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OH SURVector:
One Health Surveillance and Vector Monitoring for
cross-border pathogens

D3.1: MAP OF SAMPLING LOCATIONS (MOSQUITOES)

WP3 – FIELD WORK MOSQUITO MONITORING

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Document Information

Grant Agreement Number	101132974	Acronym	OH SURVector	
Full Title	OH SURVector: One Health Surveillance and Vector Monitoring for cross-border pathogens			
EU4Health 2023 Call	EU4H-2022-DGA-MS-IBA3			
Topic	EU4H-2022-DGA-MS-IBA-05			
Type of Action	EU4H-PJG			
Start Date	01.01.2024	Duration (in months)	36	
Service	HADEO/A/01			
EU Project Officer	Marc VANDENBROECK and Alfonso ALIBERTI, HaDEA			
Deliverable	D3.1			
Work Package	WP3 – Field work mosquito monitoring			
Date of Delivery	Contractual	30.04.2024	Actual	30.04.2024
Nature	R – Document, report	Dissemination Level	PU – Public	
Lead Beneficiary	AGES – ÖSTERREICHISCHE AGENTUR FÜR GESUNDHEIT UND ERNÄHRUNGSSICHERHEIT GMBH			

Other contributors	SZU – STATNI ZDRAVOTNI USTAV
	NNGYK – NEMZETI NEPEGESZSEGUGYI ESGYOGYSZERESZETI KOZPONT
	UVLF – UNIVERZITA VETERINARSKEHO LEKARSTVA A FARMACIE V

Document History

Version	Date	Partner	Description
V0.1	27.04.2024	Karin Bakran-Lebl, AGES	First version
V0.2	29.04.2024	Annette Nigsch, AGES	Peer review
V0.3	29.04.2024	Karin Bakran-Lebl, AGES	Second version
V0.4	29.04.2024	Jiří Černý, SZU	Peer Review
V0.5	29.04.2024	János Sztikler, NNGYK	Peer Review
V0.6	29.04.2024	Tomáš Csank, UVLF	Peer Review
V0.7	30.04.2024	Karin Bakran-Lebl, AGES	Consolidated Version
V0.8	30.04.2024	Annette Nigsch, AGES	Quality Assurance
V1.0	30.04.2024	Annette Nigsch, AGES	Final version – Ready for submission

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LIST OF ACRONYMS

hrs – hours

WNF – West Nile fever

WNV – West Nile virus

1. EXECUTIVE SUMMARY

Deliverable D3.1: MAP OF SAMPLING LOCATIONS (MOSQUITOES) contains maps of all countries and a description of the methodology and site selection for mosquito collection. Mosquitoes will be collected with special mosquito traps (BG-sentinel, BG Pro, EVS), equipped with a CO₂ release (from gas bottle, dry ice, or yeast).

The investigation and collection of mosquitoes will target the transboundary spread of pathogens, mainly West Nile virus (WNV).

The selection of sites for the mosquito collection was made individually by each country. The main criteria for sampling sites selection were previous reports of WNV (in humans or animals), high population or animal host density and/or high mosquito density.

2. METHODOLOGY AND SITE SELECTION FOR MOSQUITO COLLECTION BY COUNTRY

2.1 AUSTRIA

In Austria, for the trapping of the adult female mosquitoes BG-sentinel traps (Biogents), equipped with an additional CO₂ release (CO₂ produced by yeast, BG Generator, Biogents) and a specific lure mimicking body scent (Biogents) will be used. Traps will be operated from May – October, for 24 hrs within the first and the third week of the month.

The traps will be distributed throughout the country, with at least 1 trap per NUTS3 unit. More traps will be set up in NUTS3 units with high population density and/or previous recordings of WNV infections (humans, horses, birds). In the year 2024, traps will be set up at 68 sites.

2.2 CZECH REPUBLIC

In the Czech Republic, mosquitoes will be collected using BG Pro traps (Biogents) and EVS traps, both types baited with CO₂ from dry ice. At the 10 selected sites 20 traps will be set up every two weeks for 24 hrs from May to November.

The traps will be distributed all over the country, selected sites include sites in Prague, sites with previous WNV infections (horses, birds), rural areas and post-industrial areas.

2.3 SLOVAKIA

Mosquito collection in Slovakia is conducted with BG-sentinel traps (Biogents), equipped with an additional CO₂ release from a CO₂ gas bottle (0.5g CO₂/day). The traps will operate continuously from June to September; catch nets will be changed twice per week.

The traps will be distributed all over the country, selected sites include areas where WNV has been reported previously (birds, mosquitoes), wetlands, and areas with a higher number of hosts (raptors in captivity, horses, donkeys, and domesticated ruminants). In the year 2024, traps will be set up at 14 sites.

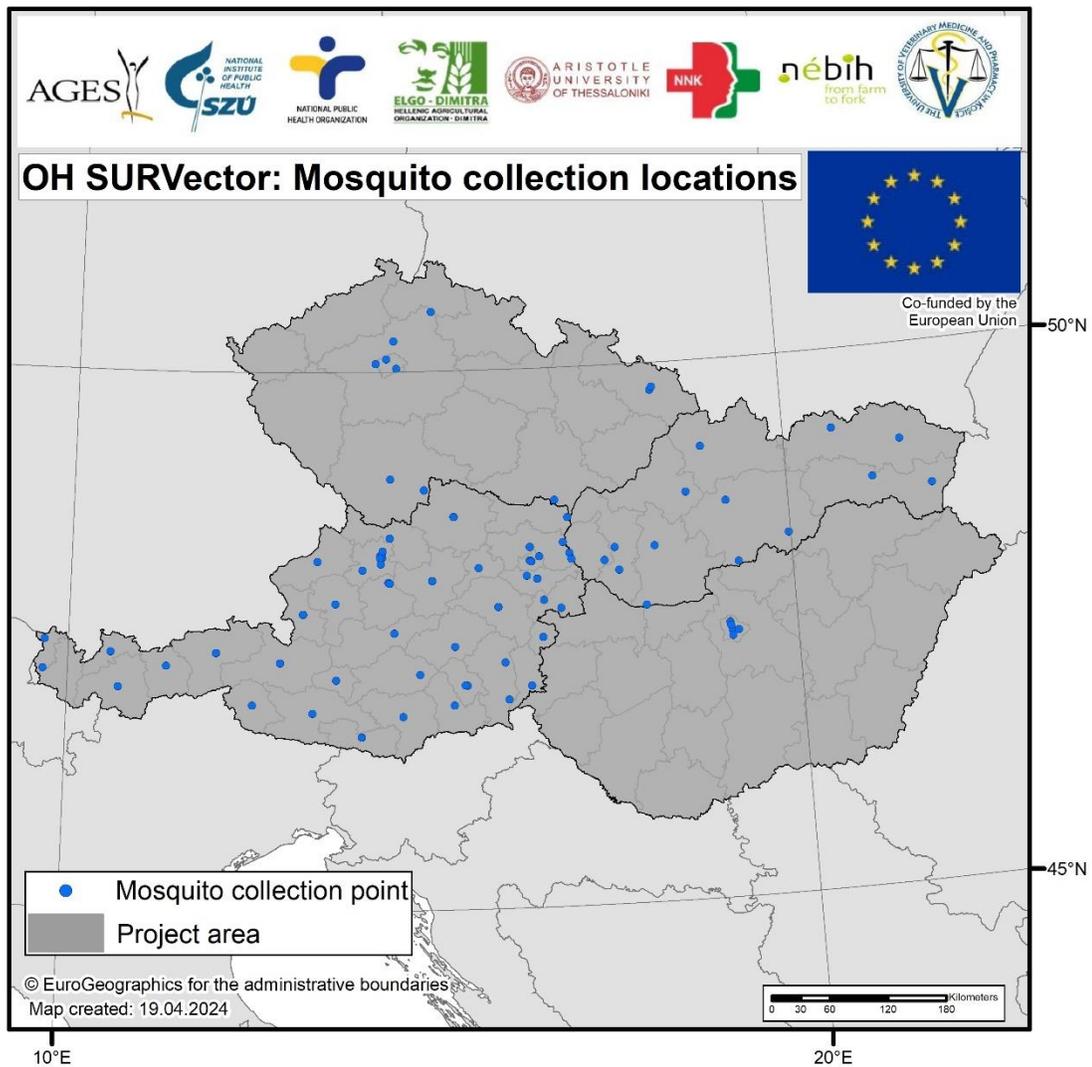
2.4 HUNGARY

In Hungary, for the trapping of the adult female mosquitoes BG-sentinel traps (Biogents), equipped with an additional CO₂ release (CO₂ gas bottle or CO₂ produced by yeast, BG Generator, Biogents). Traps will be operated for 24 hrs, twice per month, from May – October.

In a pilot study in 2024, traps will be set up at 5 sites in Budapest. Based on data of the pilot year and based on subsequent epidemiological situation the intensity of the study may be increased to cover more counties (NUTS3 level regions) of Hungary in 2025 and 2026.

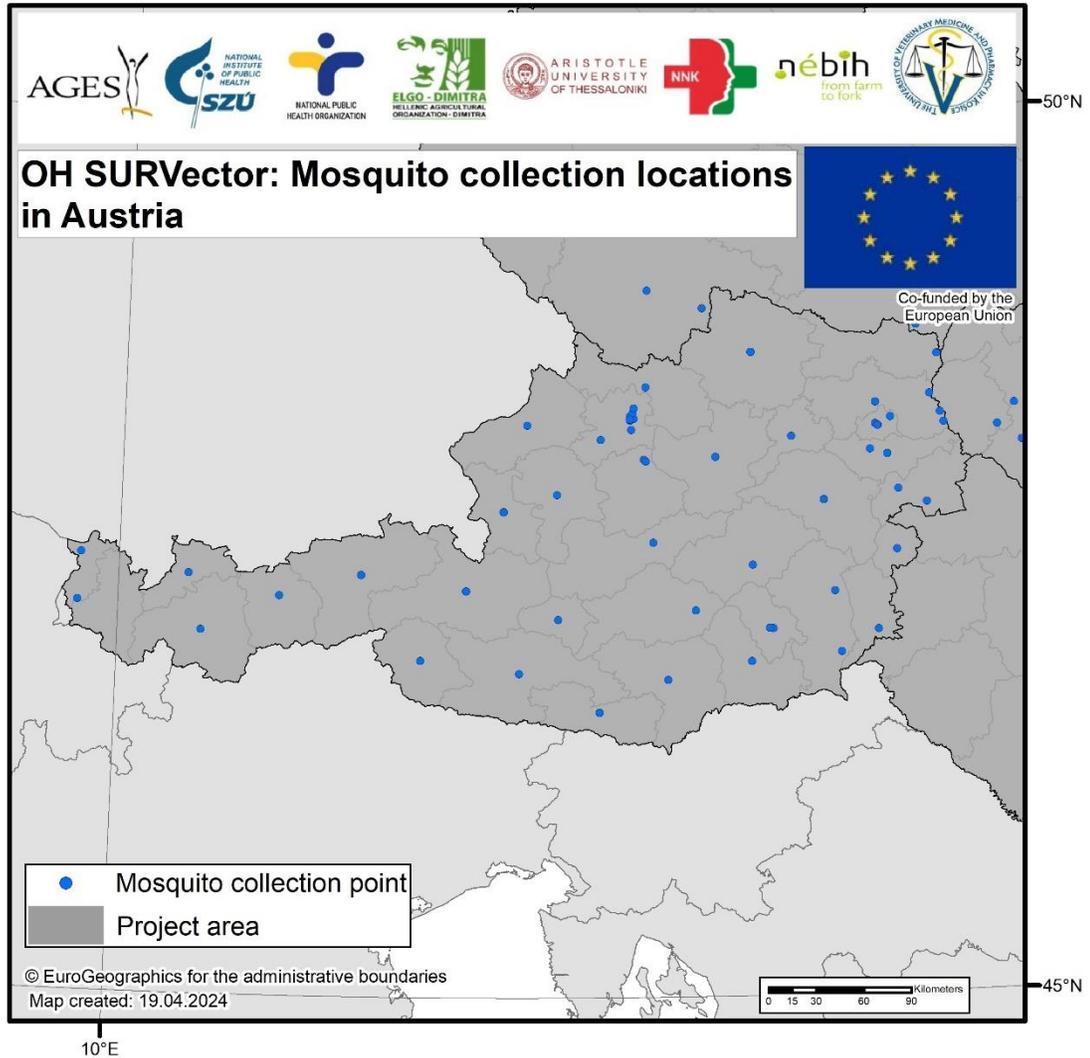
3. MAPS FOR SELECTING SITES FOR MOSQUITO COLLECTION BY COUNTRY

FIGURE 1: Mosquito sampling locations in all consortium countries.



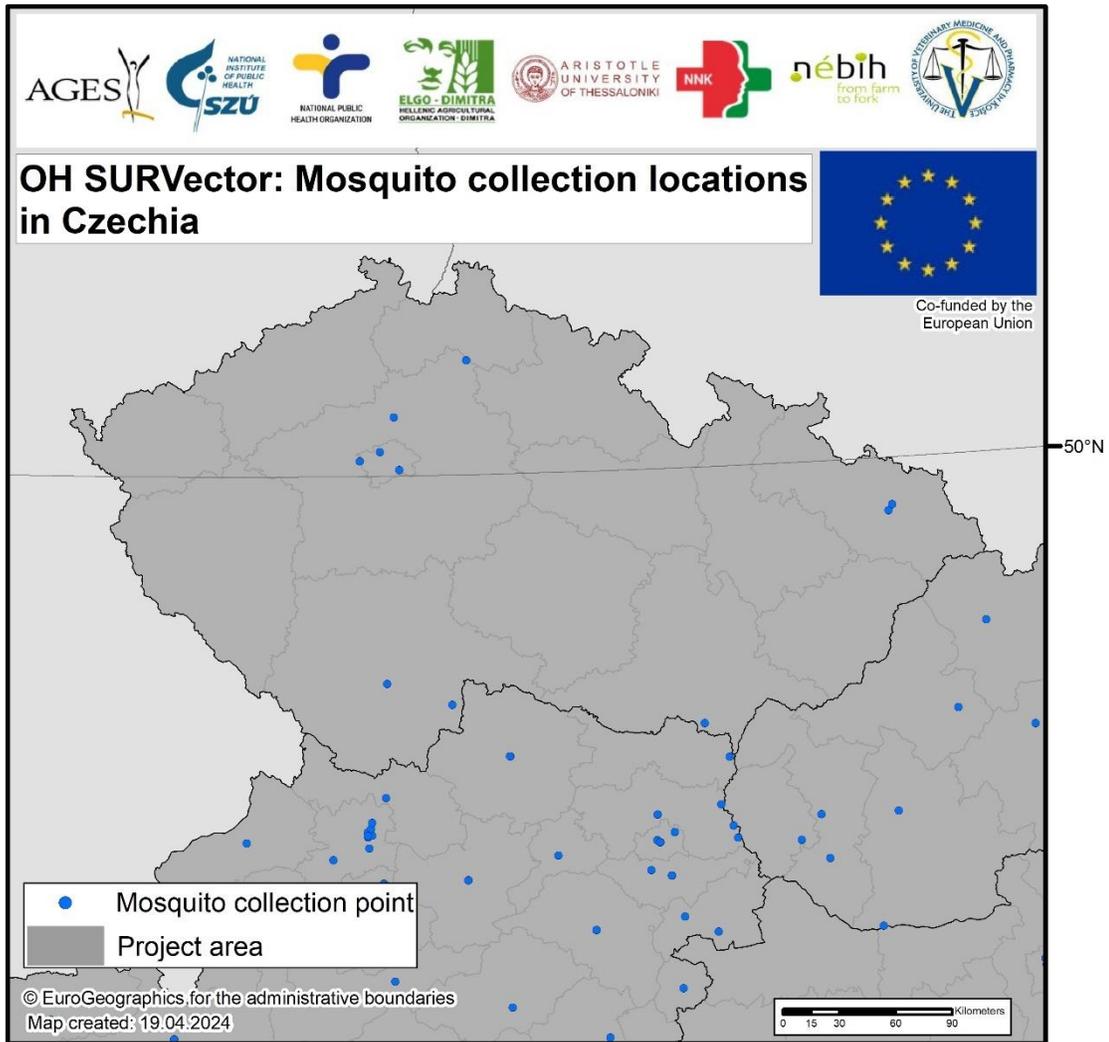
3.1 AUSTRIA

FIGURE 2: Mosquito sampling locations in Austria.



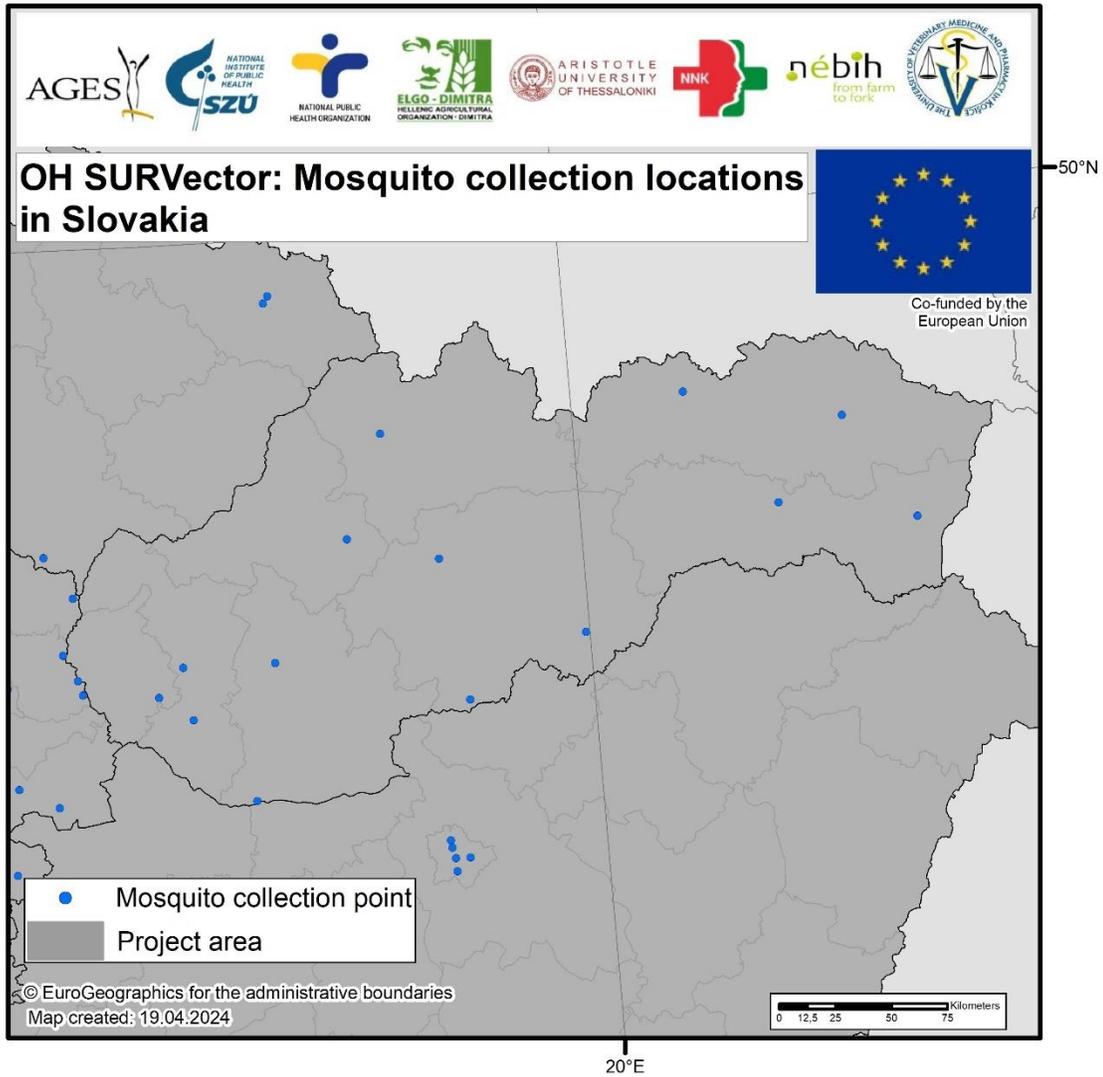
3.2 CZECH REPUBLIC

FIGURE 3: Mosquito sampling locations in the Czech Republic.



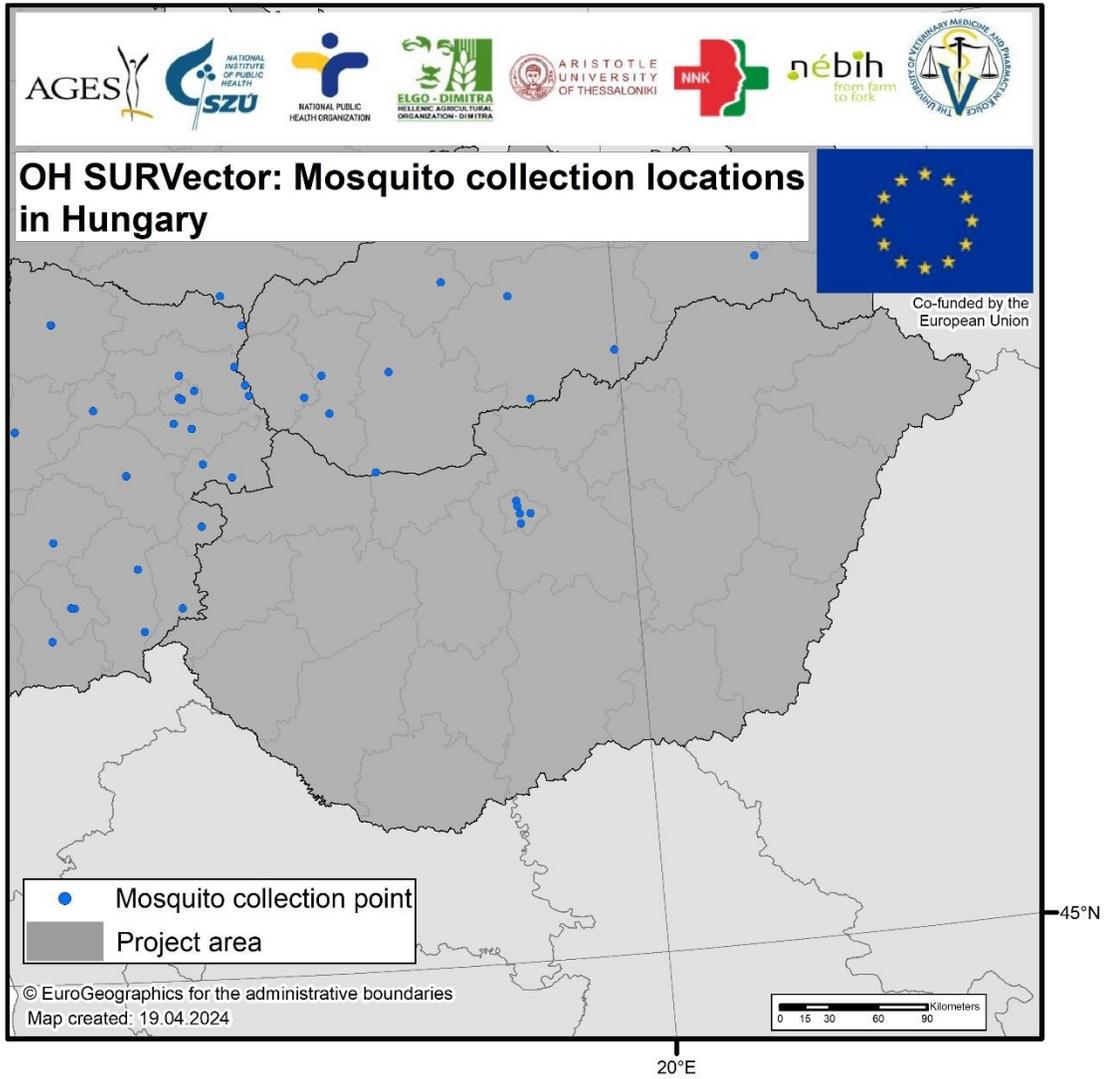
3.3 SLOVAKIA

FIGURE 4: Mosquito sampling locations in Slovakia.



3.4 HUNGARY

FIGURE 5: Mosquito sampling locations in Hungary.



4. SURVEILLANCE CARDS FOR SELECTING SITES FOR MOSQUITO COLLECTION BY COUNTRY

4.1 AUSTRIA

TABLE 1: Surveillance card for selecting sites for mosquito collection in Austria.

AUSTRIA: SURVEILLANCE CARD WNF Pathogen detection in mosquitoes in endemic regions

	Characteristics	Description
1	Surveillance component name	Pathogen detection in mosquitoes in endemic regions testing for West Nile Virus.
2	Surveillance aim	Early detection if prevalence increases; Early detection of a change of the geographic distribution; Early detection of an increase in incidence; monitor ongoing outbreaks; identify different kinds of mosquitoes that could spread the virus; when and how to act to reduce the risk of infection by applying mosquito control measures.
3	Target species and group	<i>Culex pipiens</i> ; <i>Culex modestus</i> ; <i>Culex torrentium</i> .
4	Target sector / production type	Not applicable.
5	Geographical area covered	Country-wide – focus on previously affected areas with suitable habitat for mosquitoes; <i>Culex pipiens</i> is found nationwide. (1)
6	Age group	Adult mosquitoes. (2)
7	Sampling point and strategy	<p>Areas with suitable habitat for vectors: to be preferred areas where adult female mosquitoes lay eggs on the surface of fresh or stagnant water. Areas on the outskirts of cities and population centers, bordering forests and wetlands. With host-seeking traps.</p> <p>For the trapping of the adult females will use BG-sentinel traps (Biogents), equipped with an additional CO₂ release and a specific lure mimicking body scent (Biogents). Traps will be operated from May – October, for 24 hrs within the first and the third week of the month.</p> <p>Based on a previous study (BAKRAN-LEBL et al., 2021), we estimated the minimum number of traps to detect WNV in Austria to be 59. Thus, we aim to start our project in the first year with 60 traps. We will reevaluate the necessary trap number and trapping frequency each year.</p> <p>The distribution of traps will be based on following specifications (a) at least 1 trap per NUTS3 unit, (b) focusing on urban areas and (c)</p>

		focusing on areas where previously human, horse and wild bird cases have been detected. Further, we aim to (d) incorporate information waterfowl and species of migratory birds as well and intensify the monitoring in those areas.
8	Sampling time period	The sampling period will be May to October, the time period with the greatest mosquito abundance in Austria.
9	Sampling matrix	The whole mosquito.
10	Type of disease indicators	Virus identification and isolation; Minimal Infection Rate (MIR).
11	Sampling unit	Mosquito pools – pooling unfed females by location/time, max pool of 20 [VectorNet, 2022]
12	Allocation of animal groups /animals to sampling	All trapped unfed female vector species. [VectorNet, 2022]
13	Testing protocol / Diagnostic test	PCR on pooled unfed adult females. The nucleic acid extracts will be screened by WNV and Usutu viruses (USUV)-specific reverse transcription quantitative (RT-q)PCRs. To confirm the possible presence of these two viruses, as well as to detect other flaviviruses (e.g. Bagaza virus, DENV, Barkedji virus or mosquito-specific flaviviruses), a universal flavivirus RT-PCR will be performed.
14	Design prevalence (only relevant for probability-based sampling)	Not applicable; biased (non-random or non-representative) sample of the population.
15	Level of confidence (only relevant for probability-based sampling)	Not applicable; biased (non-random or non-representative) sample of the population.
16	Contribution of the component to the surveillance system	Complementary to wild bird (passive surveillance on dead birds), human and horses (also passive surveillance).
17	Other pathogens that could be targeted with this surveillance component	In the OH SURVector project, vector pools will be tested for Usutu viruses in addition to WNV (see 13 – Testing protocol).
18	References	1) CDC Culex Species Mosquito Life Cycle Factsheet, 2022 2) TECHNICAL REPORT Field sampling methods for mosquitoes, sandflies, biting midges and ticks VectorNet project 2014–2018 3) ECDC Culex pipiens - Factsheet for experts, last updated 15 Jun 2020 BAKRAN-LEBL, K., CAMP, J. V., KOLODZIEJEK, J., WEIDINGER, P.,



		HUFNAGL, P., CABAL ROSEL, A., ZWICKELSTORFER, A., ALLERBERGER, F. and NOWOTNY, N. (2021): Diversity of West Nile and Usutu virus strains in mosquitoes at an international airport in Austria. <i>Transbound Emerg Dis</i> 1–14.
19	Additional comments	Prevalence information available: 0.005-25 % Minimum infection rate (MIR) [VectorNet, 2022]. There is currently no reliable information on the prevalence of WNV in Austria.

4.2 CZECH REPUBLIC

TABLE 2: Surveillance card for selecting sites for mosquito collection in the Czech Republic.

Czech Republic: SURVEILLANCE CARD WNF Pathogen detection in mosquitoes in non-endemic regions (Czech Republic) bordering endemic ones

	Characteristics	Description
1	Surveillance component name	Pathogen detection in mosquitoes in non-endemic regions (Czech republic) bordering endemic ones testing for WNV by RT-PCR.
2	Surveillance aim	Early detection of a spread of the virus to new areas bordering endemic ones; Early detection of the introduction of the pathogen; mosquito identification; mosquito abundancy.
3	Target species and group	<i>Culex pipiens</i> ; <i>Culex modestus</i> ; <i>Culex perexiguus</i> ; <i>Culex torrentium</i> ; <i>Culex univittatus</i>
4	Target sector / production type	Not applicable.
5	Geographical area covered	Country-wide areas with suitable habitat for mosquitoes, most often around water areas; <i>Culex pipiens</i> is found in all European countries (1)
6	Age group	Adult female mosquitoes. (2)
7	Sampling point and strategy	Areas with suitable habitat for vectors: to be preferred areas where adult female mosquitoes lay eggs on the surface of fresh or stagnant water. Areas on the outskirts of cities and population centres, bordering forests and wetlands. (2) to be conducted with <i>Encephalitis Vector Survey</i> traps (EVS trap). At least ten locations will be selected where mosquito sampling will be carried out.
8	Sampling time period	Year-round period of mosquitoes activity: from March to November. (2)
9	Sampling matrix	The whole mosquito.
10	Type of disease indicators	Presence of the pathogen RNA, identification of WNV; Minimal Infection Rate (MIR); WNV lineage determination by sequencing
11	Sampling unit	Mosquito pools; pooling females by location/time, max pool of 100 for non-endemic region with low prevalence (1 positive in 5000). [VectorNet, 2022]
12	Allocation of animal groups /animals to sampling	Not applicable

13	Testing protocol / Diagnostic test	Morphological identification of mosquito species with subsequent detection of WNV by RT-qPCR on pooled mosquito females. (2)
14	Design prevalence (only relevant for probability-based sampling)	Not applicable; biased (non-random or non-representative) sample of the population
15	Level of confidence (only relevant for probability-based sampling)	Not applicable; biased (non-random or non-representative) sample of the population
16	Contribution of the component to the surveillance system	Complementary to sentinel birds, wild birds, human, horse surveillance. Low compared to sentinel birds, wild bird, human, horse surveillance.
17	Other pathogens that could be targeted with this surveillance component	RVF, Usutu Viruses, canine dirofilarial worms, and avian malaria parasites in Europe. (1)
18	References	<p>1) ECDC Culex pipiens - Factsheet for experts, last updated 15 Jun 2020</p> <p>2) Rudolf I, Betášová L, Blažejová H, Venclíková K, Straková P, Šebesta O, Mendel J, Bakonyi T, Schaffner F, Nowotny N, Hubálek Z. West Nile virus in overwintering mosquitoes, central Europe. Parasit Vectors. 2017 Oct 2;10(1):452. doi: 10.1186/s13071-017-2399-7. PMID: 28969685; PMCID: PMC5625652.</p> <p>3) EFSA External scientific report Risk of vector-borne diseases for the EU: Entomological aspects: Part 2; Marieta Braks, Giuseppe Mancini, Marieke de Swart, Maria Goffredo First published: 10 March 2017</p>
19	Additional comments	Prevalence information available: 0.005-0,001%. Minimum infection rate (MIR) (3)

4.3 SLOVAKIA

TABLE 3: Surveillance card for selecting sites for mosquito collection in Slovakia.

SK: SURVEILLANCE CARD WNF Pathogen detection in mosquitoes in endemic regions

	Characteristics	Description
1	Surveillance component name	Pathogen detection in mosquitoes in endemic regions testing for West Nile Virus.
2	Surveillance aim	Early detection if prevalence increases; Early detection of a change of the geographic distribution; Early detection of an increase in incidence; monitor ongoing outbreaks; identify different kinds of mosquitoes that could spread the virus; when and how to act to reduce the risk of infection by applying mosquito control measures.
3	Target species and group	<i>Culex pipiens</i> ; <i>Culex modestus</i> ; <i>Culex torrentium</i> .
4	Target sector / production type	Not applicable.
5	Geographical area covered	Country-wide – previously affected areas with suitable habitat for mosquitoes; <i>Culex pipiens</i> is found in all European countries, except Iceland and Faroe Islands, and in all Middle Eastern and North African countries. (1)
6	Age group	Adult mosquitoes. (2)
7	Sampling point and strategy	Areas with suitable habitat for vectors: to be preferred areas where adult female mosquitoes lay eggs on the surface of fresh or stagnant water. Areas on the outskirts of cities and population centres, bordering forests and wetlands. (2) With host-seeking traps.
8	Sampling time period	Minimum annual time frame with the greatest diffusion of vectors: from March to November. (2)
9	Sampling matrix	The whole mosquito.
10	Type of disease indicators	Virus identification and isolation; Minimal Infection Rate (MIR).
11	Sampling unit	Mosquito pools – pooling unfed females by location/time, max pool of 10 – 50 [VectorNet, 2022]
12	Allocation of animal groups /animals to sampling	All trapped unfed female vector species. [VectorNet, 2022]
13	Testing protocol / Diagnostic test	PCR on pooled unfed adult females.

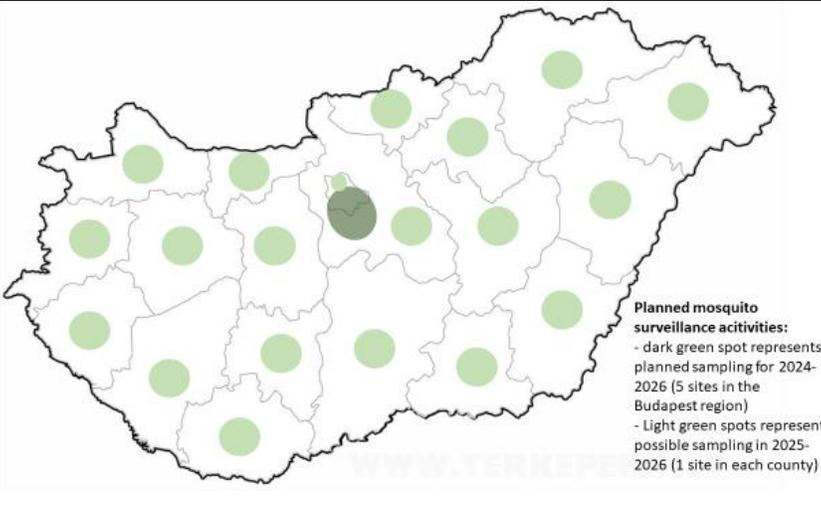
14	Design prevalence (only relevant for probability-based sampling)	Not applicable; biased (non-random or non-representative) sample of the population.
15	Level of confidence (only relevant for probability-based sampling)	Not applicable; biased (non-random or non-representative) sample of the population.
16	Contribution of the component to the surveillance system	Complementary to sentinel birds, wild bird, human, horse surveillance.
17	Other pathogens that could be targeted with this surveillance component	RVF, Usutu viruses, canine dirofilarial worms and avian malaria parasites. (3)
18	References	<p>1) CDC Culex Species Mosquito Life Cycle Factsheet, 2022</p> <p>2) TECHNICAL REPORT Field sampling methods for mosquitoes, sandflies, biting midges and ticks VectorNet project 2014–2018</p> <p>3) ECDC Culex pipiens - Factsheet for experts, last updated 15 Jun 2020</p> <p>4) Čabanová Viktória, Tichá Elena, Bradbury Richard Stewart, Zubriková Dana, Valentová Daniela, Chovancová Gabriela, Grešáková Ľubomíra, Víchová Bronislava, Šikutová Silvie, Csank Tomáš, Hurníková Zuzana, Miterpáková Martina, Rudolf Ivo. Mosquito surveillance of West Nile and Usutu viruses in four territorial units of Slovakia and description of a confirmed autochthonous human case of West Nile fever, 2018 to 2019. Euro Surveill. 2021;26(19):pii=2000063. https://doi.org/10.2807/1560-7917.ES.2021.26.19.2000063</p>
19	Additional comments	Prevalence information available: 0.005-25 % Minimum infection rate (MIR) [VectorNet, 2022]. In the southern district of Komárno in Slovakia MIR 0.46 (4)

4.4 HUNGARY

TABLE 4: Surveillance card for selecting sites for mosquito collection in Hungary.

Hungary: SURVEILLANCE CARD WNF Pathogen detection in mosquitoes in endemic regions

	Characteristics	Description
1	Surveillance component name	Pathogen detection in mosquitoes in endemic regions testing for West Nile Virus.
2	Surveillance aim	Early detection if prevalence increases; Early detection of a change of the geographic distribution; Early detection of an increase in incidence; monitor ongoing outbreaks; identify different kinds of mosquitoes that could spread the virus; when and how to act to reduce the risk of infection by applying mosquito control measures.
3	Target species and group	<i>Culex pipiens</i> ; <i>Culex modestus</i> ; <i>Culex torrentium</i> .
4	Target sector / production type	Not applicable.
5	Geographical area covered	<p>2024-2026: Area of Budapest during the first/pilot year of the study, then for 2 consecutive years. Altogether 10 CO2 traps at 5 sites at the southern outskirts of the city. The sites are designated based on cumulative epidemiological data, (previously affected areas according to communicated WNV cases) and habitat suitability for <i>Culex</i> mosquitoes.</p> <p>2025-2026: Based on data of the first/pilot year and based on subsequent epidemiological situation the intensity of the study may be increased to cover all counties (NUTS3 level regions) of Hungary. This means 20 additional traps, 1 trap/each county. (Increase of the surveillance intensity might not be implemented).</p>

		 <p>Planned mosquito surveillance activities: - dark green spot represents planned sampling for 2024-2026 (5 sites in the Budapest region) - Light green spots represent possible sampling in 2025-2026 (1 site in each county)</p>
6	Age group	Adult mosquitoes. (2)
7	Sampling point and strategy	Areas with suitable habitat for vectors: to be preferred areas where adult female mosquitoes lay eggs on the surface of fresh or stagnant water. Areas on the outskirts of cities and population centers, bordering forests and wetlands. With host-seeking traps.
8	Sampling time period	Time frame with the greatest abundance of vectors: from May to October.
9	Sampling matrix	The whole mosquito.
10	Type of disease indicators	Virus identification and isolation; Minimal Infection Rate (MIR).
11	Sampling unit	Mosquito pools – pooling unfed females by location/time, max pool of 20 [VectorNet, 2022]
12	Allocation of animal groups /animals to sampling	All trapped unfed female vector species. [VectorNet, 2022]
13	Testing protocol / Diagnostic test	PCR on pooled unfed adult females.
14	Design prevalence (only relevant for probability-based sampling)	Not applicable; biased (non-random or non-representative) sample of the population.
15	Level of confidence (only relevant for probability-based sampling)	Not applicable; biased (non-random or non-representative) sample of the population.

16	Contribution of the component to the surveillance system	Complementary to, wild bird (passiv dead birds), human, horse surveillance (passiv).
17	Other pathogens that could be targeted with this surveillance component	RVF, Usutu viruses, canine dirofilarial worms and avian malaria parasites. (3)
18	References	1) CDC Culex Species Mosquito Life Cycle Factsheet, 2022 2) TECHNICAL REPORT Field sampling methods for mosquitoes, sandflies, biting midges and ticks VectorNet project 2014–2018 3) ECDC Culex pipiens - Factsheet for experts, last updated 15 Jun 2020
19	Additional comments	Prevalence information available: 0.005-25 % Minimum infection rate (MIR) [VectorNet, 2022]. There is currently no reliable information on the prevalence in Hungary.