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

D2.1: MAP OF SAMPLING LOCATIONS (TICKS)

WP2 – FIELD WORK TICK 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	D2.1		
Work Package	WP2 – Field work tick monitoring		
Date of Delivery	**Contractual**	31.03.2024	**Actual**	15.04.2024
Nature	R — Document, report	**Dissemination Level**	PU – Public
Lead Beneficiary	SZU – STATNI ZDRAVOTNI USTAV		

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	EODY – ETHNIKOS ORGANISMOS DIMOSIAS YGEIAS
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	NFCSO – NEMZETI ELELMISZERLANC-BIZTONSAGI HIVATAL
	UVLF – UNIVERZITA VETERINARSKEHO LEKARSTVA A FARMACIE V KOSICIACH

Document History

Version	Date	Partner	Description
V0.1	14.04.2024	Katerina Kybicová, SZÚ	Table of Contents
V0.2	14.04.2024	Katerina Kybicová, SZÚ	Peer Review
V0.3	15.04.2024	Annette Nigsch, AGES	Peer Review
V0.4	15.04.2024	Annette Nigsch, AGES	Consolidated Version
V0.5	15.04.2024	Annette Nigsch, AGES	Quality Assurance
V1.0	15.04.2024	Annette Nigsch, AGES	Final version – Ready for submission

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CCHF - Crimean-Congo hemorrhagic fever
CCHFV - Crimean-Congo hemorrhagic fever virus
TBE - Tick-Borne Encephalitis
TBEV - Tick-Borne Encephalitis virus

1. Executive summary

Deliverable D2.1: MAP OF SAMPLING LOCATIONS (TICKS) contains maps of all countries and a description of the selection of tick testing locations. Apart from Austria, which will approach the collection of ticks using the Citizen Science method, the other countries will actively collect ticks either 1) by the flagging method or 2) by collecting ticks from animals.
The investigation and collection of ticks will target the transboundary spread of pathogens, mainly: 1) Crimean-Congo hemorrhagic fever virus (CCHFV) in Greece, Hungary and Austria, 2) *Borrelia burgdorferi* s.l. in Hungary, Austria, Slovakia and the Czech Republic and 3) Tick-Borne Encephalitis virus (TBEV) in the Czech Republic, Slovakia and Greece.

The selection of sites for the tick collection was made individually according to pathogen and country. Two main approaches were followed. The first was to select several sites based on previous knowledge of the presence of the pathogen in the environment or based on reported human cases of the disease at the site in recent years. The second approach is to cover the entire territory of a given country in order to obtain the most accurate information about the spread of the pathogen.
Thanks to these approaches, we obtain a comprehensive picture of the cross-border spread of tick-borne pathogens depending on latitude and longitude.

2. Methodology for selecting sites for tick collection by country

2.1 Austria

In Austria, ticks are collected using the citizen science approach with the goal to cover all 35 NUTS3 regions (see map) and a target value of 300 ticks per region per year. Information for citizen scientists is spread via the media, the AGES-homepage, and by personal communication to veterinarians, horse-keepers, farmers, and hunters. In this way, hard ticks from different geographic areas of Austria will be obtained either directly from the environment or from hosts during the tick season which usually lasts from March to November. The ticks will be identified by stereomicroscope and investigated for the presence of *Borrelia burgdorferi* sensu lato by PCR in the case of endemic hard tick species such as *Ixodes ricinus* (target value 2500 ticks per year). In the occasional detection of introduced *Hyalomma marginatum* ticks, the laboratory investigation will focus on the detection of CCHFV.
The component can contribute to the identification of Borrelia in new areas. Presence of ticks positive for Borrelia is the most important risk factor for human Lyme borreliosis. It is debated if better knowledge or systematic surveillance can inform ecosystem management to reduce transmission probability. The component can contribute to detection of introduction of the ticks in new areas. However, results cannot be used directly for estimation of detection sensitivity or confidence of freedom since this is a biased (non-representative) sample of an unknown population.

2.2 Czech Republic

In the Czech Republic, ticks will be collected using the flagging method from selected localities. For TBEV - We have identified ten locations based on the incidence of reported cases over the last decade. These sites have seen the highest number of outbreaks. Prioritizing areas with a history of virus transmission in ticks, we aim to sample around 750 to 1000 ticks per site during the spring season. Regarding *Borrelia burgdorferi* sensu lato - Taking a comprehensive approach, tick collection sites are distributed evenly throughout the Czech Republic, given that the entire country is an endemic area. Each NUT3 region will

host 10 to 15 collection sites. Our target is to gather at least 10,000 ticks during the peak tick activity in spring, covering the entire territory of the Czech Republic.
The component contributes to estimation of *Borrelia burgdorferi* s.l. and TBEV prevalence in target tick species, identification of Borrelia species in endemic areas and detection of introduction of Borrelia and TBEV in new areas, with high expected sensitivity.

2.3 Slovakia

Tick collection sites in Slovakia were selected based on data on the occurrence of tick-borne encephalitis in the human population from the Epidemiological Information System. Localities with the largest number of outbreaks in the last 5 years were selected. Two locations were specified in each NUT3 region. Three biotopes (farm, tourist area and urban greenery) were selected in each location. Collecting will be done in spring and autumn. In one year, we plan to collect approximately 2300 ticks equally from all selected locations. The same samples will be used for both TBEV and *Borrelia burgdorferi* detection.
The component can contribute to identification of introduction of Borrelia and TBEV in new areas, with high expected sensitivity. However, results cannot be used directly for estimation of detection sensitivity or confidence of freedom since this is a biased (non-representative) sample of an unknown population.

2.4 Hungary

Tick sampling locations were selected based on the localization of Lyme disease and TBE diseases between 2020 and 2022 and the number of human cases. Sampling sites were selected in the forests surrounding the suburban zone of 15 major Hungarian settlements where these diseases occurred cumulatively. Within the 15 selected sampling points, sampling will take place in or on the edge deciduous, typically oak forests, near lakes, rivers or streams and with a high density of game. Based on the above, we created detailed maps of the sampling points using different overlays.
The component can contribute to complementary human and animal surveillance (passive, citizen science).

2.5 Greece

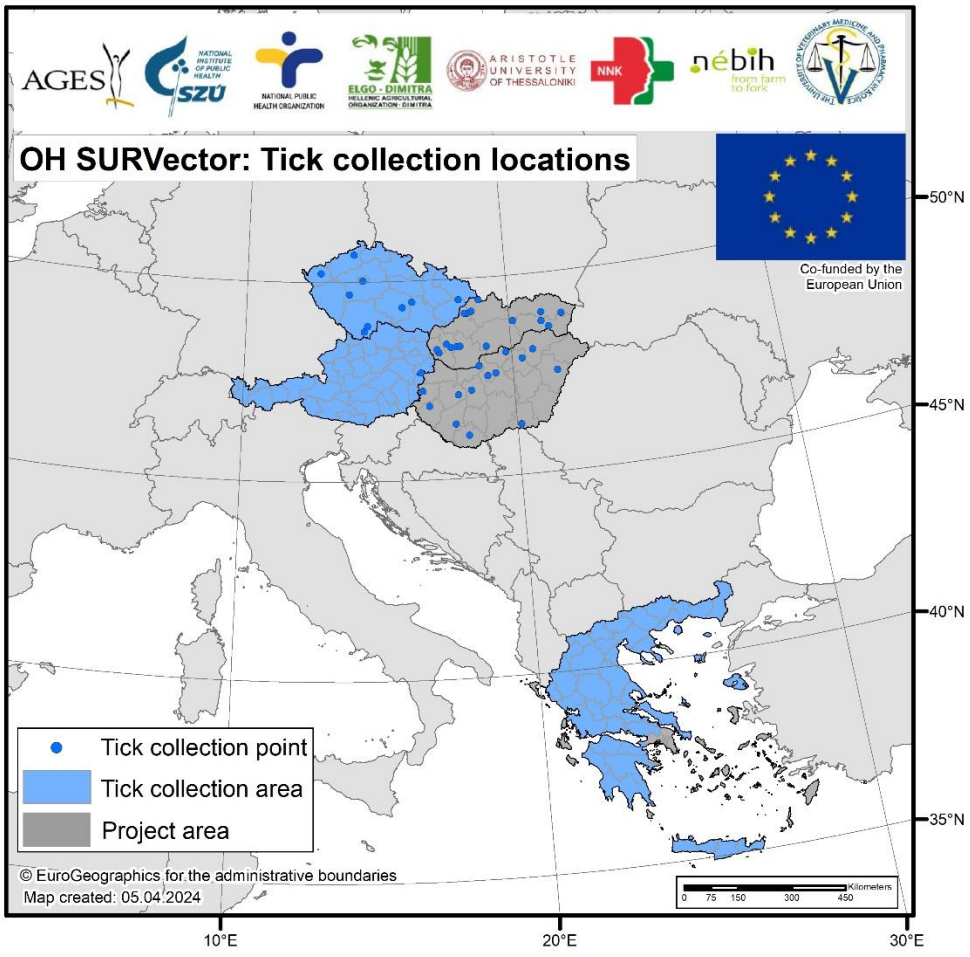
For the sampling in Greece, the main plan for the first year is to visit almost all Regions of Greece (NUTS2 level they are 13) excluding Attica (EL30) South Aegean (EL42) and Ionian islands (EL 62) because of low livestock numbers.
From the remaining part, we selected all regional units (NUTS3 level) based on 2 criteria:

1. Farms/animal density (as shown in the maps)
2. Previously recorded presence of the TBE and CCHF viruses.

We have chosen sheep and goats as they are the only grazing animals in Greece, so they will act as “tick sensors” and we will also apply flagging methods in the areas around the farms we will visit.
The component is not expected to have a high sensitivity. However, since TBE is emerging with only a few human cases recorded, and CCFV was only once introduced in Greece, the component can contribute to the early detection of introduction of the viruses in new areas. However, results cannot be used directly for estimation of detection sensitivity or confidence of freedom since this is a biased (non-representative) sample of an unknown population.

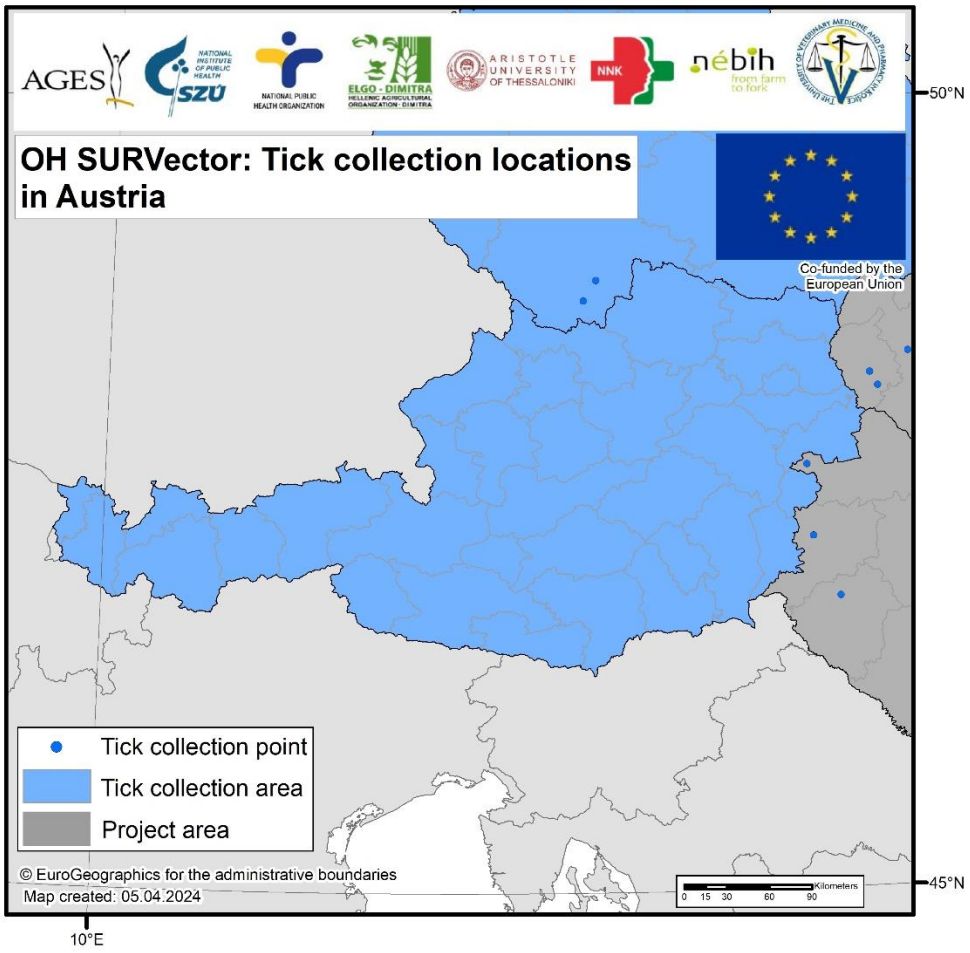
3. Maps for selecting sites for tick collection by country

Figure 1: Tick collections areas in all consortium countries



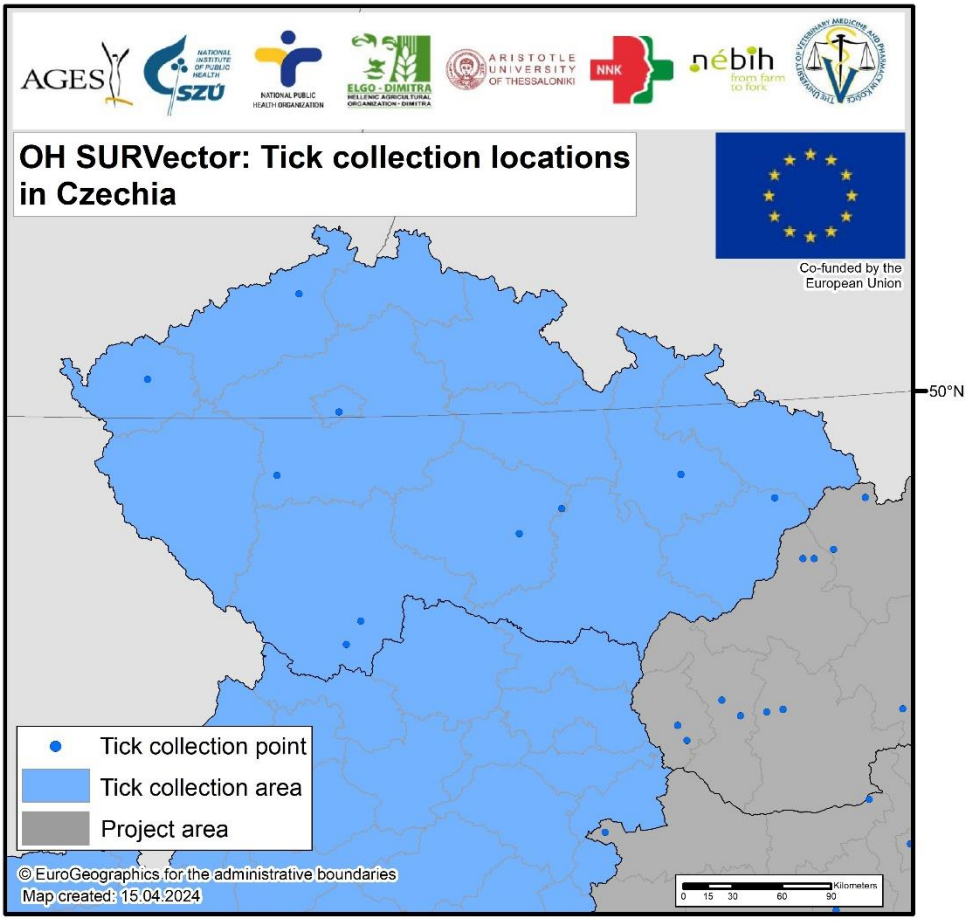
3.1 Austria

Figure 2: Tick collections areas in Austria



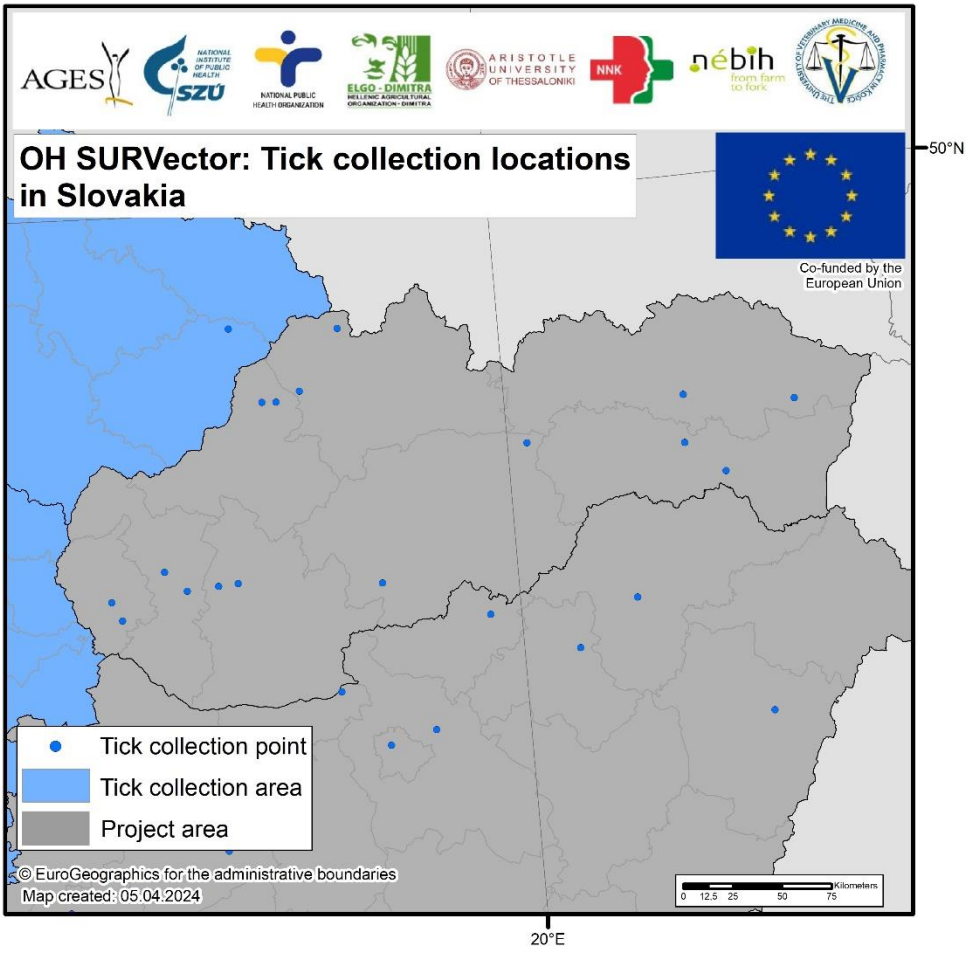
3.2 Czech Republic

Figure 3: Tick collections areas in the Czech Republic



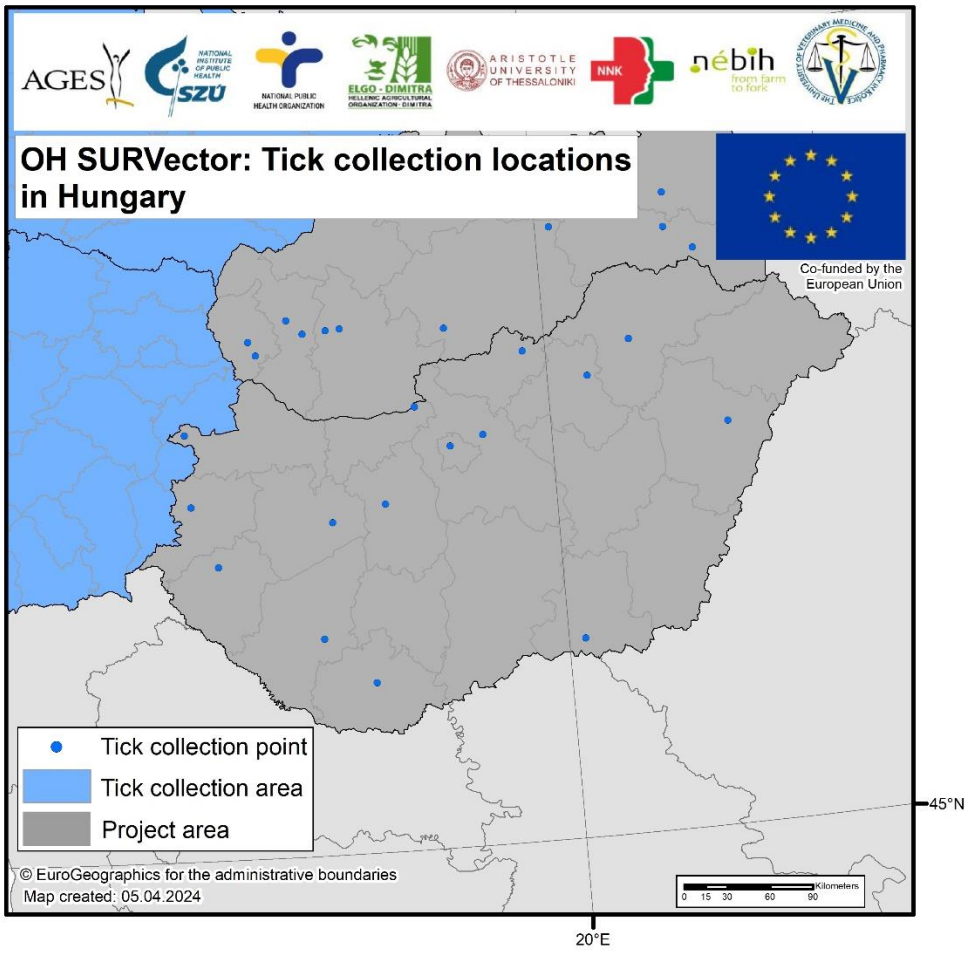
3.3 Slovakia

Figure 4: Tick collections areas in Slovakia



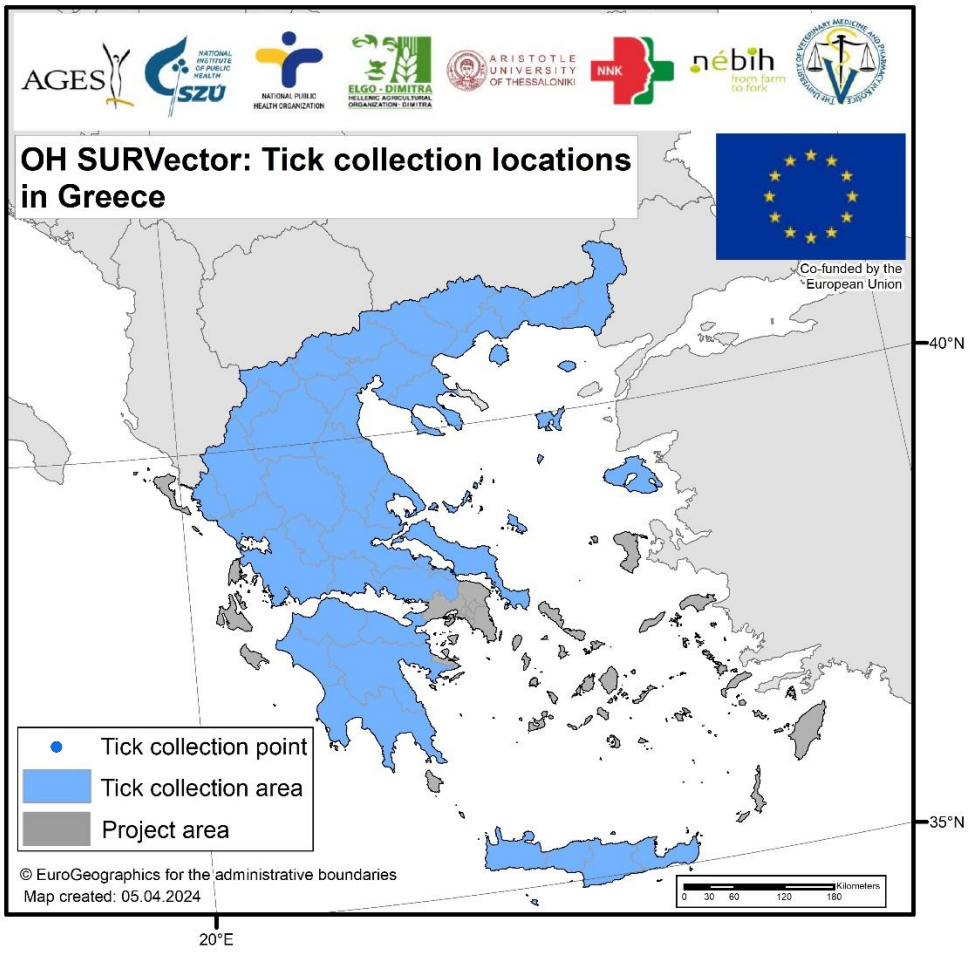
3.4 Hungary

Figure 5: Tick collections areas in Hungary



3.5 Greece

Figure 6: Tick collections areas in Greece



4. Surveillance cards for selecting sites for tick collection by country

4.1 Austria

Table 1: Surveillance cards for selecting sites for tick collection in Austria

AUSTRIA: SURVEILLANCE CARD_CCHFV_Surveillance of ticks in areas at risk of introduction and establishment of the vector

	Characteristics	**Description**
1	Surveillance component name	Surveillance of ticks in areas at risk of introduction and establishment of the vector.
2	Surveillance aim	Early detection of a change of the geographic distribution/spread of *Hyalomma spp*. ticks to new areas.
3	Target species and group	*Hyalomma* spp. collected from animals
4	Target sector / production type	Not applicable.
5	Geographical area covered	Areas where *Hyalomma marginatum* is considered to be absent, but which are at risk of introduction and establishment of the vector (climatic conditions, vegetation, neighbouring endemic areas).
6	Age group	tick stages: larvae, nymphs, adults
7	Sampling point and strategy	Collection of ticks from wildlife or livestock, including horses (1), living near or in a suitable vector habitat. Ticks will be obtained by citizen science approach.
8	Sampling time period	Vector period (spring to autumn).
9	Sampling matrix	Ticks.
10	Type of disease indicators	i) presence of competent vectors incl. ii) presence of CCHFV within them.
11	Sampling unit	Ticks collected from animals are identified individually using a stereomicroscope and identification key (2). Optionally molecular techniques can be used in addition.
12	References	1) Uiterwijk, M., Ibáñez-Justicia, A., van de Vossenberg, B., Jacobs, F., Overgaauw, P., Nijsse, R., ... & Sprong, H. (2021). Imported Hyalomma ticks in the Netherlands 2018–2020. Parasites & Vectors, 14(1), 1-12. 2) Ticks of Europe and North Africa: https://doi.org/10.1007/978-3-319-63760-0

AUSTRIA: SURVEILLANCE CARD_Borrelia burgdorferi s.l.

	Characteristics	**Description**
1	Surveillance component name	Pathogen detection in ticks high-risk regions testing for *Borrelia burgdorferi s.l.* (1)
2	Surveillance aim	Detection of a change of the geographic distribution. (2)
3	Target species and group	Hard ticks – Genus *Ixodes,* species *I. ricinus* and *I. persulcatus.* (3)
4	Target sector / production type	Not applicable.
5	Geographical area covered	Ticks will be collected in their habitats or removed from their hosts and sent in for identification and pathogen screening.
6	Age group	All tick stages: nymphs and adults. (4)
7	Sampling point and strategy	Ticks will be obtained by citizen science approach.
8	Sampling time period	Ticks, are seasonally active and more likely to be detected from March to November, considering the period of maximum incidence of the disease in humans. However, the active period tick vector varies depending on the latitude, altitude and the actual temperature. (5)
9	Sampling matrix	Whole ticks.
10	Type of disease indicators	Presence of the pathogen (identification of *Borrelia burgdorferi s.l.* by PCR) (6).
11	Sampling unit	Whole ticks.
12	References	1) Gandy, S., Kilbride, E., Biek, R. et al. Experimental evidence for opposing effects of high deer density on tick-borne pathogen prevalence and hazard. Parasites Vectors 14, 509 (2021). https://doi.org/10.1186/s13071-021-05000-0 2) TECHNICAL REPORT Field sampling methods for mosquitoes, sandflies, biting midges and ticks VectorNet project 2014–2018 3) ECDC Ixodes ricinus - Factsheet for experts, last updated 31 Jul 2014 4) Estrada-Peña, A.; Cevidanes, A.; Sprong, H.; Millán, J. Pitfalls in Tick and Tick-Borne Pathogens Research, Some Recommendations and a Call for Data Sharing. Pathogens 2021, 10, 712. https://doi.org/10.3390/pathogens10060712 5) Krawczyk AI, van Duijvendijk GLA, Swart A, Heylen D, Jaarsma RI, Jacobs FHH, Fonville M, Sprong H, Takken W. Effect of rodent density on tick and tick-borne pathogen populations: consequences for infectious disease risk. Parasit Vectors. 2020 Jan 20;13(1):34. doi: 10.1186/s13071-020-3902-0. PMID: 31959217; PMCID: PMC6971888.

4.2 Czech Republic

Table 2: Surveillance cards for selecting sites for tick collection in the Czech Republic

Czech Republic: SURVEILLANCE CARD_Lyme borreliosis_ticks risk regions_surveillance card in the Czech Republic

	Characteristics	**Description**
1	Surveillance component name	Pathogen detection in ticks *Ixodes ricinus* in high-risk regions throughout the territory of the Czech Republic where the vector is endemic (tick flagging) testing for *Borrelia burgdorferi* s.l. by real-time PCR
2	Surveillance aim	Detection of changes in the geographical distribution of the *I. ricinus* tick and the spread of other tick species (*Dermacentor reticulatus*, *Haemaphysalis concinna*) to new areas; early detection of an increase in the incidence of a disease-causing pathogen *Borrelia burgdorferi* s.l.; monitoring of the genetic diversity of *B. burgdorferi* s. l. species and its potential changes in time
3	Target species and group	Hard ticks – primary Genus *Ixodes;* species *I. ricinus.*
4	Target sector / production type	Not applicable.
5	Geographical area covered	High-risk areas where Lyme borreliosis (LB) has been previously diagnosed (by probable place of infection – i.e. exposure to tick bite) throughout the territory of the Czech Republic; preferred habitats of *Ixodes ricinus* ticks: shady and moist forests (1), along trails, forest edges, urban and rural areas.
6	Age group	Larval, nymphal and adult tick developmental stages.
7	Sampling point and strategy	The collection flag consists of white cotton cloth with medium-length hair (resembling animal fur) sized 1 × 1 m, attached to a wooden pole approx. 150 cm long. The actual collection of questing ticks will be executed by sliding the tarpaulin over the vegetation (1,2). Estimate of the number of ticks per 100 m2 per collection site. 150 locations will be selected throughout the territory of the Czech Republic, where the abundance of ticks will be monitored, we assume a sufficient number of nymphs for pathogen testing in at least 50 to 70 locations
8	Sampling time period	The period of tick activity in the Czech Republic is from March to November, with a peak in May and a second lower peak in September. In this period also the most new cases of LB have been reported. Actual tick activity in a given year depends on actual temperature and humidity, latitude, altitude. Sampling would take place only in optimal weather, i.e., not in the rain or immediately after the rain, and with no wind, only at an air temperature within the range of 14–26 °C and air humidity, ranging from 45–85% (2,3,4,5,6).
9	Sampling matrix	Whole unfed ticks.

10	Type of disease indicators	Presence of pathogen DNA in ticks (identification of *Borrelia* spirochetes).
11	Sampling unit	Single ticks, testing at least 50 nymphs per sampling location (2)
12	References	1) Široký, P., Kubelová, M., Bednář, M., Modrý, D., Hubálek, Z., & Tkadlec, E. (2011). The distribution and spreading pattern of Dermacentor reticulatus over its threshold area in the Czech Republic-How much is range of this vector expanding? Veterinary Parasitology, 183(1–2), 130–135. https://doi.org/10.1016/j.vetpar.2011.07.006 2) Vacek, Z., Cukor, J., Vacek, S., Václavík, T., Kybicová, K., Bartoška, J., Molina, S. M. (2023). Effect of forest structures and tree species composition on common tick (Ixodes ricinus) abundance—Case study from Czechia. Forest Ecology and Management, 529, 120676.

Czech Republic: SURVEILLANCE CARD_Tick-borne encephalitis virus (TBEV)_Pathogen detection in ticks in high-risk regions in the Czech Republic

	Characteristics	**Description**
1	Surveillance component name	Pathogen detection in ticks *Ixodes ricinus* in high-risk regions in the Czech Republic testing for TBEV by RT-(q)PCR.
2	Surveillance aim	Early detection if prevalence of TBEV increases in high-risk regions; Early detection of an increase in incidence tick-borne encephalitis disease cases; characterization of the local strains by sequencing of partial or in selected cases whole genome sequencing, phylogenetic analyses
3	Target species and group	Hard ticks – Genus *Ixodes*, primarily species *I. ricinus*
4	Target sector / production type	Not applicable.
5	Geographical area covered	Areas with high-risk of disease incidence; areas where TBE has been previously diagnosed (by probable place of infection) or where TBEV was detected; preferred habitats of hard ticks: shady and humid forests, along trails, forest edges (1), urban and rural areas. (2)
6	Age group	Adult and nymphal ticks.
7	Sampling point and strategy	The collection flag consists of white cotton cloth with medium-length hair (resembling animal fur) sized 1 × 1 m, attached to a wooden pole approx. 150 cm long. (1); at least ten locations will be selected where sampling will take place; 4 transects á 100 m^2 per sampling site for estimation of tick abundance; additional sampling if needed concerning the minimum of ticks for testing
8	Sampling time period	Year-round - the period of tick activity in the Czech Republic is from March to November, with a peak in May and a second lower peak in September (2). During the season, the new TBE cases human have been reported with one peak in July and sometimes the second peak in September, depending on season.
9	Sampling matrix	Whole ticks - adult and nymphal tick body.
10	Type of disease indicators	Presence of the pathogen RNA, identification of TBEV.
11	Sampling unit	Pooled flagged ticks by location/life stage – max pool of 5 adults or 10 nymphs at least 750 to 1000 individuals per sampling location.
	References	1) Hönig V, Svec P, Halas P, et al. Ticks and tick-borne pathogens in South Bohemia (Czech Republic)--Spatial variability in Ixodes ricinus abundance, Borrelia burgdorferi and tick-borne encephalitis virus prevalence. Ticks Tick Borne Dis. 2015 Jul;6(5):559-67. 2) Daniel M, Danielová V, Kříž B, Růžek D, Fialová A, Malý M, Materna J, Pejčoch M, Erhart J. The occurrence of Ixodes ricinus ticks and important tick-borne pathogens in areas with high tick-borne encephalitis prevalence in different altitudinal levels of the Czech Republic Part I. Ixodes ricinus ticks and tick-borne encephalitis virus. Epidemiol Mikrobiol Imunol. 2016 Summer;65(2):118-28. English. PMID: 27467329.

4.3 Slovakia

Table 3: Surveillance cards for selecting sites for tick collection in Slovakia

Slovakia: SURVEILLANCE CARD_Lyme borreliosis_ticks risk regions_surveillance card

	Characteristics	**Description**
1	Surveillance component name	Pathogen detection in ticks in high-risk regions where the vector is endemic (tick dragging of flagging) testing for *Borrelia burgdorferi s.l.*.
2	Surveillance aim	Detection of a change of the geographic distribution/spread to new areas; possibly early detection of an increase in incidence, i.e., early epidemic detection.
3	Target species and group	Hard ticks – Genus *Ixodes;* species *I. ricinus*
4	Target sector / production type	Not applicable.
5	Geographical area covered	Areas with high-risk of disease prevalence; areas where LB has been previously diagnosed; areas with recent tick vector expansion; preferred habitats of hard ticks: shady and humid woodlands, trails (1), clearings with grass, open fields and bushes, urban and rural areas.
6	Age group	Larvae, nymphs and adults.
7	Sampling point and strategy	Flagging a cloth in suitable vector habitat to collect questing ticks. (1)
8	Sampling time period	Ticks, are seasonally active and more likely to be detected from March to November, considering the period of maximum incidence of the disease in humans (2). However, the active period tick vector varies depending on the latitude, altitude and the actual temperature. (3)
9	Sampling matrix	Whole ticks.
10	Type of disease indicators	Presence of the pathogen (identification of *Borrelia* spirochetes) or DNA.
11	Sampling unit	Single ticks, testing at least 100 individuals per sampling location [VectorNet, 2022]
12	References	1) Salomon J, Hamer SA, Swei A. A Beginner's Guide to Collecting Questing Hard Ticks (Acari: Ixodidae): A Standardized Tick Dragging Protocol. J Insect Sci. 2020 Nov 1;20(6):11. doi: 10.1093/jisesa/ieaa073. PMID: 33135760; PMCID: PMC7604844. 2) Petrulionienė A, Radzišauskienė D, Ambrozaitis A, Čaplinskas S, Paulauskas A, Venalis A. Epidemiology of Lyme Disease in a Highly Endemic European Zone. Medicina (Kaunas). 2020 Mar 5;56(3):115. doi: 10.3390/medicina56030115. PMID: 32151097; PMCID: PMC7143858. 3) TECHNICAL REPORT Field sampling methods for mosquitoes, sandflies, biting midges and ticks VectorNet project 2014–2018

Slovakia: SURVEILLANCE CARD_Tick-borne encephalitis (TBE)_Pathogen detection in ticks in high-risk regions

	Characteristics	**Description**
1	Surveillance component name	Pathogen detection in ticks in high-risk regions.
2	Surveillance aim	Early detection if prevalence increases; Early detection of a change of the geographic distribution; Early detection of an increase in incidence.
3	Target species and group	Hard ticks – Genus *Ixodes.*
4	Target sector / production type	Not applicable.
5	Geographical area covered	Areas with high-risk of disease prevalence; areas where LB has been previously diagnosed; areas with recent tick vector expansion; preferred habitats of hard ticks: shady and humid woodlands, trails (1), clearings with grass, open fields and bushes, urban and rural areas. (2)
6	Age group	Adults and nymphs.
7	Sampling point and strategy	Flagging a cloth in suitable vector habitat to collect questing ticks. (1)
8	Sampling time period	Year-round / not limited; ticks, are most active from March to November. (2)
9	Sampling matrix	Adult and nymphal tick body.
10	Type of disease indicators	Presence of the pathogen, identification of TBEV.
11	Sampling unit	Pooled flagged ticks by location/life stage – max pool of 5 adults or 10 nymphs at least 1000 individuals per sampling location. – pooling ticks from animals – max pool of 5 half ticks (other half for virus isolation & prevalence) [VectorNet, 2022]
12	References	1) Salomon J, Hamer SA, Swei A. A Beginner's Guide to Collecting Questing Hard Ticks (Acari: Ixodidae): A Standardized Tick Dragging Protocol. J Insect Sci. 2020 Nov 1;20(6):11. doi: 10.1093/jisesa/ieaa073. PMID: 33135760; PMCID: PMC7604844. 2) TECHNICAL REPORT Field sampling methods for mosquitoes, sandflies, biting midges and ticks VectorNet project 2014–2018

4.4 Hungary

Table 4: Surveillance cards for selecting sites for tick collection in Hungary

Hungary: SURVEILLANCE CARD_Tick-borne Pathogen detection in ticks in endemic regions

	Characteristics	**Description**
1	Surveillance component name	Pathogen detection in *Ixodes ricinus* and *Hyalomma* spp. in endemic regions: testing for *Borrelia* species and Crimean-Congo haemorrhagic fever virus.
2	Surveillance aim	Early detection if seasonal prevalence changes; Early detection of a change of the geographic distribution; Early detection of an increase in incidence; when and how to act to reduce the risk of infection by applying tick control measures.
3	Target species and group	*Ixodes ricinus; Hyalomma rufipes* and other *Hyalomma* spp (1)
4	Target sector / production type	Not applicable.
5	Geographical area covered	Country-wide – focus on previously affected areas with suitable habitat for *Ixodes ricinus*.
6	Age group	Larvae, nymphs and adults of ticks (2)
7	Sampling point and strategy	Areas with suitable habitat for ticks, where questing activity and host-infestation are known. Special emphasis on areas on the outskirts of cities and population centers, bordering forests and wetlands; there is no sampling on rainy and windy days. With host-seeking traps and collection from the vegetation by the dragging-flagging method.
8	Sampling time period	Time frame with the greatest abundance of ticks, anticipating mild winters: year-round collections at monthly interval.
9	Sampling matrix	The whole tick.
10	Type of disease indicators	*Borrelia* DNA identification in *Ixodes ricinus* and Crimean-Congo haemorrhagic fever virus RNA identification in *Hyalomma rufipes*. Minimal Infection Rate (MIR).
11	Sampling unit	Tick pools (*I. ricinus*) – pooling unfed ticks by location/time, max pool of 20 [VectorNet, 2022] Individual ticks (*H. rufipes*).
12	References	1) Hornok S, Kováts D, Horváth G, Kontschán J, Farkas R. Checklist of the hard tick (Acari: Ixodidae) fauna of Hungary with emphasis on host-associations and the emergence of *Rhipicephalus sanguineus*. Exp Appl Acarol. 2020 80. 311-328. doi: 10.1007/s10493-019-00461-6. 2) TECHNICAL REPORT Field sampling methods for mosquitoes, sandflies, biting midges and ticks VectorNet project 2014–2018

4.5 Greece

Table 5: Surveillance cards for selecting sites for tick collection in Greece

Greece: SURVEILLANCE CARD Tick-borne encephalitis (TBE) and CCHF Pathogen detection in ticks

	Characteristics	**Description**
1	Surveillance component name	Detection of Tick-borne encephalitis (TBEV) and Crimean-Congo haemorrhagic fever (CCHFV) viruses in ticks in Greece
2	Surveillance aim	In Greece, TBE is an emerging disease with seven human cases recorded in 2014-2022, in north, central and south Greece. In addition, CCHF virus was introduced in north Greece in 2008 (one human case), in an area bordering Bulgaria, indicating a serious cross-border threat. Tick surveillance is not established nationwide. The surveillance aim includes: 1. Identification of TBEV and CCHFV risk areas, ie by testing for TBEV and CCHFV ticks collected from various geographic locations in Greece. The results of the study will lead to the design and implementation of targeted prevention and control measures. 2. Identification of areas at risk of TBEV and CCHFV introduction and establishment through vector surveillance (vector presence and abundance, geographical distribution and activity period of the tick vectors). 3. Early detection of the introduction/ re-introduction of TBEV and CCHFV in new areas – Identification of high-risk areas/ a change of the geographic distribution. 4. Early detection of an increase in human TBE incidence, through timely monitoring of the TBE burden in ticks. 5. Monitor ongoing outbreaks.
3	Target species and group	For TBEV: Ixodid ticks – Genus Ixodes. For CCHFV: *Hyalomma* spp. ticks (mainly *H. marginatum*), and other possibly competent tick vectors (eg *Rhipicephalus bursa*).
4	Target sector / production type	Small ruminants (sheep and goats). Production type: extensive and semi-intensive (grazing all year round) and therefore exposed to ticks.
5	Geographical area covered	In order to identify the high-risk areas, especially for TBE, in 2024, a more extensive study will be conducted covering almost whole mainland Greece, specifically, eight administrative NUTS2 regions in the mainland, and Crete Island region. For CCHF: specific attention will be given to the bordering with other Balkan countries area, in the north of Greece, since those countries are known to be endemic to the disease. According to the results of the first-year study (in 2024), a more strategic sampling plan will be implemented in 2025 and 2026, which will include the high-risk areas identified (in 2024), ie areas where ticks and/or the viruses will be present. Preferred habitats of hard ticks: small ruminants farms, shady and humid woodlands, trails (1), clearings with grass, open fields and bushes, urban and rural areas (2)

6	Age group	Adult animals will be sampled which have been exposed to ticks (grazing). All different stages of ticks will be collected.
7	Sampling point and strategy	1. Flagging a cloth in suitable vector habitat to collect questing ticks. (1) 2. Collecting ticks from sheep and goat farms (5 animals per farm randomly selected) During the first year (2024), and in order to cover all regions mentioned above (country-wide) sampling trips will be organized, in different time points. In each sampling trip approximately 20 farms per NUTS2 region will be sampled (in 4 sampling periods). Flagging will be also performed in the same areas. During the following years (2025 and 2026), the same sampling strategy will be applied, however in strategically selected areas and period in the year based on the results of the first-year study.
8	Sampling time period	Extensive study, 2024: Year-round / not limited, as tick vectors activity period has not been so far systematically recorded in Greece). Targeted studies in 2025 and 2026: depends on the results of the first-year study. Presumed period: March to November (spring to autumn); ticks, are most active from March to November. (2)
9	Sampling matrix	Ticks.
10	Type of disease indicators	Presence of the pathogen, identification of TBEV/ CCHFV. Number and % proportions of tick vectors collected per species, collection location, NUTS2 and NUTS3 region, sampling period, and year Number and % proportions of infected tick vectors collected per virus, species, collection location, NUTS2 and NUTS3 region, sampling period, and year
11	Sampling unit	Ticks, animals and flagging locations: 1. Collected ticks by flagging location 2. Collected ticks from animals
12	References	1) Salomon J, Hamer SA, Swei A. A Beginner's Guide to Collecting Questing Hard Ticks (Acari: Ixodidae): A Standardized Tick Dragging Protocol. J Insect Sci. 2020 Nov 1;20(6):11. doi: 10.1093/jisesa/ieaa073. PMID: 33135760; PMCID: PMC7604844. 2) TECHNICAL REPORT Field sampling methods for mosquitoes, sandflies, biting midges and ticks VectorNet project 2014–2018