marked in red.

4.2.2 Conclusion

Environmental monitoring: The combination of RT-qPCR with Eurl Uni and H5 assays and concentration with LVC and CP proved to be a good approach for sensitive on-site processing of environmental water samples for AIV monitoring. Water samples from the Medvedce and Zbilje lakes showed positive results for Influenza A and H5 assays, the latter especially in the autumn months, which could be related to the dynamics of wild bird migration. Removing inhibitors could help to increase sensitivity in future sampling. RNA from positive samples is analysed by HTS to obtain more information about the circulating AIV strains.

4.3 AIV in domestic pigs (SIV) (WP4)

As part of Task 1 of WP4, we have developed a comprehensive protocol and established clear criteria for the collection of nasal swabs from pigs based on a review of the currently available scientific literature. The protocol describes the required number of nasal swabs depending on the size of the pig farm and specifies the appropriate type of swabs to be used for sampling. It also includes criteria for selecting the category of pig to be sampled, describes the correct method of swab collection and provides detailed instructions for the proper storage and transport of samples to the laboratory to ensure their integrity.

In addition to the protocol for the collection of nasal swabs, we have also created specific guidelines for the collection of environmental swabs and air samples. These complementary procedures are designed to provide a more comprehensive picture of the potential presence and spread of the virus in the farm environment.

To support the sampling process, we have created a questionnaire to be completed by the farmer during the sampling visit. This questionnaire collects relevant data about the farm, the pig herd and the clinical signs observed, which can then be linked to the laboratory findings to enable more robust data analysis.

As part of Task 2, which focuses on building a network of veterinarians, we organized a training session in collaboration with the Chamber of Veterinarians specifically aimed at veterinarians specializing in pig health. During this training, we gave a presentation in which we introduced the objectives and activities of the project. We also provided a refresher course on swine flu, covering the characteristics of the virus, clinical signs in pigs and appropriate countermeasures.

To support the development of the veterinary network, we collected contact details from participants during the training, including telephone numbers, email addresses and the names of their veterinary practices. This contact database will facilitate ongoing communication and coordination for future sampling as part of the project.

In 2024 one farm was visited, and nasal swabs were taken.

Testing was conducted using real-time RT-PCR assays targeting the influenza A matrix (M) gene following protocol recommended by the World Organisation for Animal Health (WOAH).

A total of 10 nasal samples originating from farm reporting respiratory symptoms were analysed during the first year. All samples tested negative for swIAV RNA.

4.3.1 Conclusion

Within WP4, we established a standardized framework for swIAV surveillance in domestic pigs, including validated protocols for nasal, environmental, and air sampling, as well as a farm questionnaire to support epidemiological analysis. A veterinary network was initiated through targeted training in collaboration with the Chamber of Veterinarians, creating a foundation for coordinated future sampling.

In 2024, one farm was visited and 10 nasal swabs from pigs with respiratory signs were tested using WOAH-recommended real-time RT-PCR targeting the Influenza A matrix gene. All samples were negative for swIAV RNA.

The established infrastructure and network provide a solid basis for expanded surveillance in the next project phase.

5 National assessment on One Health approach

5.1 An assessment of the situation for IAV during 2024

The first year of the IAVSur - BME project has provided important insights into the ecology and zoonotic potential of influenza A viruses (IAV) in wild mammals and in water bodies in Slovenia. The virological and serological detection of IAV in red foxes and wild boars confirms that terrestrial mammals are actively involved in the epidemiological cycle of IAV. At the same time, environmental surveillance detected RNA of IAV, including subtype H5, in water samples from the habitat of migratory wild birds, demonstrating viral persistence in aquatic ecosystems. As part of the mandatory AI surveillance of wild birds and poultry in 2024, HPAI H5N1 was confirmed in Slovenia between February 2024 and April 2024 and again from 10 October to the end of 2024. According to the preliminary results, the virological detection of the H5 subtype in the environment and in mammals was in line with the occurrence of HPAI H5-positive wild birds detected in Slovenia in various locations, as expected. Although the overall detection rate remains low, the isolation of HPAI H5N1 (clade 2.3.4.4b) in a red fox and the presence of swIAV in a wild boar emphasise the ongoing risk of zoonotic spillover events. Although these events are rare, they can have serious consequences, especially in the context of viral reassortment in pigs and subsequent human exposure. Currently, interest among local veterinarians and pig owners in the diagnosis of swIAV is minimal, only one farm was tested. Efforts to inform local veterinarians about project activities and to emphasize the importance of swine influenza have not generated the expected field response in 2024. From a One Health perspective, regular surveillance of pigs as potential “mixing vessels” remains essential. A key objective of the project is to raise awareness among both professionals and the general public regarding the importance of monitoring this disease.

5.2 One Health Approach

Before the start of the project, collaboration already existed between the public health and animal health sectors, structured similarly to the current project consortium, except for the environmental sector, which was not included in the active collaboration. This cooperation primarily involved the regular exchange of information on positive zoonotic cases. In addition, an annual symposium on selected topics brings together all consortium representatives. In the past, a multidisciplinary National Zoonoses Commission was also active.

As part of the IAVSur-BME project, a meeting of partners was organized in 2024, during which it was decided to reactivate the National Zoonoses Commission. Actively bringing together experts from different disciplines is essential to exchange information on diagnostics, disease status, and to develop effective action plans in the event of disease outbreaks.

The first year of the IAVSur - BME project has demonstrated the feasibility and value of integrated IAV surveillance within One Health. Although IAVs have been detected relatively rarely, the confirmed occurrence of zoonotic strains in mammals and in the environment justifies the continuation and extension of surveillance activities. This integrated approach is crucial for early warning, preparedness and prevention of future zoonotic outbreaks and potential pandemics.

6 Risk Evaluation

Risk Domain	Observation	Risk Level	Mitigation Strategy
Wildlife IAV	Sporadic HPAI H5N1 in red fox, serological exposure in multiple species	Moderate	Continue active/passive surveillance; monitor areas with known outbreaks
Environmental IAV	Positive Influenza A and H5 detections, especially during migratory periods	Moderate	Enhance environmental sampling coverage; address PCR inhibition factors
Swine (swIAV)	Detection of H1N1 in wild boar, limited farm data so far	Low	Expand veterinary network, increase on-farm sampling, integrate genomic data
Zoonotic transmission	No human cases detected; virus with zoonotic potential isolated	Low	Strengthen communication with public health sector; simulate response scenarios

7 References

1. European Union Reference Laboratory (EURL) for Avian Influenza and Newcastle Disease Diagnostic protocols: Guidelines and Minimum Requirements for the Diagnosis of H5Nx HPAI Infections in Mammals (available at: https://www.izsvenezie.com/documents//reference-laboratories/avian-influenza/diagnostic-protocols/guidelines-diagnosis-h5nx-mammals.pdf)
2. Slomka MJ, Pavlidis T, Banks J, Shell W, McNally A, Essen S, Brown IH. 2007. Validated H5 Eurasian Real-Time Reverse Transcriptase–Polymerase Chain Reaction and Its Application in H5N1 Outbreaks in 2005–2006. Avian Dis. 51(1):373-377
3. Spackman E, Senne DA, Myers TJ, Bulaga LL, Garber LP, Perdue ML, Lohman K, Daum LT, Suarez DL. 2002. Development of a real-time reverse transcriptase PCR assay for type A influenza virus and the avian H5 and H7 hemagglutinin subtypes. J Clin Microbiol. 40(9):3256–3260.
4. WOAH. Chapter 3.9.3: Influenza A viruses of swine (2023)
5. WOAH. Practical Guide for Authorized Field Responders to HPAI Outbreaks in Marine Mammals (2024).

Appendix 1

Surveillance data submitted under the EU co-funded project EU4H-2022-DGA-MS-IBA3 (CP-g-22-04.01) by Slovenia

Grant agreement number: 101132734

Period covered: 3/1/2024 - 31/12/2024

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CONTENTS

INTRODUCTION

In the context of the EU co-funded grant agreements* (hereby called "projects") in the field of One Health surveillance for emerging and re-emerging zoonotic pathogens under the EU4Health Programme (EU4H) 2022, that support coordinated surveillance systems for cross-border pathogens that threaten the Union, awarded countries perform surveillance activities on a selection of priority pathogens.

The results related to these surveillance activities are submitted to EFSA for further analysis and visualization.

The objective of this report is to provide an overview of the data reported under the EU co-funded One Health Surveillance project in Slovenia, covering the distribution of samples collected and laboratory tests performed over time by pathogen, the host and the type of laboratory tests.

This report was automatically generated based on the data linked to results reported by Slovenia from 3/1/2024 to 31/12/2024.

*Call reference: EU4H-2022-DGA-MS-IBA3 (CP-g-22-04.01); Direct grants to Member States' authorities: setting up a coordinated surveillance system under the One Health approach for cross-border pathogens that threaten the Union.

DATA SUBMISSION

RESULTS PER SURVEILLANCE COMPONENT

Slovenia operated 1 surveillance components during the period of 3/1/2024 to 31/12/2024 . For each of the surveillance components, the number of collected samples and laboratory test results as well as the number of sampling events for arthropod vectors reported by Slovenia are shown in table 1.

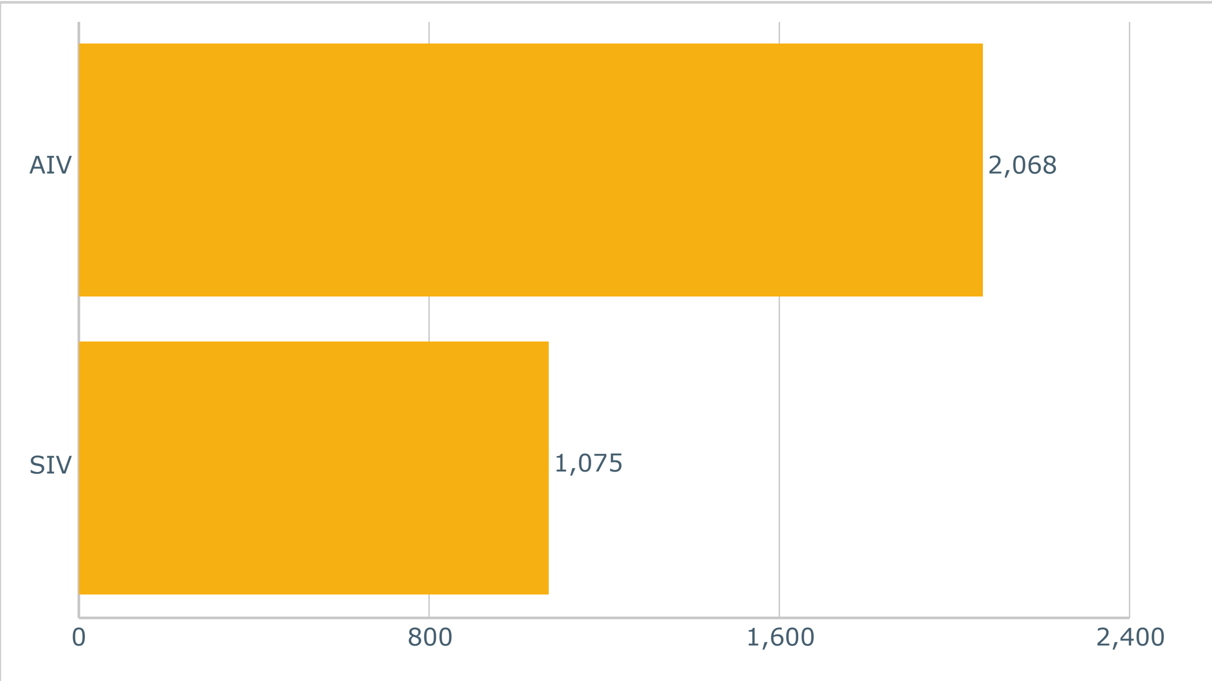
Table 1: Number of collected samples, laboratory test results and sampling events for arthropod vectors reported by Slovenia for the period of 3/1/2024 to 31/12/2024 per surveillance component.

Surveillance component ID	Surveillance component description	N sampling events	N samples	N tests
N_A	Not Available	852	1,765	3,143
Total		852	1,765	3,143

RESULTS PER PATHOGEN AND MATRIX

Slovenia submitted 3,143 laboratory test results for samples collected during the period of 3/1/2024 to 31/12/2024 to EFSA. The number of laboratory test results submitted per priority pathogen by Slovenia is shown in Figure 1.

Figure 1: Number of laboratory tests results submitted by Slovenia for samples collected between 3/1/2024 to 31/12/2024.



RESULTS PER PATHOGEN AND MATRIX DETAIL

In the context of the One Health surveillance project, different types of samples ('matrix') were analyzed, including samples from farmed animals, wild animals, vectors and environmental samples (either collected from nature or from a farm). The number of samples analyzed and the number of laboratory test results by pathogen and matrix are presented in table 2. Note that a sample can be analyzed by more than one laboratory test, hence, there can be more laboratory test results than samples.

Table 2: Number of samples and laboratory test results submitted for samples collected between 3/1/2024 to 31/12/2024 per priority pathogen and sample type.

Matrix	**Environment - Nature**		**Wild animal**		**Farmed animal**	
Pathogen	**N samples**	**N tests**	**N samples**	**N tests**	**N samples**	**N tests**
AIV	25	54	1,730	2,014		
SIV			1,043	1,065	10	10
Total	25	54	2,773	3,079	10	10

RESULTS PER TYPE OF LABORATORY TEST

Depending on the purpose of the surveillance, the samples obtained and the pathogen to be analyzed, the laboratory test(s) that are performed often vary. Table 3 includes an overview of the laboratory tests performed per priority pathogen over time by the type of laboratory test used for the analysis.

Table 3. Number of tests per priority pathogen and type of laboratory test used in the analysis submitted for samples collected between 3/1/2024 to 31/12/2024.

Pathogen	Year		Antigen detection	Antibody detection	Genomic analysis	Pathogen isolation
		Month	**N tests**	**N tests**	**N tests**	**N tests**
AIV	2024	Jan		45		
AIV	2024	Feb		30		
AIV	2024	Mar		40		
AIV	2024	Apr	30	46		
AIV	2024	May	69	76		
AIV	2024	Jun	42	39		
AIV	2024	Jul	84	67		
AIV	2024	Aug	113	102		
AIV	2024	Sep	182	88		
AIV	2024	Oct	178	108		
AIV	2024	Nov	161	135		
AIV	2024	Dec	242	189	1	1
SIV	2024	Apr	30			
SIV	2024	May	69			
SIV	2024	Jun	42			
SIV	2024	Jul	78	4		
SIV	2024	Aug	98	4		
SIV	2024	Sep	174	4		
SIV	2024	Oct	168	4		
SIV	2024	Nov	152	10		
SIV	2024	Dec	236	2		
Total			2,148	993	1	1