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Recommendations and technical specifications for sustainable surveillance of zoonotic pathogens where wildlife is implicated

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Abstract

A science-based participatory process guided by EFSA identified 10 priority zoonotic pathogens for future One Health surveillance in Europe: highly pathogenic avian influenza, swine influenza, West Nile disease, tick-borne-encephalitis, echinococcosis, Crimean Congo Haemorrhagic Fever, hepatitis E, Lyme disease, Q-fever, Rift Valley fever. The main aim of this report is to formulate recommendations and technical specifications for sustainable coordinated One health surveillance for early detection of these zoonotic pathogens where wildlife is implicated. For this purpose: (i) first, we reviewed the cornerstones of integrated wildlife monitoring that are applicable to zoonotic disease surveillance in wildlife under OH surveillance in the EU; (ii) we analysed the characteristics of the main wildlife groups and the selected pathogens relevant to surveillance aimed at early detection, and integrated with other health compartments; (iii) we proposed general recommendations for the first steps of sustainable wildlife zoonotic disease surveillance in the EU, and (iv) specific recommendations of surveillance aimed at risk based early detection of pathogens in the main wild species groups. We finally proposed (iv) a framework for integrating animal disease surveillance components (wildlife, domestic, environment) for early detection under OH approach.

Key words: Recommendations, sustainable surveillance, zoonotic pathogens, wildlife, monitoring, integrated, One Health, early detection.

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Summary

Background

The EU Commission has allocated specific resources for Member States (MS) for setting up a coordinated surveillance programmes (SPs) under the One Health (OH) approach for cross-border pathogens that threaten the Union. The *ENETWILD* consortium (www.enetwild.com) has recently reviewed and mapped surveillance systems and academic activities for emergent (and re-emergent) transboundary zoonotic disease in the EU in domestic animals, wildlife, and the environment developed by the different OH sectors (human, domestic animal, wildlife and environmental). EFSA recently guided the Animal Health and Welfare (AHAW) Network through a science-based participatory process (representatives of countries intending to apply for a direct grant, observers from the EC and ECDC, IPA countries, hearing experts and ECDC staff members) to identify priority zoonotic pathogens for the OH surveillance based on risk and surveillance criteria (feasible, implementable, beneficial, and constructive). Out of an initial list of 45 zoonotic pathogens proposed, the outcome consisted of 10 prioritized zoonotic diseases: highly pathogenic avian influenza, swine influenza, West Nile disease, tick-borne-encephalitis, echinococcosis (alveolar and cystic), Crimean Congo haemorrhagic fever, hepatitis E, Lyme borreliosis, Q-fever, and Rift Valley fever.

The main aim of this report is to formulate recommendations and technical specifications for sustainable coordinated OH surveillance for early detection of these zoonotic pathogens where wildlife is implicated. For this purpose:

1. First, we reviewed the cornerstones of integrated wildlife monitoring that are applicable to zoonotic disease surveillance in wildlife under OH surveillance in the EU;
2. We analysed the characteristics of the main wildlife groups and the selected pathogens relevant to surveillance aimed at early detection, and integrated with other health compartments;
3. We proposed (i) general recommendations for the first steps of sustainable wildlife zoonotic disease surveillance in the UE, and (ii) specific recommendations of surveillance aimed at risk based early detection of pathogens in the main wild species groups.

1. Integrating wildlife disease and population monitoring

We present the main principles, components, and steps of wildlife monitoring which are applicable to integrated wildlife population monitoring and disease surveillance under OH approach. An updated definition of integrated wildlife monitoring (or "OH integrated wildlife monitoring") is as a scheme that combines data from disease surveillance, and the monitoring of wild populations and all the biotic components of the ecosystem. Wildlife population monitoring is relevant to wildlife disease surveillance under an OH approach because (i) Wildlife monitoring provides key information for planning surveillance strategies of integrated monitoring (and therefore wildlife disease surveillance), (ii) sampling wildlife diseases, either passive or actively, requires the contribution of the wildlife sector, (iii) the analysis and interpretation of data is a crucial step that requires both population monitoring and disease surveillance contribution and expertise, and (iv) the communication to experts, professionals, and the public of the relevance of wildlife monitoring and the results obtained must be addressed commonly by the different compartments working under the OH approach. It is wise to coordinate the contribution of different sectors over the different phases of the monitoring in order not to overlap and the efforts as a function of their capacities and means. For that purpose, we recommend:

- To provide managers with information on the status and trends of wildlife populations before deciding on the appropriate design of integrated monitoring and next actions to take.
- To jointly evaluate the effectiveness of monitoring relative to the stated specific objectives. The feedback for learning about which actions lead to the success or failure of integrated wildlife monitoring is essential to specifically inform and improve monitoring in an adaptive management setting.
- Transparent detailed documentation and correct data management. Data standards are needed, which refers to the way in which we collect and record the information, following always minimum scientific-technical standards, which will also allow us to use the data from different sources and establish comparison (over space and/or time).

2. Wildlife disease surveillance under the OH approach for cross-border pathogens that threaten the European Union

The specific objectives of future coordinated zoonotic disease surveillance under the OH approach for cross-border pathogens that threaten the EU, depending on the epidemiological context, as established in EFSA discussions, are the early detection of:

- The onset and duration of the period of increased risk: This applies to known pathogens that are present in the MS but has seasonally changing risk that can vary in the time of onset of increased risk, and the severity of the risk from year to year. Therefore, surveillance is mainly targeting the risk period.
- Change in the geographic distribution/spread to new areas: known pathogen that is present in some geographic areas of the MS. Surveillance here is targeting risk areas.
- An increase in incidence, i.e., early epidemic detection: known pathogens that are endemic at a low (or constant) incidence in the MS, but epidemics may occur from time-to-time. Surveillance here is targeting risk areas, which can also be during specific risk period.
- The introduction of the pathogen: known pathogens that are not present within a MS. Surveillance can target risk period or risk areas or population at risk (e.g., those at borders, sentinel hosts, during winter migration of birds, etc.).

One can think that, a priori, targeted surveillance options for the list of selected pathogens would be the selected options to address these surveillance objectives. This approach indicates that at least 10 (actually 11) different targeted wildlife disease surveillance programs will be run in parallel. However, it is not practical to have only targeted (specific, usually based on active surveillance) surveillance programs for every disease or pathogen and a combination with general surveillance (which usually relies more on passive surveillance) is the best approach. This approach is less sustainable if the aim is to increase progressively the number of zoonotic pathogens to be harmoniously monitored in the EU. An approach incorporating also strategically general surveillance for these selected pathogens has potential to generate in a cost/effective way information that is needed to improve the current understanding, prevention, and control of certain zoonotic pathogens, but also to inform on other pathogens.

Regarding to the characteristics of selected zoonotic pathogens in wildlife that are relevant to disease surveillance, we observed that the suitability and relevance of primary objectives varied according to pathogens and their epidemiological situation in Europe:

- While most pathogens are already present in the EU, their spatial distribution and presence in potential hosts are relatively unknown, or it is known that pathogens are present in certain regions of Europe.
- Some pathogens (e.g., specific HEV subtype in wild boar and cervids) are relatively widespread, therefore, priority objectives are to detect an increase in incidence, i.e., early epidemic detection or the onset and duration of the period of increased risk. This is not at odds that where not previously detected, priorities should be detecting changes in the geographic distribution/spread to new areas, and the introduction of the pathogen.
- Some pathogens (e.g., HPAI) are probably in a phase of becoming endemic and re-incident after several incursions in the past years in Europe, a priority feasible objective in wildlife should probably progress towards detecting in increase in incidence, i.e., early epidemic detection, or the onset and duration of the period of increased risk.
- As for RVF, it was formerly regarded as an African animal disease, since 2000 RVF was detected for the first time outside the African continent, and up to date, no outbreaks have been reported in Europe. Therefore, priority objective all over Europe is early detection of the introduction of the pathogen.

The different relevance of specific objectives for different pathogens is challenging for the development of general surveillance addressing the entire range of pathogens, indicating that wildlife zoonotic disease programs should include pathogen specific approaches, design, and surveillance components, i.e., combining general and targeted activities.

We evidenced that, for the listed 11 pathogens:

- Except bats, all groups of considered species have potential to be used in disease surveillance, playing relevant epidemiological roles for the selected pathogens, and/or being useful as sentinels.
- Wild ruminants predominated as playing a relevant role (6 pathogens), being relevant as sentinels for several (4) pathogens, while only for 3 out of 6 pathogens were considered disease reservoirs.
- Wild carnivores were also relevant in terms of number of pathogens (5) they harbour, and also because of their potential role as sentinels (being in top of the trophic chain) was remarkable.
- Micromammals were relevant for 4 pathogens, as well as non waterbirds (migratory or not). To a less extent, wild boar (3 pathogens), water (3) bird and lagomorphs (2) were relevant to a low number of pathogens.
- These is lack of evidence on the epidemiological role and the utility for surveillance of West Nile disease and RVF in bats. It is relevant mentioning that bats are potentially primary hosts or reservoirs of 14.6% of the 50 pathogens included in the preliminary list of zoonosis.

3. <u>Recommendations for the first steps of sustainable zoonotic disease surveillance in wildlife in the EU.</u>

- First, zoonotic disease surveillance under the OH approach requires interdisciplinary collaboration across stakeholders in human, animal (including wildlife) and environmental health representatives at all stages of surveillance efforts (i.e., design, implementation, management, and evaluation), if not, the system will be ineffective, less sustainable, and short-lived. There is a need to conduct analysis and needs assessment of stakeholders involved in wildlife surveillance systems, at regional and national level.

- Future OH wildlife surveillance programmes in Europe should employ a combination of general (passive) and targeted (active) wildlife disease surveillance because it is cost-effective to address the surveillance of several pathogens concurrently rather than individual or separate targeted surveillance programs specific to each pathogen. Passive and active surveillance components would ideally take place simultaneously, but the final choice depends on the evaluation of their cost-effectiveness for specific hosts, pathogens, geographical and epidemiological contexts:
  - If passive surveillance is prioritized, emerging diseases will be detected, but monitoring and assessment of interventions will be limited. It requires a multi-actor passive surveillance network using available infrastructure and data sources (e.g., public participation through citizen science tools (Lawson et al., 2015) or information derived from road kills (Schwartz et al. 2020, Fenandez-López et al. 2022), and covering a broad geographical range, to ensure early detection of disease emergence.
  - When only active surveillance is prioritized, the early detection of emerging diseases may be compromised. An active sampling scheme targeting selected (prioritized) hosts and diseases must be flexible enough to enable an adaptive approach, continuously improving surveillance strategies for target populations and diseases by incorporating new information on host demography and disease prevalence (Belsare et al., 2020).
  - There is an important role of diagnostic pathology in passive surveillance for the identification of new or unexpected pathogens and diseases, which also requires choosing what additional diagnostic tests need to be carried out (such as bacterial culture, PCR for certain pathogens. Thus, it is particularly important that the countries to have, or if necessary, develop, adequate expertise and capacity in veterinary diagnostic pathology applied to general wildlife disease surveillance programmes and involve the contribution of relevant stakeholders, such as rescue centres.
  - As for active surveillance, *ante mortem* diagnostic tests are of limited value, depending on the pathogen, but especially for the host, since for many wildlife species test sensitivity and specificity have not been evaluated. Environmental detection of microbiological hazards is becoming a sensitive and cost-effective approach, but still needs to be developed for different pathogens and sampled matrices in order to become a reference technique for routine surveillance. This is relevant also to wildlife trade.
- There are multiple surveillance components (i.e., a single surveillance activity, defined by the source of data and the methods used for its collection, used to investigate the occurrence of one or more hazards in a specified population), the higher the number of them included, the higher the surveillance system sensitivity. Their selection of specific components in disease surveillance programs can be recommended for specific pathogen, hosts, epidemiological context and aims of surveillance in terms of cost-effectiveness. Under the OH approach, not only human and domestic animal, but wildlife and the environment component may need to be included in OH surveillance systems because they can serve as reservoirs of infection or infestation and/or as indicators of risk to humans and domestic animals. Therefore, an important and still needed discussion among the different compartments of OH is about the identification of criteria to guide the selection of zoonotic disease surveillance components (see section below).

- The nature, availability and sources of surveillance data may respond to different strategies, which are complementary. Under the OH approach, determining disease emergence, maintenance, and risk of transmission in multi-host communities is recommended, for which we need to focus surveillance on a diverse array of pathogens at once in a number of host community assemblages. This is the so-called "observatory approach" (see the European Observatory of Wildlife, https://wildlifeobservatory.org/). This network, ideally, should be designed under the premises of risk-based surveillance (see below) and incorporate a wide range of scenarios, and the more appropriate surveillance components to each case. Such a network addressing complex multi-host multi-pathogen systems offers the possibility to evaluate disease emergence not only when pathogens are found in new areas, but also to detect between-species jumps, and the emergence of new variants almost "in real time" (for which molecular tools are key), to report early and raise awareness about potential threats. The observatory approach therefore complements classical approaches which normally are fragmented in terms of target population (rarely entire communities of hosts) and pathogens are addressed, and opportunist spatio-temporal sources of samples/data.
- Risk-based surveillance approaches should be used and continuously informed by surveillance data as a cost-effective strategy addressing different components. This may be an especially important priority for initiating wildlife disease surveillance in settings where resources are limited. Namely, the main risk factors relate to:
    - Ecological and anthropological factors: epidemiological context (e.g., pathogen already present or not, just few incursions known, vectors present but not pathogen), risk period, variable multi-host communities, environmental and interfaces gradients, from natural areas, passing though farmland to urban and peri-urban scenarios. For a general surveillance strategy, covering all of them is an interesting initial option, which will be later improved through an adaptive process. The rapid intensification of agriculture, socioeconomic change, and ecological fragmentation have profound impacts on the epidemiology of zoonotic infectious diseases and the diverse wildlife-livestock-human interfaces must be included is surveillance strategies. These interfaces represent critical points for cross-species transmission and emergence of pathogens into new host populations.
    - Populations at risk, such as at the borders or wildlife migratory routes, in specific ecosystems/habitats (e.g., wetlands, bushlands, pasturelands), in specific interfaces (urban/peri-urban, farmland, nature/protected areas), and their combinations (stratified risk-based sampling). Traded wildlife (some are for hunting purposes) and exotic species must be considered as risky populations by definition.
    - Risk area, determined by previous risks, but also by purely biogeographic conditions, such as being in the border of EU at risk for a certain pathogen and/or vector. We recommend to map risk areas, for which it is needed to invest in the development of predictive species and pathogen modelling based on ecosystem data (e.g., mammal species richness, domestic livestock, their interfaces, and abundance, landscape changes) to map risk areas where to geographically target detection efforts. All this will allow to identify risk maps, which need to be continuously updated, for instance, about risk pathways and potential hot spots for zoonotic emerging wildlife diseases at the regional and national level.
- Where possible, initially select surveillance of wildlife at sites where:
    - Human or domestic animal surveillance is also occurring, which may help providing information on cross-species disease transmission risks.

  - Wildlife population and ecosystem monitoring are taking place, either highly available low precise data (such as hunting, which is available over large regions of Europe) or high precise density estimation data (observatory approach), ideally, a combination of both.
  - For that purpose and under the OH approach, applying the observatory approach to areas where the human and livestock interfaces are present is recommended.
- Sampling surveillance efforts for early detection, such as frequency of repeated sampling and its duration, can be adapted to specific pathogen risk and potential rate of introduction and spread of diseases within each MS.
- The efficiency of a sampling design greatly depends on the characteristics of the target population, often distributed over large regions of Europe (e.g., wild boar, rodents), even variably according to the period and year (e.g., migratory birds):
  - If the target population can be divided into different spatial units that are relatively homogenous in nature, then stratification of sampling by type would result in a more efficient sampling design and more precise prevalence/incidence/detection estimates by type.
  - As for rare species occurring at low densities often relevant as sentinels (e.g., wolves), one can maximize the number of observations by standardizing timing of surveys (time and season), when individuals are more visible increasing detection probability. Again, an adaptive sampling intensity of sampling is dependent on initial sampling results.
- Diagnostic tests should be selected on a host species-pathogen specific basis, and it must be guaranteed the sufficient capabilities of laboratories to conduct the testing of recommended sample type and methods
- Wildlife zoonotic disease surveillance sensitivity for early warning of zoonotic pathogens and cost-benefit of adopted strategies need to be continuously evaluated to be optimized.
- The continuous evaluation, including the monitoring of the implementation of agreed standards, will allow MS to take decisions based on cost-benefit as there are too many different scenarios and considerations at local level to be done.

The specific recommendations of surveillance aimed at risk-based early detection of zoonotic pathogens in the main wild species groups were:

- Farmlands (particularly outdoor) should be priority areas to be incorporated to sampling strategies for wildlife in relation to most pathogens of the list.

  <u>Hosts and pathogens</u>: all hosts and pathogens. The presence of wild ungulates, carnivores, and lagomorphs at the interface with farms, and subsequent contacts with livestock was particularly relevant as a risk and accounted for most pathogens included in the list. This risk also ranked high for other hosts, such as micromammals and birds. Wild ruminants were relevant to the highest number of pathogens (at least 6: TBE, *E. granulosus*, CCHF, HEV, Q-fever and RVF).
- It is essential to develop best possible initial mapping of pathogen (or threat) presence and distribution, at least for those already present in the EU and nearby countries, for further development of risks-based surveillance (planning and sampling). Most pathogens here listed are communicable, however the disaggregation of data sources, lack of harmonization and

interrupted data flow for these data, and neglecting published research, makes difficult to generate "live" maps on the distribution of pathogens over Europe.

Hosts and pathogens: all hosts and pathogens, considering that most pathogens already are variably present in the EU: as endemic (widely or in certain areas), few incursions only detected, or not present (RVF) but vector present.

- Currently, we are not ready to produce a complete range of good resolution maps of the spatial distribution of the wildlife/livestock interfaces in Europe, but only in some countries and for some species. If not at European level, at least countries should develop maps of the wildlife-livestock interfaces as a basis for designing future surveillance of zoonotic pathogens at such interfaces, paying special attention to outdoor livestock production (there is need to standardize the nomenclature of different types of production systems over Europe).

  Hosts and pathogens: all wildlife hosts (including domestic animals).

- The interface where direct and indirect contacts of wildlife with pets and humans occurs (outdoor recreational activities, farms, peri-urban areas, and parks) is a priority target for disease surveillance in most wildlife groups and should be considering during surveillance planning phase. The different stakeholders involved in surveillance in this interface (local veterinary services, rescue centres) and citizens should play a coordinated role.

  Hosts and pathogens: all hosts, pathogens, and vectors. Vectors that are maintained by wild hosts (ticks and mosquitoes), direct or indirect transmission, such as birds and HPAI, the presence of parasitic stages in the environment (e.g., *E. multilocularis* associated to peri-urban rodents and fox). Vector borne pathogens in the case of rodents, or for indirectly transmitted pathogens such as Q-fever in both rabbits and rodents. Micromammals may play a relevant role as sentinels at this interface.

- Regarding the risk posed by vectors where infected wildlife is present, efforts are needed to map at the finest possible resolution and at large biogeographical scales where hosts and vectors distribution overlaps, and to determine at local level, the habitat, land uses and features where both vectors and host sampling is recommended. This information will provide a solid background for sustainable vector borne zoonotic disease surveillance in the future.

  Hosts and pathogens: all hosts and those pathogens which are vector borne (and vectors).

- A gradient of wild host biodiversity should be considered in surveillance planning. The EOW (https://wildlifeobservatory.org/), involving a wide range of scenarios (potentially including or coordinated with the Natura 200 network of protected areas) offers a possibility to work on this matter with a European perspective, since diverse well distributed host communities over Europe are included.

- Hosts and pathogens: all hosts and pathogens (and vectors), of special relevance multi-host pathogens.

- Therefore, a necessary first step for design disease surveillance strategies is mapping both abundance and management schemes of wild species over Europe, using standards for data collection to incorporate wildlife abundance to disease surveillance planning (i.e., as *ENETWILD* initiative does).

  Hosts and pathogens: all hosts and pathogens.

- Wildlife zoonotic disease surveillance should target where direct contact of wildlife with hunters, and consumers of meat are present. The possible role of wildlife as a source of zoonotic cases, such as HEV, should be listed in European reports on zoonosis. This can be the basis for prioritizing surveillance strategies in wildlife.

Hosts and pathogens: all hosts and pathogens. Relevant are wild host species which are consumed and prone to carry zoonoses, such as wild boar.

- More evidence is needed on the potential role and practical use of wild species as potential sentinels for early detection of zoonosis, however their inclusion in wildlife zoonotic disease surveillance is recommended.

  Hosts and pathogens: all hosts and pathogens. Relevant are species carnivores which are on top of food chain.

- Wetlands and breeding grounds habitats/areas, and at larger scale, EU borders and where bird migration paths overlaps, especially where recent outbreaks occurred in neighbour countries are essential risk to be considered in surveillance sampling design.

  Hosts and pathogens: migratory birds and avian pathogens (HPAI). In these areas, non-migratory birds, and other species (e.g., predators) may play role as bridge hosts.

We finally proposed a frame for integrating animal disease surveillance components (wildlife, domestic animals, environment) for early detection under OH approach. In general, surveillance is aimed at demonstrating the absence of infection, determining the presence, distribution or introduction of infection, or detecting exotic diseases or emerging diseases as early as possible before they spread, cost human lives, economic, social, environmental damage and become difficult to control. An effective surveillance system may include one or more component activities that generate information on the health or disease, zoonosis in this case. Under the OH context, the early detection of zoonotic pathogens requires continuous robust and diverse components for early warning and response. Therefore, initially there is a need to select the components that are more effective to achieve the objectives and to prioritize data sources, considering the limitations of resources. Not only human and domestic animal, but also wildlife and the environment need to be included in OH surveillance systems because they can serve as reservoirs of infection or infestation and/or as indicators of risk to humans and domestic animals, and they can serve to detect pathogens earlier. This should also investigate the politics of National Reference Laboratories being the only way to (officially) report notifiable diseases, as this may limit international surveillance. Equally unvalidated and non-OIE (WOAH) approved tests may be used, producing uncertain results. One difficult area may be eDNA sampling for pathogens which may produces positive un-validated results and not occur on a potential infected premises. These aspects may not be official positive cases but would benefit from being captured in some way.

Following, some criteria to guide the selection of zoonotic disease surveillance components adapted to objectives and prioritizing cost/efficiency are identified, for which we propose the following scheme:

I. Define main targets for the respective pathogens, which is our case is early detection of:
  - Change in the geographic distribution/spread to new areas
  - The introduction of the pathogen
  - An increase in incidence, i.e., early epidemic detection
  - The onset and duration of the period of increased risk

II. To initiate the evaluation and optimization of a surveillance system, all surveillance system components need to be identified and their utility to the aims described. This step is essential and must be addressed/discussed jointly by the different health compartments for all priority pathogens.
  - Sensitivity:

- In which component of OH systems can we achieve the earliest detection?
  - Human (active/passively?)
  - Livestock (active/passively?)
  - Pets (active/passively?)
  - Wildlife (active/passive?)
  - Environment (active/passively?)
- The ability to detect at least one positive unit given that the population is truly infected (considering that the sensitivity of surveillance components depends on the level of disease in the population).

- Costs (comparing different options): economic, technical, and logistic aspects. Cost/effectiveness can be evaluated based on previous parameters.

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1. Background

1.1. Terms of Reference as provided by the requestor

This contract was awarded by EFSA to Universidad de Castilla-La Mancha, contract title Wildlife collecting and sharing data on wildlife populations, transmitting animal disease agents, contract number OC/EFSA/ALPHA/2016/01 – 01.

The terms of reference for the present report (specific contract 10, task 7. *Ad hoc* requests in systematic literature review, scientific and technical advice on targeted wildlife surveillance), are, as indicated in deliverable 2.5 "Formulating recommendations and technical specifications for sustainable surveillance of zoonotic pathogens where wildlife is implicated". The deliverable had to consist of a scientific report listing recommendations as in ToR.

1.2. Scope of the report

The *ENETWILD* consortium (www.enetwild.com) implemented an EFSA funded project in which the main objective has been the harmonization and collection of information regarding the geographical distribution and abundance of wildlife and wildlife diseases throughout Europe.

The EU-Commission has allocated specific resources for EU Member states (MS) for setting up a coordinated surveillance programmes (SPs) under the One Health (OH) approach for cross-border pathogens that threaten the Union. In this context, the tasks requested by EFSA to *ENETWILD* under specific contract 10 are to identify, describe and learn lessons from existing coordinated/collaborative disease surveillance. *ENETWILD* has recently reviewed and mapped surveillance systems and academic activities for emergent (and re-emergent) transboundary zoonotic disease in the EU in domestic animals, wildlife, and the environment developed by the different OH sectors, namely, human, domestic animal, wildlife and environmental (*ENETWILD*-consortium et al. 2022a, b).

The OH subgroup of EFSA's Animal Health and Welfare (AHAW) Network has guided a science-based participatory process (representatives of countries intending to apply for a direct grant, observers from the EC and ECDC, IPA countries, hearing experts and ECDC staff members) based on risk and surveillance (feasible, implementable, beneficial, and constructive) criteria to identify priority zoonotic pathogens for the OH surveillance. Out of an initial list of 45 zoonotic pathogens proposed, the outcome consisted of 10 prioritized zoonotic pathogens, i.e., Highly Pathogenic Avian Influenza, Swine Influenza, West Nile Disease, Tick-Borne-Encephalitis, Echinococcosis (alveolar and cystic), Crimean Congo Haemorrhagic Fever, Hepatitis E, Lyme Borreliosis, Q-fever, Rift Valley Fever.

To address next steps, this report formulates recommendations and technical specifications for sustainable coordinated OH surveillance of these zoonotic pathogens where wildlife is implicated. For this purpose, first we review the cornerstones of integrated wildlife monitoring that are applicable to zoonotic disease surveillance in wildlife under OH surveillance in the EU. Thereafter we analyse the characteristics of the main wildlife functional groups and the selected pathogens relevant to surveillance aimed at early detection and integrated with other health compartments.

By wildlife, we refer to feral animals, captive wild animals with the aim to be released (game animals), and wild animals. Feral animal is an animal of a domesticated species that now lives without direct human supervision or control.

1.3. Emerging infectious zoonotic diseases and wildlife in Europe

1.3.1. The European scenario

Europe is the Western part of the Eurasian supercontinent. It extends from Iceland in the West to the Ural Mountains in the East and from Artic Islands in the North to Mediterranean coastal areas in the South. Throughout Europe, habitat change has been significant during the last 3000 years, with deforestation as a historically dominating feature (Kaplan et al. 2009). Land use changes are still going on at a high rate, and it is estimated that annually 0.5% of the whole European territory changes its use between categories such as pasture, agriculture, forest, or urban and industrial. In the last 60 years however, deforestation has been reversed and forest surface has grown in most if not all European countries (Fuchs et al. 2015). These massive changes in habitat, along with agricultural intensification and human population growth (>742 million inhabitants in 2018, 34/km^2, 74% urban; http://www.worldometers.info/world-population/europe-population/) have had significant effects on the European wildlife communities.

Biodiversity loss due to human-mediated habitat change has been more intense in Europe than in other less densely or more recently populated regions of the world. In general terms, opportunistic species that benefit from anthropogenic habitat change such as the red fox (*Vulpes vulpes*) or some urban and coastal bird species have seized the opportunity represented by these changes and have greatly increased their numbers. Rural abandonment and growing woodland and scrubland habitats, along with agricultural intensification, favour the population growth of the native Eurasian wild boar (*Sus scrofa*) and several wild ruminants (Milner et al. 2006, Massei et al. 2015), often leading to overabundance and conflicts with agriculture including sanitary risks (Gortázar et al. 2006). Large predators are recovering almost at a European wide level due to this population explosion of their prey (Chapron et al. 2014). By contrast, specialist species and lowland species which are more susceptible to modern agriculture and habitat loss are in general terms declining (Donald et al. 2001). These changes imply that a few actors, including several carnivores, most ungulates and relatively few highly adaptable bird species, become the main wildlife species to consider at the European wildlife-livestock interface and this may be leading to some vector (ticks) overabundance. Driven by the changes in habitat and animal populations, as well as in human behaviour, there is an emergence and/or re-emergence of infections shared between wildlife and livestock and considering that some of them are zoonotic (e.g., tick borne), an increased impact of wildlife health on human health.

Infectious diseases also pose a threat to wildlife populations and biodiversity and are perceived by the society as real risks to wildlife conservation. Recently, *ENETWILD* has reviewed the endangered wildlife hosts in Europe that may be affected by the selected pathogens. We elaborated a list of potential endangered wildlife hosts distributed in Europe for each pathogen, which were sorted following their taxonomic classification and hosts species were classified as a function of their conservation status (*ENETWILD* consortium 2022c).

1.3.2. The One Heath perspective in Europe

Europe is highly human-density populated area and has a major dairy, beef, and pork production, it also holds significant poultry, sheep, and goat populations. In 2016, half of the EU-28 livestock units (LU, a reference unit which facilitates the aggregation of livestock from various species and age as per convention, based on nutritional requirements) consisted of cattle, one quarter of pigs and one sixth of poultry. Improved monitoring of livestock and large-scale trends are needed to elucidate interfaces and evaluate broad-scale risks in Europe, for which high-resolution data and discriminating among farming systems would be required. As illustrative of the need for better, harmonized, and standardized data in the domestic compartment, recent literature suggests low reliability when predicting the wild boar-pig interface (irrespective of farming type) at European scale (*ENETWILD*-consortium et al. 2020, 2021).

It is well known that the majority of emerging infectious diseases are zoonoses with the predominant source shown to be wildlife (Jones et al, 2008). The abovementioned massive changes in habitat and human population growth have had significant effects on European wildlife communities. Rural abandonment and growing woodland and scrubland habitats, along with agricultural intensification, favour the population growth of a few successful species, including several carnivores, most ungulates and relatively few highly adaptable bird species. Some of them, as discussed in this report, are the main wildlife species to be considered at the European wildlife interfaces (with domestic animals and humans).

Until now wildlife diseases have garnered national authority's attention mainly when a communicable disease is involved. A few shared diseases have a strong impact on the European economy, with implications beyond the wildlife and livestock sectors. Among other actions, reverting the current abundant wildlife population trends requires management options which are not always easily accepted among stakeholders and the public. In fact, Europe is the historical source of animalism, and the so-called Bambi-syndrome (an objection against killing of animals perceived as "cute" and little or no objection to the suffering of animals perceived as less desirable, Martínez-Jauregui et al. 2020, Brock 2015) generates strong debate wherever wildlife is harvested for hunting purposes or culled as a management tool or an intervention for disease control. However, many reports clearly highlight the new challenge played by wildlife diseases for the One Health perspective in Europe. As stated above, Europe is a highly populated continent with a huge number of livestock and pet animals, but also, in the last decades, a significant increase in many wild species abundance and distribution. This is the heritage of centuries of human activities (practical and cultural) that is still in progress, and we are facing a new era where increased rural protection areas and rewilding, with the consequent increase in many wild species, will result in a more fragmented landscape with an increment of suburban areas that will boost the overlapping of wild and domestic animals and of animals and humans for potential pathogen transmission. Land use and climatic changes are also reshaping vectors distribution and abundance and, apart from the case of sandflies and leishmaniasis, also mosquito driven infections, such as West Nile Virus, has increased in the last decades due the introduction of new mosquito species. Ticks and tick-borne diseases are a health issue of greater concern as it has been shown that up to 75% of pathogens found in ticks collected from dogs are of sylvatic origin (Zanet et al. 2020) and that a high prevalence of zoonotic *Babesia* species, with wildlife as reservoir, have been found in ticks collected from humans (Battisti et al. 2020). The spread of *E. multilocularis* towards many new countries all across Europe, up to the Scandinavian peninsula represents another example of the new scenario where the introduction of a competent alien reservoir, the raccoon dog, along with the natural movement of foxes and the transport of domestic dogs that can act as competent definitive host have resulted in an expanded range, exacerbated by the increase of the red fox and rodent populations

It is not the exception that climatic change in Europe is an issue for the emergence of pathogens at animal interfaces. For instance, climatic changes let some vectors, such as sand flies, to survive also in continental and climate areas (Ferroglio et al. 2005), and subsequently favour the transmission *Leishmania*. This is an expanding zoonotic vector-borne disease that is important in lagomorphs, and also for wild canids and domestic dogs. Lagomorphs (hares and the European wild rabbit) has been recently demonstrated to be a maintenance host for *Leishmania infantum* (Jiménez et al. 2014).

To sum, it is of great concern the impact and increase of wildlife-sourced zoonoses on human populations as globalisation, climate change and ecosystem alterations bring people and wildlife into closer contact. Importantly, emerging infectious diseases in Europa have arisen in wildlife, from within the country and abroad. For these reasons, Europe need to implement a general wildlife health surveillance system or zoonosis integrated into OH surveillance to enhance the early detection and characterization of microbial agents potentially involved with emerging diseases in free-ranging wildlife populations. Domestic animals and humans.

The long-term commitment to One Health practices and policy development is essential to sustainable progress towards inter-sectoral collaboration, but challenged by 'short-term' project funding models and mechanisms

1.3.3. Wildlife diseases in zoonotic disease surveillance programs in the European Union

A recent report by *ENETWILD* (*ENETWILD*-consortium et al. 2022a, b) described and mapped the main existing structures and systematic initiatives, for surveillance of zoonoses (transboundary, emerging and re-emerging) in domestic animals and wildlife in the EU. It included all types of surveillance activities even if only one sector was involved (human, domestic animals, wildlife and/or environment), excluding foodborne diseases and antimicrobial resistance. Results based on a questionnaire on official surveillance (distributed by EFSA to Members and Observers of EFSA Animal Health and Welfare Network), explored (i) the general organization of the surveillance programs (SPs), and (ii) the target pathogens, host species and methods for surveillance. Data was collected at SP level, normally several of them per country, each coordinated by one or multiple institutions belonging to one of different health sectors (animal health, public health, environmental authorities), with variable objectives and focusing on different pathogens (of different nature and epidemiological characteristics).

The analysis of the questionnaire on official surveillance revealed that the integration between sectors is not generalized, which is a necessary step to develop OH surveillance for such multi-host transboundary zoonotic pathogens. SPs are mainly applied and funded at the national level, however a OH approach ideally requires an international approach since pathogens, risks and determining factors cross borders. Despite the relevance of wildlife in SPs, wildlife surveillance still seems to be unrepresented. A relevant exercise to evaluate and improve future European SPs was to compare the actual sampled hosts/reservoirs species in SPs and the primary hosts/reservoirs for the selected pathogens (even though for some pathogens are not completely known yet). Domestic animals are among those more frequently sampled by SPs, but they are not always the preferential or main hosts for most pathogens of the list, and the opposite occurs for wildlife. Wild mammals predominated as the main potential reservoirs for the selected list of pathogens. Some of them, such as wild ungulates and certain carnivores, are widely distributed all over the continent and are involved in conflicts including shared diseases with livestock and humans. This situation requires a common transboundary approach over Europe. A relevant proportion included wild birds as main hosts (about 30%), many of which are migratory and may carry pathogens all over Europe and beyond. This reinforces the need of coordinated SPs in the continent as pathogens cross borders.

Among the main recommendations for further implementing OH surveillance in this report we remarked:

- The integration between sectors (human, animal, and environment health) is a necessary step to develop OH surveillance. Moreover, more efforts should be made to plan surveillance and coordinate and integrate approaches at an international level.
- Considering the specifics of each pathogen group, hosts (reservoirs), potential source, access, types of samples and costs (normally lower for passive surveillance), a general framework needs to be developed to design best strategies (active and passive surveillance) shared among sectors.
- The sampling design of the reviewed SPs predominantly included risk-based sampling (vs random and random stratified), which requires relevant prior knowledge.
    - Therefore, a structured approach is needed to determine priorities for surveillance and the approach to be used in European surveillance schemes to achieve a higher benefit-cost ratio with existing or reduced resources.
    - Transnational research and collaboration of sectors (i.e., governmental and supranational health institutions and Academia) and countries based on their respective expertise would help to this aim (need for data and expertise sharing).
    - High quality (spatially precise) information for livestock at European level is needed to assess risks (such as the interface with wildlife) and subsequent risk-based sampling. However, this information is not available at European level at sufficient resolution and must be openly shared by countries.
    - Wildlife population monitoring (integrated surveillance) is also essential to develop risk-based surveillance.
- Comparison of the actual sampled hosts and the primary known reservoir species for the selected pathogens is needed to evaluate and improve future European SPs. Overall, a first exercise revealed that wildlife, the main reservoir host for most zoonotic pathogens, is underrepresented in current SPs. Wildlife under-represented in current surveillance schemes, particularly mammals, namely rodents and bats, and to a less extent, wild ungulates, and carnivores, should be included in SPs.
    - There is a need to involve more wildlife and environmental institutions to increase feasibility of surveillance. These institutions have the technical ability, knowledge, and expertise to develop active and passive surveillance and can also provide means and logistics, which, however, need improvement.
    - Concerning passive surveillance, wildlife disease professionals can assess clinical signs and pathology, the preliminary clinical-pathological diagnosis guides the correct selection of samples/organs and of pathogens to be tested. Testing of animals found dead or with clinical signs provides a higher chance of detecting the pathogens as compared with testing of healthy animals. Passive surveillance is very important for the early detection of new diseases/pathogens and finding dead wild animals may be the first indication of introduction of a pathogen.
    - Regarding active surveillance, the hunting sector, as well as wildlife management and environmental agencies have access to samples from apparently healthy animals, which may carry subclinical/unapparent infections.
    - For all the above, guidelines/protocols, means and reliable diagnostic tests are needed, since these are not normally evaluated on wildlife species.

2. Integrating wildlife disease and population monitoring

This section presents the main principles, components, and steps of wildlife monitoring which are applicable to integrated[2] wildlife population monitoring and disease[3] surveillance under OH approach. Wildlife monitoring, broadly speaking, refers to the planned, normally regular, observation and recording of information to show how wild species, population, community, ecosystem, or disease parameters progress over time, usually (and recommended) following a long-term approach (Barroso et al. 2021). Wildlife monitoring is often used to refer only to wildlife population monitoring; however, this review proposes a broader approach, illustrated in Figure 1. The classical concept of integrated wildlife monitoring[2] (IWM, Cardoso et al. 2022), under the OH approach, needs to be extended to include the whole biotic component of the ecosystem. An updated definition of **integrated wildlife monitoring** (or "OH integrated wildlife monitoring") is as *a scheme that combines data from disease surveillance, and the monitoring of wild populations and all the biotic components of the ecosystem.* For instance, it is well known the impact of diseases goes beyond individuals and species, shaping host communities, which in turn has direct and/or indirect impact on disease-risks. At the highest level, **Integrated OH monitoring** also includes the abiotic environment, and the human, and domestic animal components.

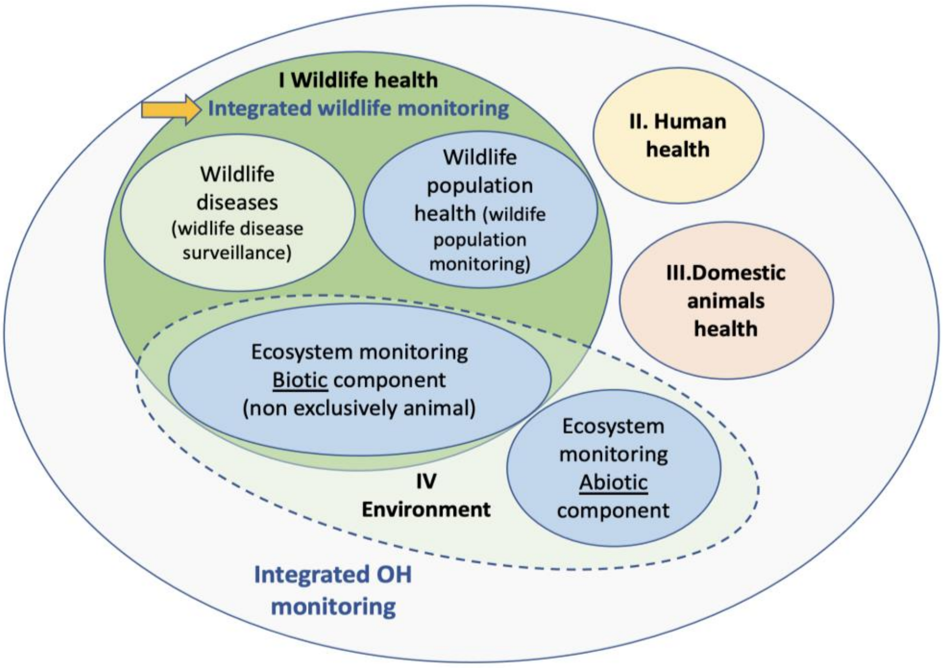

Figure 1. An extended definition of integrated wildlife monitoring (or "OH integrated wildlife monitoring") as a *scheme that combines data from disease surveillance, and the monitoring of wild populations and the biotic components of the ecosystem*.

[2] Definition of integrated monitoring (recently reviewed by Cardoso et al. 2022) a scheme that combines data from passive and active disease surveillance with population monitoring.
[3] "Wildlife disease surveillance" is used here in a broad sense since may also refer to pathogen surveillance in wildlife, given that infection with pathogen(s) may not always produce visible clinical signs associated with disease in a given species or at a given point of time.

From a holistic OH point of view, wildlife disease surveillance is also part of monitoring domestic animal, human and the environmental health (including population/ecological aspects of the biotic component), and all these aspects should not be independently addressed. The robustness of any monitoring system is enhanced by the variety of its data sources and number of components, and always, a systematic, planned, and rigorous approach is essential. This holistic approach becomes more complex, but also more necessary as new components and sectors (human, domestic animal, wildlife, environment) need to work together.

Wildlife population monitoring is relevant to wildlife disease surveillance under a OH approach because:

- Wildlife monitoring provides **key information for planning surveillance strategies** of integrated monitoring (and therefore wildlife disease surveillance). Cost-effective risk-based approaches, as well as the determination of where to perform sampling, need to consider population parameters, such as wildlife host community composition, distribution, abundance, and behaviour (e.g., migratory routes in birds). Determining the spatial distribution and characteristics of the animal interface where wildlife is involved is particularly relevant for the surveillance of transboundary emergent pathogens, such as those at the wildlife/livestock interface (*ENETWILD*-consortium et al. 2021, 2022). Wildlife monitoring is a key component of OH disease surveillance, both at local and large geographical scales. An example of a combined approach incorporating local precise population estimations over a network of representative areas in Europe, is the European Observatory of Wildlife (EOW, https//wildlifeobservatory.org/). Such as network of monitoring areas presents an enormous potential to assess population and epidemiological trends of wildlife zoonotic diseases, being also extraordinary network of (sentinel) host communities for early detection of pathogens. It also may address what is going on at the interfaces of wildlife with domestic animals and humans.
- **Sampling wildlife diseases**, either passive or actively[4], requires the contribution of the wildlife sector (managers, wildlife professionals, conservationists, hunters, rescue centres), which is the sector responsible for population monitoring. No strategy aimed at improving the sensitivity of early detection will succeed without the participation of the wildlife sector. This is relevant for routine surveillance, but also in case of urgent epidemiological contexts, such as outbreaks and rapid spread of diseases. In such situations, there is a need to react in terms of intensive disease surveillance, geographical delimitation of the problem (e.g., local distribution of wildlife hosts) and decision making for outbreak constraint.
- The **analysis and interpretation** of data is a crucial step that requires both population monitoring and disease surveillance expertise. Data analysis should be based on coordinated distribution of roles and proper data management. By jointly addressing the analysis and interpretation of population and epidemiological data, an **adaptive approach[5]** will be possible. Wildlife monitoring should be flexible enough to adapt and be able to introduce changes in the objectives and/or on how monitoring is performed in order to improve the system. Wildlife disease surveillance may need to shift target species, areas, or risks-based

[4] Active surveillance is defined as investigator-initiated provision of health-related data, while passive surveillance refers to observer-initiated provision of health-related data (https://www.fp7-risksur.eu/terminology/glossary).

[5] Adaptive monitoring: An essential characteristic of monitoring is that, through its application and evaluation, it improves itself, while it informs the stakeholders and sectors involved. Wildlife monitoring must be is informative to adopt an adaptive management mode making informed decisions, continually adjusting to objectives and resources, and making the model more efficient, effective, or practical over time.

design to continuously improve early detection because the epidemiological context changes or the results of the surveillance program may not be completely satisfactory. All these adjustments must also consider variable regional contexts. Fluctuations in population size and disease lead to bias in surveillance-based estimates of prevalence and the power to detect disease (Walton et al, 2016). Therefore, neglecting such integration may lead to poorly designed surveillance and ultimately to incorrect assessments of the risks posed by disease in wildlife, and subsequently to domestic animals and humans.

- The **communication** to experts, professionals, and the public of (i) the relevance of wildlife monitoring (inc. disease surveillance), (ii) the role of different stakeholders, and (iii) the results obtained must be addressed commonly by the different compartments working under the OH approach. Otherwise, there can be a risk of losing interest by the sectors involved in wildlife monitoring, and their engagement to support routine monitoring as well as during disease outbreaks. A lack of coordination among sectors participating in wildlife monitoring and delivering incorrect messages to stakeholders may lead to unsuccessful policies.

2.1. Integrated wildlife monitoring: a systematic and rigorous approach

Only the information collected through adequately executed monitoring of wildlife can be later analysed, interpreted, and finally become useful for further management of wildlife and their diseases with a technical and scientific basis. All monitoring, including wildlife disease surveillance, must guarantee a correct design and subsequent statistical analysis, and, ultimately, the usefulness of the results obtained, otherwise it would not be truly monitoring/surveillance, it would be for example simply data collection, therefore:

- The objectives of monitoring should be clear and defined, but it is also a process that can be adapted to changing contexts (e.g., host, vectors, and pathogens distributions, new emergent threats, etc.);
- The design (e.g., random, stratified, risk based, and their combinations), their components (what to monitor), and applied methodology should be appropriate;
- There should be a coordinated distribution of activities and roles;
- Throughout the process, the way samples/data are collected, processed, analysed, and reported follows minimum scientific-technical standards (including data standards), which will also allow establishing comparisons (standardized harmonized monitoring), including data compatibility between health sectors, and the reporting of negative samples.

While wildlife monitoring is a systematic and rigorous approach; it should not at odds with the fact that it can be applied routinely and easily. Its design must be as simple as possible, with simple measures or protocols, unequivocal as far as possible, and replicable (https://wildlifeobservatory.org/guides-and-population-density-cards). Wildlife monitoring must be informative and flexible to adopt an adaptive management mode making informed decisions, continually adjusting to objectives and resources, and making the model more efficient, effective, or practical over time. In this way, monitoring does not lose its true essence being useful for early detection of changes and subsequent informed management. Applying minimum standards for the statistical design of monitoring will not only ensure a minimum level of rigor and thus usefulness of the results, but also improve cost-effectiveness in the long-term (Yoccoz et al. 2001, Williams et al. 2002).

2.2. Recommendations for integrated wildlife population monitoring and disease surveillance

The objectives of integrated wildlife monitoring will determine which population and diseases related variables to measure, such as, the pathogens to detect and the range of hosts (incl. the environment), and how to do it. To do this, alternative objectives must be considered, as well as technical capabilities and resources available by different sectors. Integrated wildlife monitoring requires multidisciplinary teamwork over the different phases of the process, information gathering, a design appropriate to the objectives, selection of methodologies, subsequent application/sampling by a team/s of people or sectors that carry it out, analysis, and dissemination.

The questions of interest to be answered by integrated wildlife monitoring will determine the scale at which the monitoring will initially be set out, as well as the frequency and nature of the sampling, and, therefore, the sensitivity, accuracy, and precision considered sufficient for the surveillance estimates. From this starting point, it will be possible to calculate the resources needed to carry out the sampling by the different sectors, at least initially with the possibility for adjustments as monitoring progresses. Because resources are scarce, the methods and specific objectives will be readjusted to what is can be affordable and useful. It is advisable that different sectors coordinate their contribution over the different phases of the monitoring in order to avoid overlap of efforts in relation to capacities and means. For that purpose, WE RECOMMEND:

- To provide managers with **information on the status and trends of wildlife populations** before deciding on the appropriate design of integrated monitoring and next actions to take. This includes these types of wildlife population/ecological parameters:
    - Distribution in terms of occurrence and occupancy[6], inc. early detection, for instance, for alien invasive species that may host exotic diseases, or new spatial range for any host species
    - Community and host species richness and composition
    - Abundance, preferably density
    - Behaviour

Occupancy surveys or 'presence-absence surveys' involve sampling methods that require multiple visits to sites during an appropriate time-period when a species may be detected. The patterns of detection and non-detection (presence/absence) over repeated visits permits estimation of detection probability and the parameter of interest, proportion of sites occupied. The information collected is essential to plan wildlife disease monitoring. The visits to sites can be used to co-ordinately collect samples, such as sera, tissue, vectors, or environmental samples. Observers developing wildlife population monitoring may also be key to the passive surveillance by warning about the presence of death or sick animals or manifesting signs of disease. European frameworks, such as *ENETWILD* (www.enetwild.com; funded by EFSA), can already provide national and European managers with information on the status and trends of wildlife populations.

[6] Extent of occurrence is defined as the area contained within the shortest continuous imaginary boundary that can be drawn to encompass all the known, inferred, or projected sites of present occurrence of a taxon, excluding cases of vagrancy. Area of occupancy is defined as the area within its extent of occurrence, which is occupied by a taxon, excluding cases of vagrancy (e.g., the sum of the occupied grids). The measure reflects the fact that a taxon will not usually occur throughout the area of its extent of occurrence, which may contain unsuitable or unoccupied habitats. Definitions by the International Union for the Conservation of Nature (IUCN, 2001).

- To **jointly evaluate the effectiveness of monitoring** relative to the stated specific objectives. The feedback for learning about which actions lead to the success or failure of integrated wildlife monitoring is essential to specifically inform and improve monitoring in an adaptive management setting. This is useful to evaluate proactive or preventive actions under OH approach when the impacts are still minor, to be more effective in the response and to save health, economic, social, and environmental costs.
- **Transparent detailed documentation and correct data management**: In all these phases (design, sampling, analysis, communication), it is essential to have the documentation and correct shared data management by all sectors involved in integrated wildlife monitoring. All aspects of the monitoring program documented and stored in a defined, coordinated, and accessible place that reflects the objectives of the monitoring program, its design, protocols and data collection methods, analytical techniques, standards. Monitoring programs must be adaptable and may change as new techniques (e.g., eDNA) develop and more information becomes available, which will be reflected in this documentation.
    - A field **data storage and management** system is required to ensure that the integrity, traceability, and original quality of the data are maintained, so population and disease data can be paired at any stage. Essential and feasible data collection requirements should be determined before a programme is initiated to achieve wildlife integrated monitoring goals. As for wildlife disease surveillance, at least a minimum level of data should be collected; for example, data should be recorded on the disease incident or sampling event, date, latitude and longitude coordinates, observation of mortality or sickness, specimen identification numbers, animal species, laboratory identification numbers, and diagnosis(es) with associated detection method (WOAH 2014, 2015).
    - The traditional notebooks or printed forms continue to be the main working format; however, a system is needed that transcribes this data into an electronic format. The recent development of **Information Technologies** has allowed the appearance of more and more APPS of great value applied to facilitate the collection and management of information (e.g., SMART, citizen science app such as *iMammalia*), since the data is digitized from the field, it is easier to take certain formats of data, such as images, and involving new groups willing to use these apps. However, we must be aware that apps are tools available to monitoring programs, and their design and use must respond to the objectives and approaches of the program. In other words, the apps must be compatible with an appropriate design and methodology, and do not replace the need to set up a well-studied monitoring program with clear objectives. If not, we run the risk that the information collected will not be useful for decision-making regarding management of wildlife populations and diseases, which must have a technical and scientific basis.
    - **Data standards**, which refers to the way in which we collect and record the information, following always **minimum scientific-technical standards**, which will also allow us to use the data from different sources and establish comparison (over space and/or time).

3. Wildlife disease surveillance under the OH approach for cross-border pathogens that threaten the European Union

As summarized in Figure 2, this section, which is central in this report, analyses the characteristics of hosts and pathogens relevant to wildlife disease surveillance under the OH approach for cross-border pathogens that threaten the EU. First, the objectives (agreed with EFSA) are stated, which focus on early detection of pathogens under different circumstances (epidemiological, risk, spatial and temporal contents). Thereafter, we describe (i) the characteristics of selected zoonotic pathogens in wildlife and (ii) the characteristics of the main wild species groups and their environments which are relevant to early detection in disease surveillance. These aspects are then integrated (iii) to characterize the risks relevant to early detection for the selected zoonotic pathogens in the main wild host groups, as a basis to develop cost-effective strategies of wildlife disease surveillance aimed at early detection of zoonotic pathogens. Finally, in the next section of the report, we provide general recommendations on wildlife disease surveillance aimed at early detection of pathogens, as well as host by pathogen specific recommendations.

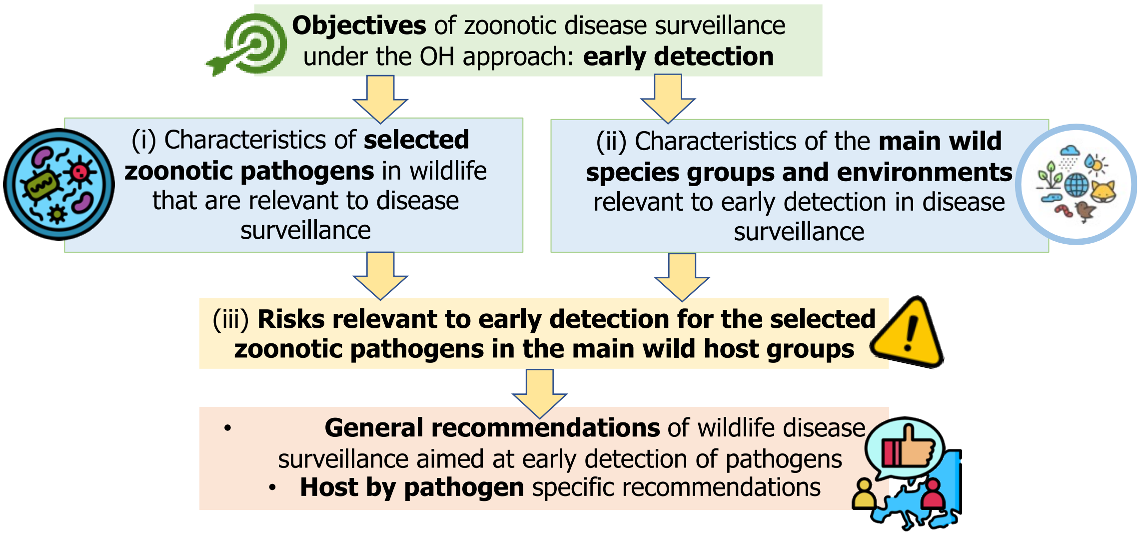

Figure 2. Scheme on how this section addresses the elaboration of recommendations on wildlife disease surveillance under the OH approach for cross-border pathogens that threaten the EU.

3.1. Objectives of zoonotic disease surveillance under the OH approach

Surveillance is key to obtain reliable information and subsequently prevent or control diseases, whether to monitor endemic infections already in place (e.g., Ryser-Degiorgis 2013) or to detect introduction of emerging or re-emerging diseases (e.g., Lipkin 2013), such as several zoonotic pathogens considered in the present report. Carrying out disease surveillance of wildlife contributes to early detect the presence of transmissible pathogens and to quickly adopt counter measures (Morner et al. 2002).

The **general objectives** that normally wildlife disease surveillance programs incorporate are (WOAH 2014, 2015):

- To provide information on wild animal prevalence/incidence of pathogen detection, morbidity, and mortality;
- To identify changes in patterns of disease occurrence over time, space, and other considered factors;
- To assist in early detection of pathogen presence/disease outbreaks, including those linked to emerging diseases.

The specific objectives of future zoonotic disease[7] surveillance under the OH approach and considering the list of 10 zoonotic pathogens proposed (11 if we consider *E. granulosus* and *E. multilocularis* separately), **are mainly focused on early detection of such pathogens**, most of them being already present in the EU with variable distribution patters.

We are primarily focusing on a relatively reduced number of zoonotic pathogens in wildlife, which can be subject to both general and targeted surveillance. General or scanning wildlife disease surveillance (often referred to as "passive" surveillance) aims at detecting any disease and pathogens in wildlife, rather than obtaining precise statistical data on one or a few pathogens, such as pathogen prevalence estimates. For this latter purpose, targeted wildlife disease surveillance focuses on one or more particular pathogens in one or more wild animal species and is typically used to obtain statistical data on prevalence, age and sex distribution of infection, or geographic distribution of the pathogen. Although general surveillance often is referred to as "active" surveillance, actually, general surveillance can be active or passive (similarly to targeted surveillance). Recent definitions put the emphasis on the fact that active surveillance is investigator-initiated provision of health-related data, while passive surveillance refers to observer-initiated provision of health-related data (https://www.fp7-risksur.eu/terminology/glossary). Therefore, in this report for general and targeted surveillance both active and passive surveillance can be applied.

The **specific objectives** of future coordinated zoonotic disease surveillance under the OH approach for cross-border pathogens that threaten the Union, depending on the epidemiological context, as established in EFSA discussions, are **the early detection of**

- The onset and duration of the period of increased risk: This applies to known pathogens that are present in the MS but have seasonally changing risk that can vary in the time of onset of increased risk, and the severity of the risk from year to year. Therefore, surveillance is mainly targeting the risk period.
- Change in the geographic distribution/spread to new areas: Known pathogen that is present in some geographic areas of the MS. Surveillance here is targeting risk areas.
- An increase in incidence, i.e., early epidemic detection: Known pathogens that are endemic at a low (or constant) incidence in the MS, but epidemics may occur from time-to-time. Surveillance here targets risk areas, which can also be during specific risk period.
- The introduction of the pathogen: known pathogens that are not present within a MS. Can be targeting risk period or risk areas or population at risk (e.g., those at borders, sentinel hosts, during winter migration of birds, etc.).

The first three objectives are to identify spatial-temporal trends in disease occurrence and are essential to critically assess the impact of any intervention (Gortázar et al. 2015).

One can think that, *a priori*, targeted surveillance options for the list of selected pathogens would be the selected options to address these surveillance objectives. This approach indicates that at

[7] Aimed at both pathogen and disease detection, referred to as disease surveillance in a broad sense.

least 10 (actually 11) different targeted wildlife disease surveillance programs will be run in parallel. However, it is not practical to have only targeted (specific, usually based on active surveillance) surveillance programs for every disease or pathogen and a balance with general surveillance (which usually relies more on passive surveillance) is the best approach. This approach is less sustainable if the aim is to increase progressively the number of zoonotic pathogens to be harmoniously monitored in the EU. An approach incorporating also strategically general surveillance for these selected pathogens has the potential to generate in a cost/effective way information that is needed to improve the current understanding, prevention, and control of certain zoonotic pathogens, and also to inform on other pathogens. Under this approach, this section addresses (Figure 3):

- The characteristics of selected zoonotic pathogens in wildlife relevant to disease surveillance;
- The characteristics of the main wild species groups relevant to early detection in disease surveillance;
- The specifics of disease surveillance for the selected zoonotic pathogens in the main wild species groups relevant to early detection in disease surveillance as a basis for developing both general and targeted surveillance as a complementary and cost/effective approach, potentially scalable to other zoonotic pathogens not prioritized so far.

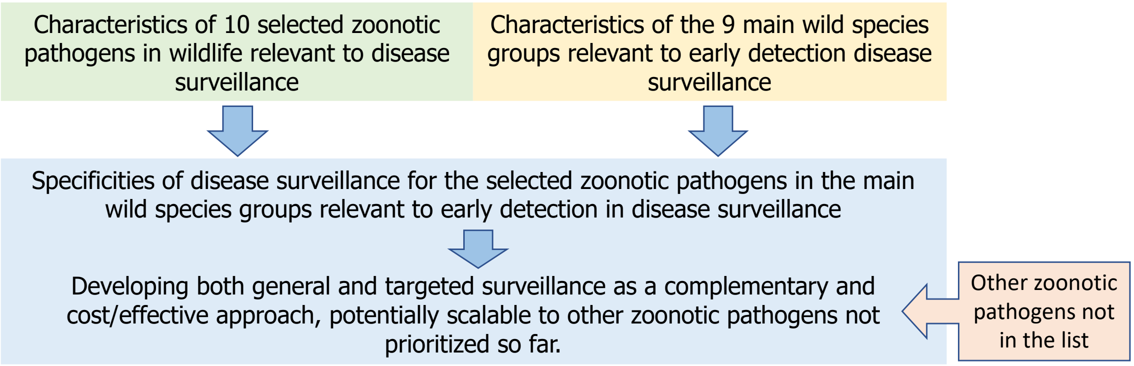

Figure 3. It is not practical to conduct only targeted surveillance programs for every disease or pathogen and an approach balanced with general surveillance may also inform or incorporate other pathogens by taking advantage of the general surveillance component.

Finally, it is worth mentioning that even when different MS would make different pathogen choices, the fact that they agreed on priorities and criteria for the final selection of a list of 10 pathogens is highly relevant for OH surveillance.

3.2. Characteristics of selected zoonotic pathogens in wildlife that are relevant to disease surveillance

3.2.1. General characteristics

The main aim of this report is to formulate recommendations and technical specifications for sustainable coordinated OH surveillance for early detection of a list of prioritized zoonotic pathogens where wildlife is implicated. Next, we analyse the characteristics of the main wildlife groups and the selected pathogens relevant to surveillance aimed at early detection and integrated with other health compartments. The analysed characteristics of the selected pathogens in wildlife relevant to zoonotic surveillance aimed at early detection are show in Table 1.

Table 1. Characteristics of the selected pathogens in wildlife relevant to disease surveillance.

Characteristics of the selected pathogens in wildlife
• Susceptible population
• Objectives of the surveillance specific to the pathogen in wildlife for early detection
• Wildlife as possible source of infection
• Wildlife as sentinel for surveillance
• Syndromic surveillance in wildlife
• Transmission
• The role of vectors
• Main transmission routes in the wild and at the interfaces with humans, animals, and environment
• Known risks as a basis for risk-based sampling (cost-effective) in wildlife disease surveillance
• Target areas for surveillance
• Interfaces at risk where wildlife is involved
• Risk period
• Wildlife populations at risk (target population for surveillance)
• Disease surveillance
• Types of surveillance that can be implemented in wildlife
• Surveillance components[8] for early detection in wildlife
• Diagnostic tests of choice and sample matrix

The main groups of terrestrial vertebrate wild hosts, as well as potential wild sentinel species for the 10 selected pathogens (*E. granulosus* and *E. multilocularis* were considered separately) is shown in Table 2; and Tables 3 to 13 summarise the main characteristics of surveillance aimed at early detection of each pathogen in the list, respectively.

[8] A single surveillance activity (defined by the source of data and the methods used for its collection) used to investigate the occurrence of one or more hazards in a specified population.

Table 2. The main groups of terrestrial vertebrate wild hosts, as well as potential wild sentinel species for the 10 elected list of pathogens (*E. granulosus* and *E. multilocularis* were considered separately). Vector borne pathogens are indicated in colour (for Q-fever, a relevant role in transmission by ticks is not clear).

Pathogen	Main groups of terrestrial vertebrate wild hosts	Wild sentinels
HPAI	Wild birds, mainly waterfowl as primary reservoirs and birds those in close contact	Waterfowl
Swine Influenza	Wild boar	Carnivores?
West Nile Disease	Birds and mammals (also reptiles)	Passive: Falcons, corvids; active: sparrows, pigeons, but ideally wild bird should be determined locally on the basis of seroprevalence studies
TBE	*Ixodes* and Small mammals are the main reservoir. Larger mammals, birds and reptiles can support viral maintenance indirectly	Rodents (*M. arvalis*). Non competent hosts such as wild ungulates (antibodies).
E. granulosus	Large canids as definitive host; Ungulates - intermediate host	Wolf (adult forms), wild boar, roe deer (locally, the ungulate species may vary)
E. multilocularis	Small canids (red fox, raccoon dog) - Definitive hosts; Rodents - intermediate host	Red fox, among murids, *Apodemus* spp
CCHF	Ticks are true reservoir (migratory birds from Africa harbouring CCHFV-infected ticks). Amplifying wild vertebrate hosts	Red deer (serology, long life span), ticks (*Hyalomma*, pathogen detection)
Hepatitis E	Wil boar and other Ungulates for specific subtype. Wild mammals and birds for other specific subtypes. Environment (water)	Wild boar and red deer for specific subtype
Lyme Borreliosis	Wild mammals (e.g., hedgehogs, voles, wood mice, red fox, reindeer, and birds)	Canids. Hedgehogs, squirrels, and blackbirds (tested by PCR in central Europe, spp. which tends towards synurbization)
Q-fever	Wild ruminants, micromammals, lagomorphs, environment	Rodents, predator (foxes) species could act as indicators for the presence of *C. burnetii* in rodents
Rift Valley Fever	Wild ruminant ungulates are potential reservoir where endemic (specially where their density is high) abroad Europe. Although not yet identified, bats and rodents may be implicated, but their epidemiological role in virus transmission and maintenance is not clear	No data available on the susceptibility of European wild ruminants to RVFV, or the capacity of the virus of causing a detectable viraemia. Need to be tested in rodents

Table 3. The main characteristics for surveillance aimed at early detection of Highly Pathogenic Avian Influenza (**HPAI**).

Highly pathogenic Avian influenza				
Objectives of the surveillance in wildlife for early detection	**Susceptible population**		**Transmission**	
	Wildlife: possible source of infection	**Wild sentinel species**	**(Main) transmission routes in the wild and at the interfaces with humans and animals**	**Vector borne?**
- The primary objectives used to be: 1. Change in the geographic distribution/spread to new areas 2. The introduction of the pathogen - However, as this pathogen is becoming endemic and recurring, priority objectives in wildlife should determination of: 3. An increase in incidence, i.e., early epidemic detection 4. The onset and duration of the period of increased risk	Avian species - True maintenance reservoirs. Mainly waterfowl and those in close contact	Aquatic migratory birds, mainly waterfowls, gulls, and shorebirds	Wild: Direct and indirect contact. The virus is shed in the faeces and respiratory secretions of infected birds and may persist in water Interfaces with human and domestic: mainly indirect contact. The virus can survive for long periods of time in cold temperatures; therefore, it can be persistent in the environment and/or be carried by fomites	Not relevant
Surveillance components in the wild				**Diagnostic tests of choice and sample matrix**
Syndromic surveillance in the wild	**Active**	**Passive**	**Comments**	
Clinical signs: variable clinical, normally no overt clinical signs (in waterbird), respiratory signs, such as ocular and nasal discharges, coughing, dyspnoea, swelling of the sinuses and/or head, apathy, reduced vocalization, marked reduction in feed and water intake, cyanosis of the unfeathered skin, wattles and comb, incoordination and nervous signs and diarrhoea. Death: Massive die-offs in some bird species especially in wetlands during the winter	Active surveillance in living and hunted birds	Passive surveillance of cadavers (mainly susceptible birds)	Environmental samples (faeces, water, air, mud, and swabs of surfaces) in wild areas can also be implemented	PCR; ELISA; virus neutralization

Table 4. The main characteristics for surveillance aimed at early detection of **Swine Influenza**.

Swine Influenza				
Objectives of the surveillance in wildlife for early detection	**Susceptible population**		**Transmission**	
	Wildlife: possible source of infection	**Wild sentinel species**	**(Main) transmission routes in the wild and at the interfaces with humans and animals**	**Vector borne?**
- The primary objectives used to be: 1. Change in the geographic distribution/spread to new areas 2. The introduction of the pathogen - However, as this pathogen is becoming widespread in pigs over Europe and it is found concomitantly in wild boar, priority objectives in wildlife should determine: 3. An increase in incidence, i.e., early epidemic detection 4. The onset and duration of the period of increased risk	Wild boar -True maintenance reservoir	Apart from wild boar, potentially carnivores, but need to be tested	Wild: Direct contact Interfaces with human and domestic: Mainly indirect contact (sporadic human-to-human, but not sustained)	No
Surveillance components in the wild				**Diagnostic tests of choice and sample matrix**
Syndromic surveillance in the wild	**Active**	**Passive**	**Comments**	
Clinical signs: fever, respiratory distress, nasal discharge. Death: Mortality rates generally low	Active surveillance in wild boar	Passive surveillance on hunted wild boar or animals found dead	Considering the role of domestic and wild *Sus scrofa* species in the IAVs' ecology, surveillance against these viruses in the wild boar population needs to be implemented	PCR, culture, haemagglutination inhibition test

Table 5. The main characteristics for surveillance aimed at early detection of **West Nile virus**.

<table>
<tr><th colspan="5">West Nile Virus</th></tr>
<tr><th rowspan="2">Objectives of the surveillance in wildlife for early detection</th><th colspan="2">Susceptible population</th><th colspan="2">Transmission</th></tr>
<tr><th>Wildlife: possible source of infection</th><th>Wild sentinel species</th><th>(Main) transmission routes in the wild and at the interfaces with humans and animals</th><th>Vector borne?</th></tr>
<tr><td rowspan="3">- The two priority objectives where WNV is not endemic, or only detected occasionally, are:
1. Change in the geographic distribution/spread to new areas
2. The introduction of the pathogen
3. An increase in incidence, i.e., early epidemic detection
- However, in endemic areas associated to human cases, priority objective every year should be:
4. The onset and duration of the period of increased risk</td><td rowspan="3">Birds - true maintenance reservoir. Reptiles, amphibians, and mammals may also play a role</td><td rowspan="3">Wild birds, mainly falcons, corvids, sparrows, and pigeons, but targeted species should be determined locally based on seroprevalence studies</td><td>Wild: Indirect contact through vectors</td><td>Yes (mosquitos).</td></tr>
<tr><td rowspan="2">Interfaces with human and domestic: Indirect contact through vectors</td><td>Culex spp.</td></tr>
<tr><td>Culex species are widely distributed throughout Europe</td></tr>
</table>

<table>
<tr><th colspan="4">Surveillance components in the wild</th><th rowspan="2">Diagnostic tests of choice and sample matrix</th></tr>
<tr><th>Syndromic surveillance in the wild</th><th>Active</th><th>Passive</th><th>Comments</th></tr>
<tr><td>Clinical signs: in birds and horses causes inapparent infection, mild febrile illness, meningitis, or encephalitis. Death: in birds, as especially high mortality in some species, like corvids</td><td>Active vector surveillance and active surveillance on birds should for an earlier detection and more abundant species should be targeted, like sparrows and pigeons</td><td>Passive surveillance of birds (mainly dead crows and falcons)</td><td>Focus on vector surveillance and active surveillance of birds for an early detection</td><td>Direct (vectors and birds): In-vivo and in-vitro culture; Indirect (birds and mammals): ELISA; haemagglutination inhibition, plaque reduction neutralisation and microtiter virus neutralization</td></tr>
</table>

Table 6. The main characteristics for surveillance aimed at early detection of **Tick-Borne Encephalitis** (**TBE**).

Tick Borne Encephalitis				
	Susceptible population		**Transmission**	
Objectives of the surveillance in wildlife for early detection	**Wildlife: possible source of infection**	**Wild sentinel species**	**(Main) transmission routes in the wild and at the interfaces with humans and animals**	**Vector borne?**
- Priority objectives for this pathogen where it is present or is absent (Southern Europe), are to detect: 1. Change in the geographic distribution/spread to new areas 2. The introduction of the pathogen - Where endemic, priorities are: 3. An increase in incidence, i.e., early epidemic detection 4. The onset and duration of the period of increased risk	Small mammals are the main reservoir. Larger mammals, birds and reptiles can support viral maintenance indirectly	Rodents (*M. arvalis*) and other microtinae locally present and abundant, and ungulates (antibody response), such as roe deer	Wild: Indirect contact through vectors	Yes (ticks)
			Interfaces with human and domestic animals: Indirect through vectors	*Ixodes spp.*
				In Europe main vectors are: *I. ricinus* and *I. persulcatus*
Surveillance components in the wild				**Diagnostic tests of choice and sample matrix**
Syndromic surveillance in the wild	**Active**	**Passive**	**Comments**	
Clinical signs infrequent in wild animals.	Vector surveillance. Susceptible wild hosts as well, small mammals as they are mainly asymptomatic it is the best way to detect pathogen circulation	Vector surveillance captures of small mammals or during pest control	Vector or domestic animal surveillance seems to be the most efficient way for early detection	Direct (in vectors and small mammals): PCR; immunohistochemistry; virus isolation. Indirect (non-competent hosts such as wild ungulates): ELISA; serum neutralization.

Table 7. The main characteristics for surveillance aimed at early detection of ***Echinococcus granulosus*.**

Echinococcus granulosus				
Objectives of the surveillance in wildlife for early detection	**Susceptible population**		**Transmission**	
	Wildlife: possible source of infection	**Wild sentinel species**	**(Main) transmission routes in the wild and at the interfaces with humans and animals**	**Vector borne?**
- Widespread pathogen, therefore main objectives are: 1. An increase in incidence, i.e., early epidemic detection 2. The onset and duration of the period of increased risk - However, where still not detected (e.g., following the expansion of wolves): 3. Change in the geographic distribution/spread to new areas 4. The introduction of the pathogen	Wild and domestic canids- Definitive host; Ungulates - intermediate host	Wolf (adult forms), wild boar, roe deer (locally, the ungulate species may vary)	Wild: Through direct contact, by ingestion of contaminated prey or contaminated pasture Interfaces with human and domestic: Accidental ingestion of contaminated food or wate	No
Surveillance components in the wild				**Diagnostic tests of choice and sample matrix**
Syndromic surveillance in the wild	**Active**	**Passive**	**Comments**	
Clinical signs: definitive hosts have no clinical signs; in intermediate hosts weakness, apathy, anorexia, and ascites. Death: of the intermediate host in severe cases	Active surveillance in the definitive host	Passive surveillance in hunted wild canids and ungulates, but also dead animals	Surveillance is needed to evaluate the role of expanding jackal population in the increased risk	Intestinal scrapping technique; sedimentation and counting technique; PCR; coproantigen ELISA

Table 8. The main characteristics for surveillance aimed at early detection of ***Echinococcus multilocularis***.

Echinococcus multilocularis				
Objectives of the surveillance in wildlife for early detection	**Susceptible population**		**Transmission**	
	Wildlife: possible source of infection	**Wild sentinel species**	**(Main) transmission routes in the wild and at the interfaces with humans and animals**	**Vector borne?**
- Priority objectives for this pathogen, present, still absent in south-western Europe, are: 1. Change in the geographic distribution/spread to new areas 2. The introduction of the pathogen - Where endemic, priorities are: 3. An increase in incidence, i.e., early epidemic detection 4. The onset and duration of the period of increased risk	Carnivores (fox and racoons)- Definitive host; Rodents - intermediate host.	Red fox, among murids, *Apodemus* spp	Wild: Through direct contact, by ingestion of contaminated prey or contaminated pasture Interfaces with human and domestic: Accidental ingestion of contaminated food or water	No
Surveillance components in the wild				**Diagnostic tests of choice and sample matrix**
Syndromic surveillance in the wild	**Active**	**Passive**	**Comments**	
Clinical signs: definitive hosts have no clinical signs; in intermediate hosts weakness, apathy, anorexia, ascites. Death: of intermediate host in severe cases	Active surveillance in the definitive host. Surveillance is needed to evaluate the role of the local jackal population in the increased risk	Passive surveillance in hunted wild canids and rodents, but also dead animals	Surveillance is needed to evaluate the role of the expanding jackal population in the increased risk	Intestinal scrapping technique; sedimentation and counting technique; PCR; coproantigen ELISA

Table 9. The main characteristics for surveillance aimed at early detection of Crimean Congo Haemorrhagic Fever (**CCHF**).

Crimea Congo Haemorrhagic Fever				
	Susceptible population		**Transmission**	
Objectives of the surveillance in wildlife for early detection	**Wildlife: possible source of infection**	**Wild sentinel species**	**(Main) transmission routes in the wild and at the interfaces with humans and animals**	**Vector borne?**
CCHF is considered the most widespread tickborne viral haemorrhagic disease in the world, but its presence unknown in many regions of Europe, therefore priority objectives are four, depending on previous reporting or not, and prevalence in a given region: 1. Change in the geographic distribution/spread to new areas 2. The introduction of the pathogen 3. An increase in incidence, i.e., early epidemic detection 4. The onset and duration of the period of increased risk	Ticks are the true reservoir. Migratory birds from Africa harbouring CCHFV-infected ticks may be a source of infection and spreader. Wild vertebrate hosts mainly play a role as amplifier	Red deer, ticks. Most wildlife is asymptomatic, some wild birds do develop symptoms, especially ostriches	Wild: Indirect contact (vector)	Yes (ticks).
			Interfaces with human and domestic animals: indirect contact through vectors	Ticks in EU: *Hyalomma marginatum*, *H. anatolicum*, *H. rufipes* and *H. asiaticum*
				All EU countries harbour tick species, some species are more relevant in certain areas
Surveillance components in the wild				**Diagnostic tests of choice and sample matrix**
Syndromic surveillance in the wild	**Active**	**Passive**	**Comments**	
Clinical signs: None, wild animals are susceptible but do not develop clinical signs	Vector surveillance. Active surveillance in wild ruminants is the only indicator of circulation and should be implemented in targeted areas after confirmed circulation of the virus	Surveillance of ticks in wild migratory birds during ringing sessions and in other wild vertebrate hosts, hunted animals or during captures	Wild ruminants are relevant for the epidemiology of disease, but since EU is not endemic, early detection is likely to occur through active vector surveillance and surveillance of domestic ruminant herds	In red deer and other vertebrate hosts: serology (indirect immunofluorescence test; IgG-sandwich and IgM-capture ELISA) is a good indicator of prevalence; In ticks antigen detection (PCR, Virus isolation) is the preferred method

Table 10. The main characteristics for surveillance aimed at early detection of Hepatitis E virus (**HEV**).

Hepatitis E (subtype 3)				
Objectives of the surveillance in wildlife for early detection	**Susceptible population**		**Transmission**	
	Wildlife: possible source of infection	**Wild sentinel species**	**(Main) transmission routes in the wild and at the interfaces with humans and animals**	**Vector borne?**
- Specific subtype is widespread in wild boar and cervids, therefore priority objectives are to detect: 1. An increase in incidence, i.e., early epidemic detection 2. The onset and duration of the period of increased risk - However, where not previously detected, and other that wild boar and der species, priority should be: 3. Change in the geographic distribution/spread to new areas 4. The introduction of the pathogen	Wild Boar and other Ungulates - True maintenance reservoir. Wild mammals and birds for other specific subtypes. Environment (water)	Cervids and other wild ruminants (wild boar and red deer). Shellfish as ecosystem indicator	Wild: direct contact and indirect transmission through environment, food Human and domestic animal interfaces: direct contact and indirect transmission through environment	No
Surveillance components in the wild.				**Diagnostic tests of choice and sample matrix**
Syndromic surveillance in the wild	**Active**	**Passive**	**Comments**	
Clinical signs infrequent in wild animals	Active surveillance in wild ungulates	Passive surveillance in hunted animals or animals found dead	Active and passive surveillance in humans in relation to the risk groups (hunters, vets, pig farmers, residents of areas bordering the forest - interface)	Antibody ELISA in serum; RT-PCR - liver, spleen, faeces, blood

Table 11. The main characteristics for surveillance aimed at early detection of **Lyme borreliosis**.

Lyme borreliosis				
Objectives of the surveillance in wildlife for early detection	**Susceptible population**		**Transmission**	
	Wildlife: possible source of infection	**Wild sentinel species**	**(Main) transmission routes in the wild and at the interfaces with humans and animals**	**Vector borne?**
Widespread (rarely found in southern Europe) with marked local variations which makes the relevance of objectives specific to local context: 1. Change in the geographic distribution/spread to new areas 2. The introduction of the pathogen 3. An increase in incidence, i.e., early epidemic detection 4. The onset and duration of the period of increased risk	Wild mammals and birds, such as European hedgehogs (*Erinaceus europaeus*); Voles (*Clethrionomys glareolus, Apodemus* spp.); Red fox (*Vulpes vulpes*); Reindeer (Rangifer tarandus); Wood mice (*A. sylvaticus*); Yellow-necked field mice (*Apodemus flavicollis*)	Canids, hedgehogs, squirrels, and blackbirds (tested by PCR in central Europe, spp which tends towards synurbization); rodents in farmland	Wild: Indirect contact through vectors	Yes (ticks)
			Interfaces with human and domestic: Indirect through vectors	*Ixodes ricinus* and *Argas spp*
				In Europe *Ixodes ricinus* is the primary vector
Surveillance components in the wild.				**Diagnostic tests of choice and sample matrix**
Syndromic surveillance in the wild	**Active**	**Passive**	**Comments**	
Clinical signs infrequent in wild animals	Vector surveillance. Susceptible wild hosts as well, as they are mainly asymptomatic it is the best way to detect pathogen circulation	In animals found dead. Vector surveillance in ringing sessions or through surveillance of hunted or capture loss (e.g., rodents) animals	Vector, domestic and peri-domestic and farmland wild animal surveillance seems to be the most efficient way for early detection	Direct (mainly for the vector): PCR; immunofluorescence; bacterial culture. Indirect (for the wild susceptible hosts): Indirect fluorescent antibody; ELISA

Table 12. The main characteristics for surveillance aimed at early detection of **Q-fever**.

Q fever				
Objectives of the surveillance in wildlife for early detection	**Susceptible population**		**Transmission**	
	Wildlife: possible source of infection	**Wild sentinel species**	**(Main) transmission routes in the wild and at the interfaces with humans and animals**	**Vector borne?**
Confirmed Q-fever cases, with variable incidence, in most European countries, which makes the relevance of objectives specific to national context: 1. Change in the geographic distribution/spread to new areas 2. The introduction of the pathogen 3. An increase in incidence, i.e., early epidemic detection 4. The onset and duration of the period of increased risk	Wild ruminants, micromammals, lagomorphs, environment	Ruminant, rodent´s predator species (such as red foxes) could act as indicators for the presence of *Coxiella burnetii* in rodents, the environment (e.g., rodent burrows)	Wild: Air-borne; vector. It is usually acquired by inhalation of an aerosol from an infected animal or the ground, but may be rarely acquired by tick bite or by crushing a tick Interfaces with human: air-borne; contaminated milk and sheep wool and direct contact with infected animals; Domestic and wild animals: exposure/inhalation of birth material, faeces, and contaminated milk	Yes (ticks) Proved competent vectors by experimental infection: *Dermacentor andersoni, Hyalomma aegyptum, H. asiaticum, Ornithodoros hemsi, O. Moubata, Ixodes holocylus, Haemaplysalis humerosa* They seem to play a minor role in the epidemiology
Surveillance components in the wild				**Diagnostic tests of choice and sample matrix**
Syndromic surveillance in the wild	**Active**	**Passive**	**Comments**	
Clinical signs: in ruminants might cause abortion and reproductive disorders but mostly asymptomatic. Death: in lambs, kids, and calves	Active surveillance in wild ruminants. Since it is mainly asymptomatic it is the best way to detect pathogen circulation	Passive surveillance in dead ruminants, mainly lambs, kids, and calves where reproductive disorders are suspected	Human surveillance seems to be the most efficient way for early detection, followed by investigation of animal/environmental sources	PCR, ELISA, complement fixation test

Table 13. The main characteristics for surveillance aimed at early detection of **Rift Valley Fever**.

Rift Valley Fever				
Objectives of the surveillance in wildlife for early detection	**Susceptible population**		**Transmission**	
	Wildlife: possible source of infection	**Wild sentinel species**	**(Main) transmission routes in the wild and at the interfaces with humans and animals**	**Vector borne?**
While it was formerly regarded as an was regarded as an African, animal disease, since 2000 RVF was detected for the first time outside the African continent, and up to date, no outbreaks have been reported in Europe. Therefore, priority objective all over Europe is: **1. Early detection of the introduction of the pathogen**	Wild ruminant ungulates are potential reservoirs where endemic, abroad Europe. Although not yet identified, bats and rodents may be implicated, but mostly as a dead-end host	No data are available on the susceptibility of European wild ruminants to RVFV, or the capacity of the virus of causing detectable viremia. Need to be tested in rodents	Wild: Indirect contact through vectors Interfaces with human: direct and indirect contact with wildlife and vectors; Domestic animals: indirect contact through vectors	Yes (mosquitos) Vectors in EU: *Aedes albopictus, Aedes caspius, Aedes detritus, Aedes japonicus, Aedes vexans, Culex pipiens* and *Culex theileri* All EU countries harbour RFV vectors
Surveillance components in the wild				**Diagnostic tests of choice and sample matrix**
Syndromic surveillance in the wild	**Active**	**Passive**	**Comments**	
Clinical signs (type of disease indicators): mainly reproductive ones, such as abortions in ruminants; Death: in lambs, kids, and calves	Vector surveillance; Active surveillance in wild ruminants	Passive surveillance: testing in animals found dead (particularly lambs and calves)	Wildlife ruminants are relevant for the epidemiology of disease, but since EU is not endemic, early detection is likely to occur through active vector surveillance and surveillance of domestic ruminant herds	Vectors: Antigen detection in pooled samples; Wild animals: Antigen detection in organs of animals found dead or serology in the blood of live sampled animals

We observed that the appropriateness of the primary objectives, (i.e., to detect 1. changes in the geographic distribution/spread to new areas, 2. the introduction of the pathogen, 3. an increase in incidence, 4. the onset and duration of the period of increased risk varied according to the pathogen and their epidemiological situation in Europe, which may be very variable regionally.

In summary:

- While most pathogens are already present in the EU, their spatial distribution and presence in potential hosts are relatively unknown, or it is known that pathogens are present in certain regions (more or less restrictively) of Europe.
- Some pathogens (e.g., specific HEV subtype in wild boar and cervids) are relatively widespread therefore priority objectives are to detect an increase in incidence, i.e., early epidemic detection or the onset and duration of the period of increased risk. Where not previously detected, priorities should be to detect changes in the geographic distribution/spread to new areas, and the introduction of the pathogen.
- Some pathogens (e.g., HPAI) are probably in a phase of becoming endemic and recurring after several incursions and local maintenance in the past years in Europe. A priority feasible objective in wildlife should probably progress towards detecting increase in incidence, i.e., early epidemic detection, or the onset and duration of the period of increased risk.
- RVFV was formerly regarded as an animal disease present only in Africa but in 2000 RVF was detected for the first time outside the African continent, up to date no out-breaks have been reported in Europe. Therefore, priority objective all over Europe is early detection of the introduction of the pathogen.

The different relevance of specific objectives for different pathogens is challenging for the development of general surveillance suitable for the entire range of pathogens, indicating that wildlife zoonotic disease programs should include pathogen specific approaches, design, and surveillance components, i.e., combining general and targeted activities.

Table 14 details the roles of the different host groups as primary maintenance reservoirs (dark green), secondary reservoirs (light green) or not susceptible to infection (white), indicating the potential role of the various host species as sentinel species (dotted cells).

Figure 4 shows the relevance of wildlife groups for the selected pathogens, measured as number of pathogens for which a given host group is relevant as: 1) reservoir and/or sentinel species, 2) only sentinel, 3) only reservoir. For the listed 11 pathogens the following applies:

- Except for bats, all groups of considered species have potential to be used in disease surveillance, playing relevant epidemiological roles for the selected pathogens, and/or being useful as sentinels.
- Wild ruminants predominated as playing a relevant role (6 pathogens), being relevant as sentinels for several (4) pathogens, while only for 3 out of 6 pathogens were considered disease reservoirs.
- Wild carnivores were also relevant in terms of the number of pathogens (5) they may play a role for, and their role as sentinel (being in top of the trophic chain) was remarkable.
- Micromammals were relevant of 4 pathogens, as well as non waterbirds (migratory or not). To a less extent, wild boar (3 pathogens), water (3) bird and lagomorphs (2) were relevant to a lower number of pathogens.

- Bats, apparently, are not relevant for the listed pathogens. However, these is lack of evidence on their epidemiological and utility for surveillance of West Nile disease and RFV.

It arises from these results that there a diversity of host groups (also highly diverse internally within groups), whose epidemiological particularities may vary locally and as a function of the prevailing host community. For instance, a given species may play different epidemiological roles, and utility for surveillance even for the same pathogens depending on the context (e.g., wild rabbits and Q-fever). Therefore, a better characterization of the importance and roles of wildlife for specific pathogen surveillance may still requires further research and evaluation to local contexts.

Table 14. Roles of the different host groups as primary maintenance reservoirs (dark green), secondary reservoirs (light green) or not susceptible to infection (white), indicating the potential role as sentinel species (dotted cells).

Pathogen	Wild ruminants	Wild boar	Wild carnivores	Wild lagomorphs	Micromammals	Bats	Waterbirds	Other migratory birds	Non migratory birds
HPAI									
Swine Influenza									
West Nile Disease						¿?			
TBE									
E. granulosus									
E. multilocularis									
CCHF									
Hepatitis E									
Lyme Borreliosis									
Q-fever									
Rift Valley Fever					¿?	¿?			

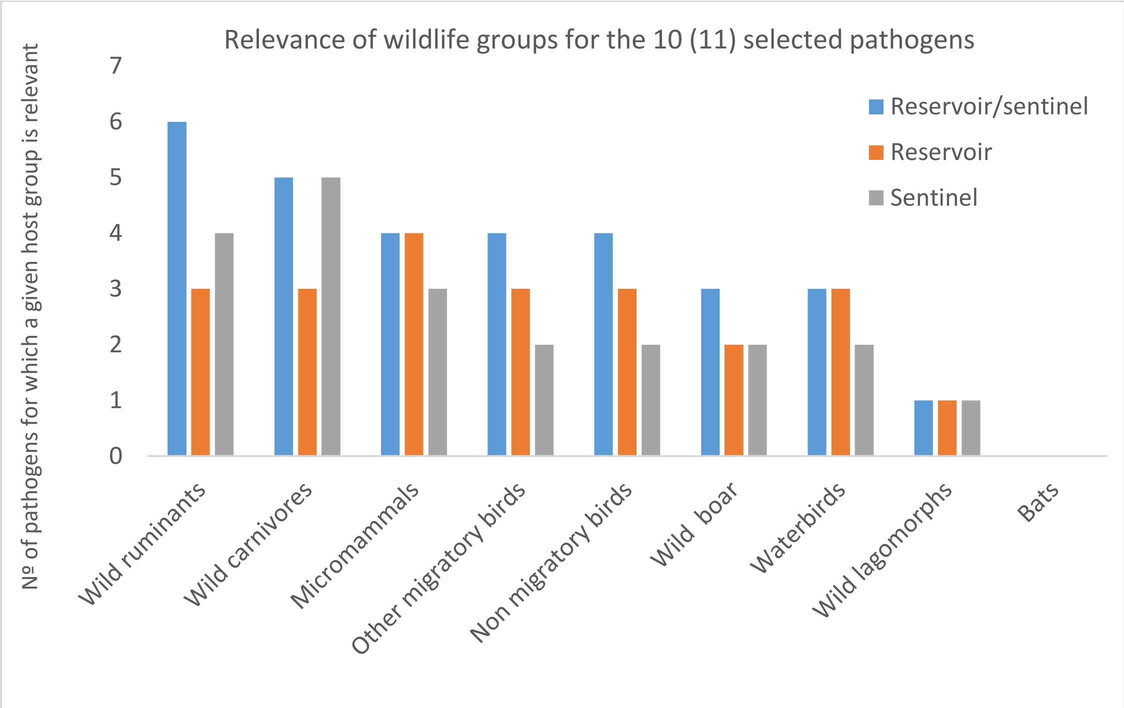

Figure 4. The relevance of wildlife groups for the selected pathogens (measured as number of pathogen for which a given host group is relevant as reservoir or sentinel) according to their role: 1) reservoir and/or sentinel species, 2) only sentinel, 3) only reservoir.

3.2.2. Risks to be considered in early detection surveillance of zoonotic pathogens in wildlife

We scored risks to obtain indicators of their respective relevance for a general surveillance approach but targeting this specific group of pathogens (11), i.e., integrating both general and targeted surveillance as a complementary and cost/effective approach.

The main risks to be considered in disease surveillance for each pathogen, including a score per risk as an overall indicator of its relevance in the surveillance of the 10 pathogens, shown in Table 15. For each pathogen, risks were classified and scored as a function of their relevance for risk-based surveillance as follows:

- No relevant at all or risk not applying (N.A.) = 0
- Optional/not too relevant for risk-based surveillance =1
- Recommended for risk-based surveillance = 2
- Highly recommended for-risk based surveillance = 3

For better visualizing, the total score per risk and the number of times if was selected (regardless of the score) for each pathogen are represented in Figure 5. The risk factors which are mainly bird-specific are also represented separately only for birds (Figure 6).

Table 15. The main risks to be considered in disease surveillance for each pathogen, including the scores per risk (risk are ranked decreasingly), and the total sum per risk as an overall indicator of its relevance in the surveillance of the 11 pathogens

Risk factors of relevance for surveillance design and targeting	HPAI	Swine Influenza	West Nile Disease	TBE	*E. granulosus*	*E. multilocularis*	CCHF	Hepatitis E	Lyme Borreliosis	Q-fever	Rift Valley Fever	Score total
Pathogens, morbidity and/or mortality has been reported in livestock and/or wildlife (listed groups, and others sharing pathogens), as well as zoonotic cases in humans. If endemic, when unusual episodes	3	3	3	3	3	3	3	2	3	3	3	32
INTERFACE Farming Direct/indirect contact with livestock/poultry (inc. gamebird) (outdoor or extensive more relevant)	3	3	3	1	3	2	1	3	1	3	1	24
INTERFACE Direct/Indirect contact with pets, humans: outdoor recreational activities, farms and peri-urban areas & Parks	2	1	2	3	1	2	3	1	3	3	3	24
High diversity of hosts (groups and species). But dilution effect (opposite situation: pests) (e.g. Natura 2000 network)	3	3	1	1	1	1	1	3	2	2	1	19
High host density and/or intensive game management of listed grous inc. artificial feeding and game release (e.g. ungulates, birds: include Anseriformes & Galliformes)	2	3	2	1	2	1	1	3	1	1	1	18
Mammal sentinels: abundant and widely distributed species (e.g. red fox, rats) often subject to population control (e.g. exotic spp. such as raccoon, Am. mink, rodents)	0	3	1	1	2	2	3	1	3	2	¿?	18
Where? At the borders of EU, with special attention to geographical borders of the continent: eastern Europe, the Balkans, southern mediterranean region. Pathogen specific (e.g. migration crosspaths)	3	1	1	1	1	1	3	1	1	1	3	17
Vectors for target pathogens (vector borne) are presen (macroscale and microhabitat, e.g. *Ixodes*)	0	0	3	3	0	0	3	0	2	2	3	16
Areas susceptible to rapid environmental change nearest to human setlements or domestic animals	1	1	2	2	1	2	2	1	2	1	1	16
INTERFACE Direct contact with hunters and consumers of meat, regions where meat is commercialized	3	3	0	0	3	1	0	3	0	1	0	14
Highy diverse waterbird community. Natura 2000 network, a large proportion of wetlands of international importance has been designated as Ramsar sites	3	3	2	1	0	0	2	1	0	0	0	12
Pest & exotic birds in URBAN/PERI-URBAN Parks, such as pigeons (often subject to control programs)	3	3	2	1	0	0	2	1	0	0	0	12
When and where unusual or expected (cyclic) population explosion (e.g. voles)	1	0	0	3	0	1	0	0	3	2	1	11
Overwintering bird colonies and landfills leading to intermingling of different species, and high aggregation and densities	3	2	2	0	0	1	0	3	0	0	0	11
Wetlands and breeding grounds, at the borders of EU, cross-border regions and where different migration paths overlaps, when outbreaks have been recently detected in neighbour countries	3	3	3	0	0	0	1	0	0	0	0	10
Bird sentinels: can be set in natural/artificial water bodies, or song birds and/or zoo birds (e.g., at feeders)	3	2	3	0	0	0	1	0	0	0	0	9
Adapt to changes in birds migration risk maps (Euro Bird Portal EBP)	3	3	2	0	0	0	1	0	0	0	0	9
Definitive or intermediate hosts are present (for heteroxenous parasites)	0	0	0	0	3	3	0	0	0	0	0	6
Species capable of performing large migrations that could result in a cross-border spread (e.g. *Nyctalus* and *Pipistrelus* bats)	3	0	1	0	0	0	2	0	0	0	3*	6

Score for risks

Geographical risks	
Interfaces/host diversity as a risk	
Epidemiological risks	
0	No relevant at all or N.A.
1	Optional/not too relevant
2	Recommended
3	Highly recommended

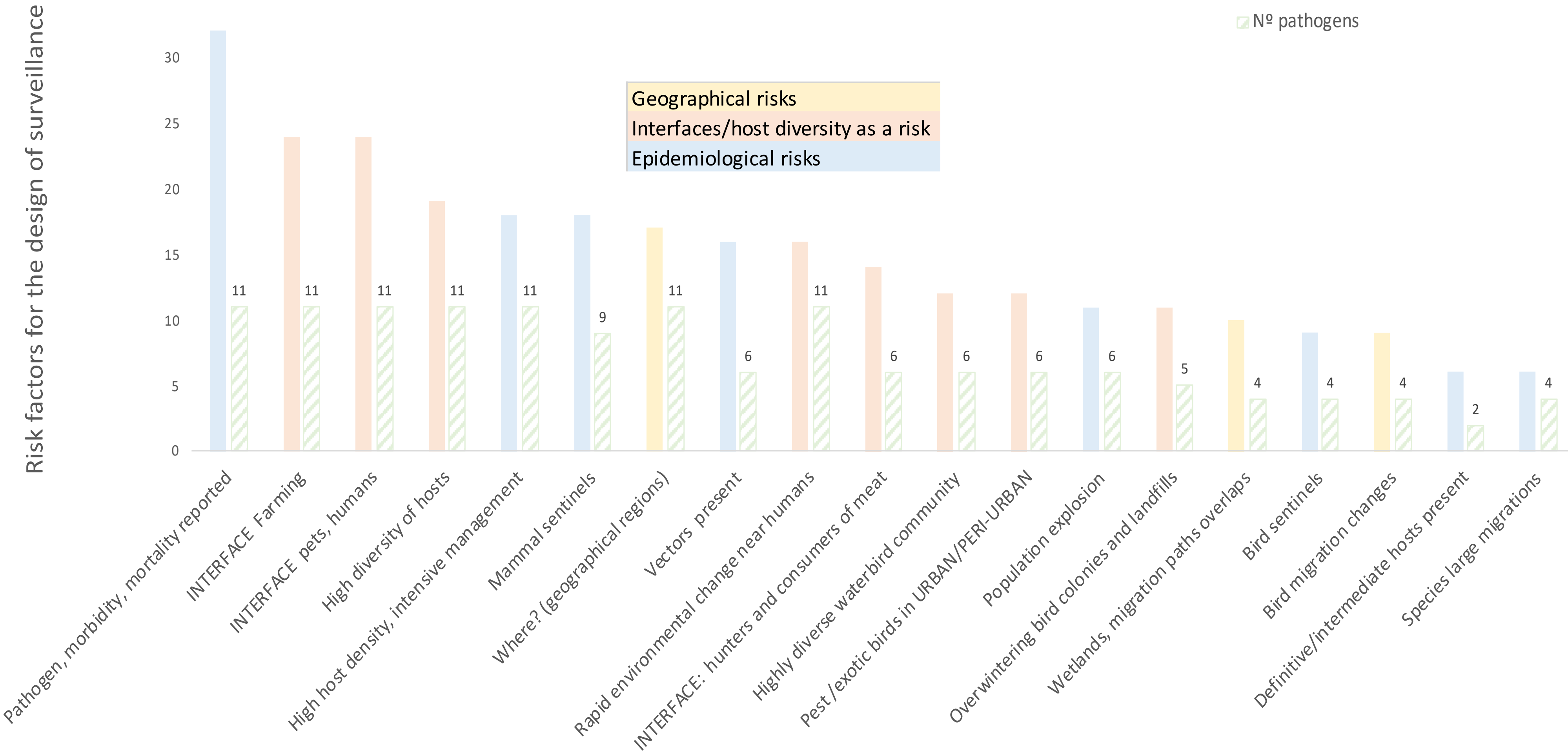

Figure 5. The total score per risk (colour indicate the type of risk), and the number of times each risk was selected (regardless of the score, when > 0) for each pathogen are represented (striped bars). The risk factors which were bird-specific are also represented separately.

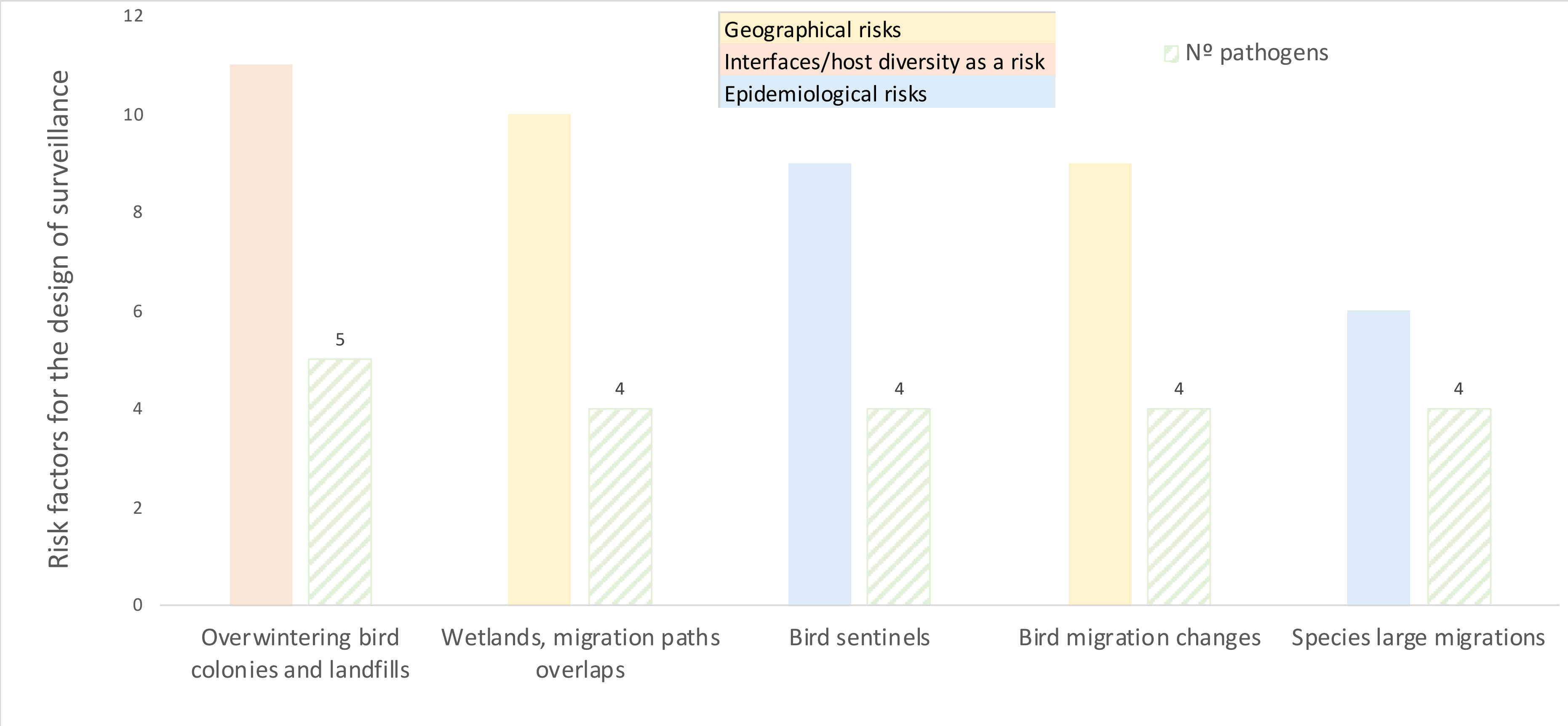

Figure 6. The total score per risk (colour indicate the type of risk), and the number of times each risk was selected (regardless of the score) for each pathogen are represented (striped bars) for factors which were bird-specific.

Overall, based on the score:

- The more relevant risk factor for subsequent surveillance design and early detection was the previous reporting of the pathogen, morbidity, or mortality in the area, following by the existence of interfaces and frequent contact between wildlife and domestic animals (farmed, pets) and humans.
- They were followed by factors related to the existence of high diversity and density of wild hosts, including their intensive management, normally for game purposes. Next factors in the ranking indicated that for surveillance design it should be considered where mammals may act as sentinels for a given pathogen.
- The geographical region is next relevant factors, the risk a specific pathogen is more likely to be found in a geographic region of Europe, with special attention to where pathogen may enter through the borders (with special attention to eastern Europe, the Balkans, southern Mediterranean region). This is pathogen specific.
- The present of vectors had medium ranking; however, this is explained by the fact that not all pathogens are vector borne, and this risk was always characterized as priority for targeting surveillance for such vector borne pathogens.
- The existence of areas susceptible to rapid environmental change nearest to human settlements or domestic animals also ranked in the mid. Interestingly, it was considered a risk for all 11 pathogens. Urbanization is characterized by rapid intensification of agriculture, socioeconomic change, and ecological fragmentation, which can impact on the epidemiology of zoonotic diseases. There is scientific evidence for the drivers and epidemiology of emerging wildlife-borne zoonoses in urban landscapes, where anthropogenic pressures can create diverse wildlife-livestock–human interfaces. These interfaces represent a critical point for cross-species transmission and emergence of pathogens into new host populations.

Regarding bird-specific factors (Figure 6), all factors were relevant for 4 to 5 different pathogens, and in terms of scoring:

- Overwintering bird colonies and landfills leading to intermingling of different species, and high aggregation and densities ranked the highest.
- Closely followed by the presence of wetlands and breeding grounds, at the borders of EU, special attention cross-border regions and where different migration paths overlaps, when outbreaks have been recently detected in neighbour countries.
- Where birds can be used as sentinels for disease surveillance, and when/where unusual migrations (e.g., in case of extreme winter conditions) were also referred as relevant.

3.3. Characteristics of the main wild species groups relevant to early detection in disease surveillance

The vast majority of human pathogens have emerged from animal populations (Gut et al. 2022). Because of the extraordinary diversity of both animal hosts and the pathogens they harbour, understanding which animal and virus groups are more likely to source dangerous zoonoses is an important public health aim. The ecology of wildlife species and the pathogens to which they are exposed lead to conditions that should be considered for surveillance aimed at early detection of pathogens. A recent analysis (Guth 2019) found that mammalian reservoir hosts most closely related to humans harbour zoonoses of lower impact in terms of mortality relative to more phylogenetically distant hosts. These results were consistent with phylogenetic trends in virulence that have been reported in cross-species pathogen emergences in other systems (Longdon et al. 2011, Farrell et al. 2019).

This section presents the main characteristics of relevance for zoonotic disease surveillance (aimed at early detection) of the main wildlife host groups present in Europe (Table 16).

Table 15 and Figure 5 in the previous sections showed the relevance of wildlife groups for the selected pathogens, measured as number of pathogen for which a given host group is relevant as: 1) reservoir and/or sentinel species, 2) only sentinel, 3) only reservoir. In the following sections we describe the main characteristics of each host group which are relevant to early detection in zoonosis disease surveillance.

Table 16. Considered list of the characteristics of the main wildlife groups relevant to zoonotic disease early detection surveillance.

Characteristics of the main groups of wildlife hosts
• Ecological characteristics relevant to epidemiology
o Diversity and distribution
o Current knowledge of populations in Europe
o Host ecology/epidemiology
• Risks to be considered in disease surveillance design
o Used environments and established interfaces
o Role in long-distance spread / cross-border spread
o Role in spread or maintenance if pathogen was introduced by other host specie
o **Host specific risk factors for surveillance of zoonosis**
• Characteristics of surveillance for early detection
o Feasibility of surveillance
o Main surveillance needs
o Priority pathogens under surveillance
o Active Surveillance specific to priority diseases
o Passive Surveillance specific to priority diseases
o Type of sample material to collect
o Diagnostic tests

3.3.1. Characteristics of **wild ruminants** relevant to early detection in disease surveillance

The Table 17 shows the main characteristics relevant to surveillance in European **wild ruminants** aimed at early detection of zoonotic disease in wildlife. European wild ruminants belong to two main families, cervids and bovids, and both share several infections with domestic animals, mainly ruminants (Putman and Apollonio et al. 2010). Populations of wild cervids and bovids occur in almost all European countries in almost all habitats and land uses (agriculture, protected areas, hunting grounds, peri-urban). A few species are widespread (roe deer and red deer), some are scattered (fallow deer and mouflon) and other very localized or associated to certain habitats (Chinese water deer, musk ox). The increasing availability of continental-scale abundance models relying on hunting bag and other population data (e.g., those being produced by *ENETWILD*) makes it possible to use their predicted local abundance and distribution to design risk-based surveillance of zoonotic diseases in the wild (such as active risk based, or enhanced passive surveillance). In general, population trends are increasing and expanding. Regarding the cervids, the most abundant one at the European scale is probably the roe deer.

Deer, which belonging to the subfamily cervinae, such as red deer and fallow deer, do participate in the epidemiology of many relevant shared infections including bluetongue, tuberculosis, and a large list of tick-borne diseases. In Europe, a CWD-like disease (Chronic wasting disease, CWD, is a fatal neurodegenerative, contagious prion disease affecting members of the Cervidae family) was first diagnosed in 2016 in a wild reindeer in Norway (Benestad et al. 2016) and in red deer in 2019 (Vikøren et al 2019). These cases are thought to represent sporadic prion disease as has been described in other species, rather than contagious CWD, although European deer species are probably susceptible to classical CWD; the North American White-tailed deer is susceptible and an invasive species expanding in Finland. As of April 2022, no additional cases have been found amongst the reindeer tested.

Regarding wild bovids, their distribution is patchier and less dense, but they are locally relevant for infections at the interface, sometimes as a source of infection (e.g., *Brucella melitensis* spill-over from Alpine ibex to cattle, Mick 2014) and sometimes as victims of spill-over from livestock (e.g., sarcoptic mange in Iberian ibex and Cantabrian chamois).

Therefore, ungulate ruminants are essential actors of the epidemiology of a wide range of pathogens, playing different reservoirs roles, often context dependent, even for the same pathogen (see section above about the specific list of 10 pathogens). Their roles vary from true maintenance, spill over, or dead-end hosts. Their phylogenetic closeness to ruminant livestock species makes sharing most pathogens possible, and therefore they potentially play a crucial role for those zoonotic, in some cases former reverse zoonoses. They can participate as reservoir hosts of different vector borne pathogens. However, for certain vector borne diseases (e.g., TBE for ticks) the level of viraemia after natural infection is too low to infect vectors (e.g., the absence of the pathogens in the ticks collected from them is usual), although they play a role in the survival and abundance of vector populations (such as ticks or mosquitoes, amplifying effect).

The **main risks associated to wild ruminants** to be considered in disease surveillance design for early detection are (Table 17):

- Used environments and established interfaces: wild ruminant species share pastures with livestock (lowlands, alpine pastures, bushlands) and can occupy peri-urban or urban green areas.
- Role in long-distance spread or cross-border spread in a setting comparable to the EU: most wild ruminants usually disperse over short distances and have a limited role in rapid spreading of disease (except for vectorial), although exceptional cases of longer dispersions occur

(Kropil et al. 2015). Some migratory species in northern areas (reindeer, moose) can travel tens or hundreds of kilometres and have potential for long-distance spread. As they are hunted and consumed, anthropogenic spread through game meat and/or poor hygiene and biosecurity by hunters is a risk.

- Role in spread or maintenance if pathogen was introduced by other host species: their large distribution and continuity of populations favours medium to long-term spread in case of non-vector borne diseases and disease persistence in case the disease is introduced in other species (ruminant or not, wild, or domestic, e.g., Bonardi et al. 2020 for HEV). The wild ruminant/livestock interface in Europe, although variable, considering all species potentially involved (domestic and wild), is also extensively distributed and almost a continuum in many regions, which makes difficult to prevent disease maintenance and spread at the interface, and subsequently to humans in the case of zoonosis. Particularly in case of slow hidden diseases which often are not early detected, or in case of vector-borne pathogens. To sum, in general, limited role in initial spreading, but high importance in subsequent maintenance (e.g., bluetongue) as many wild and domestic ruminant species overlap in distribution, they interact and share pathogens which can be maintained by the ungulate host community.
- Risk factors relevant to design general surveillance include:
    - Where direct and indirect contact with livestock (outdoor or extensive more relevant, however), however there is need to better map and characterize wild ungulate/livestock interface in Europe (*ENETWILD*-consortium et al. 2021);
    - Where pathogens have been reported in both in livestock and wild ungulates, or at least in one compartment, as well as zoonotic cases in humans;
    - Where high densities and intensive game management occur, artificial feeding and game release;
    - Where vectors for target pathogens are present;
    - At the borders of EU, with special attention to geographical borders of the continent: eastern Europe, the Balkans, southern Mediterranean region;
    - Where high diverse ungulate community occurs;
    - Passively in sick animals detected by hunters, rangers, citizen science (special attention to unusual mortality or morbidity);
    - Preferably adults for active surveillance, calves can be interesting for disease incidence
    - Where there is direct contact with hunters and consumers of meat;
    - Where definitive hosts are present for heteroxenous parasites (e.g., large carnivores).
- Regarding the characteristics of disease surveillance for early detection in wild ruminants:
    - **Active surveillance is feasible** since most species and populations are hunted, or to a less extent, shot or captured for population control purposes.
        - Surveillance of hunted/captured/shot specimen is useful for early detection or changes in incidence, i.e., early epidemic detection, and to detect pathogens which do not manifest clinical symptoms.
        - As a routine, active surveillance is key to monitor already established endemic pathogens, their maintenance and possible fade out (absence), and are

particularly relevant for retrospective studies to elucidate past introduction/circulation of emergent zoonotic pathogens.

- In addition to surveillance of ungulate pathogens, wild ruminants can contribute as sentinels for others (such as several vectors borne pathogens, e.g., TBE).
- The active components of national active surveillance programs must representatively cover the different regions and be organized as risk based to be cost effective and sensitive (see section on risks below). Consider the contribution and inclusion of hunting grounds (public, private) and the network of European protected areas (Natura 2000 Network).

  - **Passive surveillance is also feasible in most species** for sick and naturally found dead animals, including road kills. Found dead could be reported via APP (e.g., *iMammalia*), communicated and transported to Vet authorities. Passive surveillance is also biased toward sick animals, so has an increased chance of detection, but an inability to provide a robust estimate of prevalence.
    - Passive surveillance is essential for early detection of the introduction of the pathogen, the onset and duration of the period of increased risk; change in the geographic distribution/spread to new areas and increase in incidence, i.e., early epidemic detection.
    - Passive surveillance can be enhanced: for instance, by increasing effort to detect and collect road kills (Grilo et al. 2020; Fernández-López et al. 2022), which can be risk based (e.g., attending to areas of more likely introduction of pathogens).

- Type of sample material to collect includes blood/organ samples/swabs/ectoparasites from hunted animals, which can be sampled quite easily during hunting season or dead animals any time, faeces could be collected directly from individuals or by active searching. High quality blood for serum extraction can be taken from the endocranial venous sinuses by puncture in hunted/dead wild ungulates. Blood, swabs and ectoparasites can be collected from live trapped hosts. Environmental sampling through molecular techniques is promising, both for host and pathogen presence. Non-invasive sampling techniques based on the analysis of oral fluid specimen have been used for wild boar. Techniques to preserve serum at room temperature (Blood Collected on Filter Paper) has been tested successfully but may require testing for specific pathogens and hosts.
- Serological and pathogen detection test available and validated in most cases. May need testing for some specific host and pathogen dyads. Environmental sampling through molecular techniques already are in use and are promising (Barasona et al. 2017).

Table 17. The main characteristics relevant to surveillance in European **wild ruminants** aiming at early detection of zoonotic disease in wildlife.

Echinococcosis (E. granulosus), **Crimean Congo Haemorrhagic Fever, Hepatitis E, Q-fever ruminants, Rift Valley Fever**		
Ecological characteristics relevant to epidemiology		
Diversity and distribution	**Current knowledge of populations in Europe**	**Host ecology/epidemiology**
Populations of wild cervids and bovids occur in almost all European countries (a total of 15 species) in almost all habitats and land uses (agriculture, protected areas, hunting grounds, peri-urban). A few species are widespread (roe deer and red deer), some are scattered (fallow deer and mouflon) and other very localized or associated to certain habitats (e.g., alpine). Several exotic species introduced in the past currently are increasing their range. Wild horses (not ruminant ungulates) are only present in a few locations as a consequence of rewilding programs.	Presence data available for most countries (atlases, citizen science). Scattered abundance data (mostly hunting bags) available at country level. Continental-scale abundance models relying on hunting bag and other population data are being produced by *Enetwild*. Overall positive trend.	Most species are gregarious, do not use dens. Herbivorous diet. Typically, browsers or grazers. Are main prey of large carnivores and relevant as intermediate hosts for pathogens. Ungulate ruminants are essential actors of the epidemiology of a wide range of pathogens, playing different reservoirs roles, often context dependent, even for the same pathogen. Their roles vary from true maintenance, passing by spill over, to dead-end hosts. Phylogenetically close to ruminant livestock species, sharing most pathogens. They do not seem to play a direct role in the maintenance of certain vector borne (e.g., TBE for ticks), because, generally, the level of viraemia after natural infection is too low to infect vectors (e.g., the absence of the pathogens in the ticks collected from them is usual). Nevertheless, they play a role in the ecology of such vector borne pathogens, being a key host in granting survival and abundance of vector populations (amplifying effect). Wild ruminants' movements may also potentially introduce infected ticks into new areas, even when they are not competent hosts (TBE).

Risks to be considered in disease surveillance design for early detection			
Used environments and established interfaces	**Role in long-distance spread/cross-border spread in a setting comparable to the EU**	**Role in spread or maintenance if pathogen was introduced by other host species**	**Risk factors for general surveillance**
These species share pastures with livestock (lowlands, alpine pastures, bushlands) and can occupy peri-urban or urban green areas.	Roe deer: only disperse over short distances; red deer, fallow deer: nearly no dispersal, seasonal migration of males in a small, scaled space use. Limited role in rapid spreading of disease (mainly vectorial) but contribute to medium to long-term spread in case of non-vector borne diseases. Migratory species (reindeer, moose) can travel tens/hundreds of kilometres and have potential for long-distance spread. Anthropogenic spread through game meat and/or poor hygiene by hunters.	Limited role in spreading, high importance in maintenance (e.g., bluetongue) as many wild and domestic ruminant species overlap in distribution, they interact and share pathogens which can be maintained by the ungulate host community	1) Where direct and indirect contact with livestock (outdoor or extensive more relevant, however, there is need to better define and characterize wild ungulate/livestock interface in Europe); 2) where pathogens has been reported in both in livestock and wild ungulates, or at least in one compartment, as well as zoonotic cases in humans; 3) where high densities and intensive game management occur, artificial feeding and game release, 4) where vectors for target pathogens are present, 5) at the borders of EU, with special attention to geographical borders of the continent: eastern Europe, the Balkans, southern Mediterranean region, 6) where high diverse ungulate community occurs, 7) passively in sick animals detected by hunters, rangers an citizen science (special attention to unusual mortality or morbidity), 8) preferably adults for active surveillance, calves can be interesting for incidence, 9) where there is direct contact with hunters and consumers of meat, 10) where definitive hosts are present for heteroxenous parasites (e.g., large carnivores).

Characteristics of surveillance for early detection		
Feasibility of surveillance	**Active Surveillance specific to priority diseases**	**Passive Surveillance specific to priority diseases**
Active monitoring is feasible since most species and population are hunted, or to a less extent, shot or captured for population control purposes. Passive surveillance also feasible in most species for sick and naturally dead found animals, including road kills. Found dead could be reported via APP (e.g., *iMammalia*), communicated and transported to Vet authorities.	ACTIVE surveillance of hunted/captured/shot specimen needed for early detection of changes of incidence, i.e., early epidemic detection, following a risk-based approach. Also, active surveillance as routine is key to monitor already established endemic pathogens, their maintenance and possible fade out (absence), and are particularly relevant for retrospective studies to elucidate past introduction/circulation of emergent zoonotic pathogens (this includes sentinels). The active component of national active surveillance programs must representatively cover the different regions and be organized as risk based to be cost effective and sensitive (see section on risks). Consider the contribution and inclusion of hunting grounds (public, private) and the network of protected areas (Natura 2000 network).	PASSIVE surveillance is essential for early detection of the introduction of the pathogen, the onset and duration of the period of increased risk; change in the geographic distribution/spread to new areas and increase in incidence, i.e., early epidemic detection. Most listed pathogens rarely develop symptoms or fatal cases in wild ruminants. The role of hunters is essential for passive surveillance.

Type of sample material to collect	Diagnostic tests
Blood/organ samples/swabs/ectoparasites from hunted animals might be sampled quite easily during hunting season or dead animals any time, faeces could be collected directly from individuals or by active searching. High quality blood for serum extraction can be taken from the endocranial venous sinuses by puncture in hunted/dead wild ungulates. Blood, swabs and ectoparasites can be collected from live trapped hosts. Environmental sampling through molecular techniques is promising, both for host and pathogen presence. Non-invasive sampling techniques based on the analysis of oral fluid specimen have been used for wild boar. Techniques to preserve serum at room temperature (Blood Collected on Filter Paper) has been tested successfully but may require testing for specific pathogens and hosts.	Serological and pathogen detection test available and validated in most cases. May need testing for some specific host and pathogen dyads. Environmental sampling through molecular techniques is promising.

3.3.2. Characteristics of **wild boar** relevant to early detection in disease surveillance

The Table 18 shows the main characteristics relevant to surveillance in European **wild boar** aimed at early detection of zoonotic disease in wildlife. This species (including a few feral pig populations) shows positive trends (except in the East due to ASF effects and control policy) and is expanding towards northern latitudes, favoured by milder winters among other factors. Its distribution and relative abundance are relatively well known thanks to presence data and hunting bag, data available for most countries. Continental-scale models relying on hunting bag data have been produced by FAO and *ENETWILD* (e.g.,*ENETWILD*-consortium et al. 2022d). Their flexible and generalist ecology and behaviour mean this species is exposed to many pathogens from many host taxa in many different ways and to interact with other wildlife, domestic animals, and humans, even in urban areas. Is generally only predated by wolves. As other ungulates, wild boar may play a role in the ecology of several vector borne pathogens by enhancing the survival and abundance of vector populations (amplifying effect).

The **main risks associated with wild boar** to be considered in disease surveillance design for early detection are:

- Used environments and established interfaces: this species can approach cities (and increasing problem over Europe) and human settings to feed on garbage (synurbanization is marked in this species across Europe). Visit crops and can interact with domestic pigs in farms or in outdoor conditions. Can mate with them and produce fertile hybrids.
- Role in long-distance spread or cross-border spread in a setting comparable to the EU: wild boar, particularly males (25% of yearling population) may disperse over moderate distances (tens of km), females do so only seldom (but there are exceptional cases of long dispersion in the literature). The population is still increasing and spreading. Role in long-distance spread is generally limited (as indicative, in the order of few tens km/year for ASF). Anthropogenic disease spread through game meat and/or poor hygiene by hunters may occur.
- Role in spread or maintenance if pathogen was introduced by other host species: Important role as a host (spread and maintenance) of many pathogens (Meier and Ryser-Degiorgis 2018, Ruiz-Fons et al. 2018), which may have previously been introduced by other species. Wild boar interact with other ungulates and share pathogens which can be maintained by the ungulate host community. The wild boar/livestock/human interface in Europe, although variable and acquiring different shapes is extensively distributed and almost a continuum over many regions of Europe, which makes difficult to prevent their maintenance and spread (*ENETWILD*-consortium et al. 2021).
- Risk factors relevant to design general surveillance and the main characteristics of disease surveillance for early detection are the same listed for other wild ungulates (see above), with special attention to the interfaces where domestic pig/wild boar/human interaction takes place. However, its ecology, predator and scavenging behaviour makes the exposure of this species to risk factors quite specific (see sections below).
- Regarding the characteristics of disease surveillance for early detection in wild boar, both **Active and passive surveillance are feasible** since most wild boar population are hunted, or to a less extent, shot or captured for population control purposes, and their utility is similar to what above commented for wild ruminants (also similar types of sample material to collect).
- Serological and pathogen detection test available and validated in most cases as in domestic pigs. Environmental sampling through molecular techniques is promising (e.g., for HEV).

Table 18. The main characteristics relevant to surveillance in European **wild boar** aiming at early detection of zoonotic disease in wildlife.

<table>
<tr><th colspan="4">Highly Pathogenic Avian Influenza, Echinococcosis (E. granulosus, E. multilocularis), Hepatitis E</th></tr>
<tr><th colspan="4">Ecological characteristics relevant to epidemiology</th></tr>
<tr><th>Diversity and distribution</th><th>Current knowledge of populations in Europe</th><th colspan="2">Host ecology/epidemiology</th></tr>
<tr><td>A single ubiquitous species in Europe: the wild boar Sus scrofa. Positive trend contrasted by ASF in the east, still expanding towards northern latitudes due to milder winter climatological conditions among other factors. Focal presence of free ranging feral pigs.</td><td>Presence and hunting bag data available for most countries. Continental-scale models relying on hunting bag data have been produced by FAO and Enetwild. Overall positive trend in spite of ASF.</td><td colspan="2">Gregarious, social units represented by matriarchal sounders or groups of subadults, adult males are solitary. Do not use a den. Omnivorous and very plastic, can feed on garbage and carcasses. Its ecology and behaviour make this species to be exposed to many pathogens from many host taxa in many different ways. Is typical prey of wolves. The same species as pig, sharing potentially all pathogens. May play a role in the ecology of several vector borne pathogens by granting survival and abundance of vector populations (amplifying effect).</td></tr>
<tr><th colspan="4">Risks to be considered in disease surveillance design for early detection</th></tr>
<tr><th>Used environments and established interfaces</th><th>Role in long-distance spread / cross-border spread in a setting comparable to the EU</th><th>Role in spread or maintenance if pathogen was introduced by other host species</th><th>Risk factors for general surveillance</th></tr>
<tr><td>Can approach cities (and increasing problem over Europe) and human settings to feed on garbage. Visit crops and can interact with domestic pigs in farms or in outdoor conditions. Can mate with them and produce fertile hybrids.</td><td>Male wild boar (25% of the yearling population) may disperse over moderate distances (tens of km), females do only seldom. The population is still increasing and spreading. Role in long-distance spread is generally limited (as indicative, in the order of few tens km/year for ASF). Anthropogenic spread through game meat and/or poor hygiene by hunters.</td><td>Important role as a host (spread and maintenance) of many pathogens. Wild boar interact with other ungulates, and share pathogens which can be maintained by the ungulate host community.</td><td>1) Where direct and indirect contact with livestock (outdoor or extensive more relevant, however, there is need to better define and characterize wild ungulate/livestock interface in Europe); 2) where pathogens has been reported in both in livestock and wild ungulates, or at least in one compartment, as well as zoonotic cases in humans; 3) where high densities and intensive game management occur, artificial feeding and game release, 4) where vectors for target pathogens are present, 5) at the borders of EU, with special attention to geographical borders of the continent: eastern Europe, the Balkans, southern Mediterranean region, 6) where high diverse ungulate community occurs, 7) passively in sick animals detected by hunters, rangers an citizen science (special attention to unusual mortality or morbidity), 8) preferably adults for active surveillance, calves can be interesting for incidence, 9) where there is direct contact with hunters and consumers of meat, 10) where definitive hosts are present for heteroxenous parasites (e.g., large carnivores).</td></tr>
</table>

<table>
<tr><th colspan="3">Characteristics of surveillance for early detection</th></tr>
<tr><th>Feasibility of surveillance</th><th>Active Surveillance specific to priority diseases</th><th>Passive Surveillance specific to priority diseases</th></tr>
<tr><td>Active monitoring is feasible since most species and population are hunted, or to a less extent, shot or captured for population control purposes. Passive surveillance also feasible in most species for sick and naturally dead found animals, including road kills. Found dead could be reported via APP (e.g., iMammalia already set up for this), communicated and transported to Vet authorities.</td><td>ACTIVE surveillance of hunted/captured/shot specimen needed for early detection of changes of incidence, i.e., early epidemic detection. Also, active surveillance as routine is key to monitor already established endemic pathogens, their maintenance and possible fade out (absence), and are particularly relevant for retrospective studies to elucidate past introduction/circulation of emergent zoonotic pathogens (this includes sentinels, e.g., TBE). The active component of national active surveillance programs must representatively cover the different regions and be organized as risk based to be cost effective and sensitive (see section on risks). Consider the contribution and inclusion of hunting grounds (public, private) and the network of protected areas (Natura 2000 Network).</td><td>PASSIVE surveillance is essential for early detection of the introduction of the pathogen, the onset and duration of the period of increased risk; change in the geographic distribution/spread to new areas and increase in incidence, i.e., early epidemic detection. However, listed pathogens rarely develop symptoms or fatal cases in wild boar. The role of hunters is essential for passive surveillance.</td></tr>
</table>

<table>
<tr><th>Type of sample material to collect</th><th>Diagnostic tests</th></tr>
<tr><td>Blood/organ samples/swabs/ectoparasites from hunted animals might be sampled quite easily during hunting season or dead animals any time, faeces could be collected directly from individuals or by active searching. High quality blood for serum extraction can be taken from the endocranial venous sinuses by puncture in hunted/dead wild boar. Blood, swabs and ectoparasites can be collected from live trapped hosts. Environmental sampling through molecular techniques is promising, both for host and pathogen presence. Non-invasive sampling techniques based on the analysis of oral fluid specimen have been used for wild boar. Techniques to preserve serum at room temperature (Blood Collected on Filter Paper) has been tested successfully but may require testing for specific pathogens and hosts.</td><td>Serological and pathogen detection tests available and validated (those of pigs). Environmental sampling through molecular techniques is promising.</td></tr>
</table>

3.3.3. Characteristics of wild **terrestrial carnivores** relevant to early detection in disease surveillance

Table 19 shows the main characteristics relevant to surveillance in European **wild terrestrial carnivores** aiming at early detection of zoonotic disease in wildlife. This diverse group includes some widespread species such as red fox and badger and several exotic species introduced in the past currently increasing their range (i.e., raccoon, raccoon dog, American mink). There are limited abundance data and hunting statistics are only available for some countries where they are huntable (red fox, badger), which is collected by *ENETWILD*. Limited or no information on populations of small carnivores. Most species declined in the past but are currently recovering. Typically, predators or scavengers are definitive hosts for many pathogens (e.g., cestodes). Their epidemiological roles vary from true maintenance, passing by spill over, to dead-end hosts, and as predators, they can contribute as sentinels of multi-host pathogens, depending on the characteristics of all ecological systems. Phylogenetically close to carnivore pet species (dogs, cats, ferrets) and carnivore species farmed for fur (ferrets, minks), potentially sharing most pathogens. Key ecological role with impact on disease epidemiology since they contribute to control prey species, promoting cascading effects (e.g., control of herbivores, vectors).

The **main risks associated to wild carnivores** to be considered in disease surveillance design for early detection are:

- Used environments and established interfaces: Some species approach cities and even become urban settlers (e.g., red foxes, raccoons). Can frequent farms to prey upon poultry, livestock, or pets (e.g., golden jackals in eastern Europe). Wolves and wildcats can breed with the domestic form and produce fertile hybrids. We note that, although not included in the group, marine carnivores (pinnipeds) are present mainly in Northern Sea coasts, and are potentially susceptible to terrestrial carnivore pathogens, and can even become infected by non-exclusively mammal pathogens included in the list, such as Influenza A viruses (Zohari et al. 2014, Bodewes et al. 2015, Krog et al. 2015).
- Role in long-distance spread or cross-border spread in a setting comparable to the EU: Red fox present continuous distribution over most European territory, which may favour disease spread over long distances. For instance, in the case of rabies in red fox, incursions from other regions account for less than 1% of cases but allow for re-emergence of disease (Baker et al. 2020). Wolf and racoon dog are long distance dispersers that may spread pathogens over long distances may potentially play a very important role in long distance spreading of pathogens through dispersal (e.g., *Echinococcus* and wolf).
- Role in spread or maintenance if pathogen was introduced by other host species: Limited but may occur since many pathogens are shared by different carnivore species. Their predatory or scavenging habits makes them highly exposed to pathogens recently introduced in the animal community (e.g., HPAI in dead birds). May potentially play a very important role in long distance spreading of pathogens introduced by other species through dispersal.
- Risk factors relevant to design general surveillance are:
    - Where direct and indirect contact with domestic carnivores, including areas where fur farms are present (e.g., farm escapes), and sites where pets hang around (dogs);
    - Where pathogens have been reported in both in wild and domestic carnivores, as well as zoonotic cases in humans;
    - Where vectors for target pathogens are present;

    - At the borders of EU, with special attention to geographical borders of the continent or where some species are naturally expanding (e.g., golden jackal);
    - Where highly diverse carnivore community composition occurs;
    - Where carnivores are intensively managed for hunting purposes (e.g., feeder stations in eastern Europe);
    - Passively in sick animals detected by hunters, rangers and citizen science;
    - Where intermediate hosts (e.g., ungulates, lagomorphs, rodents) are present for heteroxenous parasites;
    - Abundant and widely, ecologically opportunistic distributed species are relevant as sentinels (e.g., red fox).
- Regarding the characteristics of disease surveillance for early detection in wild carnivores:
    - **Active surveillance** is more feasible in most common (such as red fox, badger, some small mustelids) and exotic species (American mink, raccoon, raccoon dog), which are hunted or subject to population control.
        - Active surveillance of hunted/captured/shot specimen is needed for early detection of changes of incidence, i.e., early epidemic detection.
        - Abundant species, such as red fox are useful for active and passive surveillance.
        - Also, routine active surveillance is key to monitor already established endemic pathogens, their maintenance and fade out, and are particularly relevant for retrospective studies to elucidate past introduction/circulation of emergent zoonotic pathogens.
        - Active monitoring of hunted/shot/captured specimen needs to follow a risk-based approach, especially for those highly available species, in which a cost-effective sampling designs can be performed. The active component of national active surveillance programs must representatively cover the different regions and be organized as risk based to be cost effective and sensitive (see section on risks). Consider the contribution and inclusion of hunting grounds (public, private) and the network of protected areas (Natura 2000 network).
        - Wild carnivores can contribute to active surveillance (antibody detection) as sentinels for prey diseases (e.g., red fox and rodent pathogens).
    - **Passive surveillance**
        - Useful for early detection, changes of incidence (e.g., red fox as sentinel) and recent introduction of pathogens.
        - Complementary to active surveillance since pathogens of the list rarely develop symptoms or are fatal in wild carnivores. It includes road kills, although possibly not sufficient in less abundant more evasive species and should be enhanced. For that purpose, found dead animals could be reported via apps (like *iMammalia*) and transported to veterinary authorities.
        - Abundant and widely, ecologically opportunistic distributed species are relevant as sentinels also in passive surveillance (e.g., red fox).
- The type of sample to collect include blood/organ samples/swabs/ectoparasites from hunted animals might be sampled quite easily during hunting season or dead animals any time, faeces could be collected directly from individuals or by active searching. High

quality blood for serum extraction can be taken from the endocranial venous sinuses or from the heart by puncture in hunted/dead animals. Blood, swabs and ectoparasites can be collected from live trapped hosts. Environmental sampling through molecular techniques is promising, both for host and pathogen presence. Wild carnivores usually defecate in latrines, easily identifiable, which can be sampled for pathogen detection and host identification. The non-invasively collected faecal samples can provide information about the presence of viruses specific to the host and viruses derived from their prey as well (e.g., HEV). Techniques to preserve serum at room temperature (Blood Collected on Filter Paper) has been tested successfully but may require testing for specific pathogens and hosts.

- Diagnostic tests: Some serological and pathogen detection tests available and validated in most common carnivores' species (e.g., red fox) or in their domestic counterparts (cats, wolves). However, multi-host test's reliability needs to be tested in some specific host and pathogen dyads, as well as the sensitivity/specificity of PCR vs other tests (immunopathology, pathogen isolation). Environmental sampling for molecular techniques testing is promising.

Table 19. The main characteristics relevant to surveillance in European **wild terrestrial carnivores** aiming at early detection of zoonotic disease in wildlife.

<table>
<tr><th colspan="4">Highly Pathogenic Avian Influenza, Echinococcosis (E. granulosus, E. multilocularis), Hepatitis E</th></tr>
<tr><th colspan="4">Ecological characteristics relevant to epidemiology</th></tr>
<tr><th>Diversity and distribution</th><th>Current knowledge of populations in Europe</th><th colspan="2">Host ecology/epidemiology</th></tr>
<tr><td>Diverse number of species (26), some are widespread (e.g., red fox and badger). Populations of large carnivores are more continuous and abundant in eastern Europe but are expanding in other regions. Several exotic species introduced in the past currently increasing their range (raccoon, raccoon dog, American mink).</td><td>Presence data are available but incomplete for most species and countries (atlases, citizen science). Large predator distribution well known (few initiatives of coordinated cross-boundary monitoring of large carnivores). Limited abundance data of different types are available at country level. Hunting statistics for huntable species (red fox, badger) collected by ENETWILD. Limited or no information on populations of small carnivores. Most species declined in the past but are currently recovering.</td><td colspan="2">Solitary or with small social units. Use dens. Typically, predators or scavengers and definitive host for pathogens (e.g., metazoan). Their roles vary from true maintenance, passing by spill over, to dead-end hosts. As predators they can contribute as sentinels of multi-host pathogens, depending on the characteristics of all ecological systems. Phylogenetically close to carnivore pet species (dogs, cats, ferrets) and carnivore species farmed for fur (ferrets, minks), potentially sharing most pathogens. Key ecological role with impact on disease epidemiology since they contribute to control (abundant) prey species, promoting cascading effects and structuring diverse communities (dilution effect).</td></tr>
<tr><th colspan="4">Risks to be considered in disease surveillance design for early detection</th></tr>
<tr><th>Used environments and established interfaces</th><th>Role in long-distance spread / cross-border spread in a setting comparable to the EU</th><th>Role in spread or maintenance if pathogen was introduced by other host species</th><th>Risk factors for general surveillance</th></tr>
<tr><td>Approach cities in search of protection or human-derived food. Some species become urban settlers (e.g., red foxes, raccoons). Can frequent farms to prey upon poultry, livestock, or pets. Wolves and wildcats can breed with domestic cats and produce fertile hybrids.</td><td>Wolf and racoon dog are long distance dispersers. i.e., may spread pathogens over long distances. Racoons and racoon dogs, wild cats, wolf, and golden jackal are still spreading their range. Red fox present continuous distribution over most European territory, which may favour disease spread over long distances.</td><td>Limited (but host species/pathogen specific) role as a host (spread and maintenance) of pathogens. Some species may play a very important role in long distance spreading.</td><td>1) Where direct and indirect contact with domestic carnivores, including areas where fur farms are present, 2) where pathogens has been reported in both in wild or domestic carnivores, as well as zoonotic cases in humans, 3) where vectors for target pathogens are present; 4) at the borders of EU, with special attention to geographical borders of the continent or where some species are naturally expanding (e.g. golden jackal), 5) where high carnivore community compositions, 6) where artificially fed; 7) passively in sick animals detected by hunters, rangers an citizen science, 8) where intermediate hosts (e.g. ungulates, lagomorphs, rodents) are present for heteroxenous parasites, 9) abundant and widely, ecologically opportunistic distributed species are relevant as sentinels (e.g. red fox), taking advantage of population control for exotic species (e.g. raccoon, American mink)</td></tr>
</table>

<table>
<tr><th colspan="4">Characteristics of surveillance for early detection</th></tr>
<tr><th>Feasibility of surveillance</th><th colspan="2">Active Surveillance specific to priority diseases</th><th>Passive Surveillance specific to priority diseases</th></tr>
<tr><td>Active monitoring is feasible in most common (such as red fox, badger, some small mustelids) and exotic species (American mink, raccoon, raccoon dog), which are hunted or subject of population control. Passive surveillance also feasible for sick and naturally found dead animals, including road kills, although possibly not sufficient in less abundant and more evasive species. Found dead could be reported via APP (e.g., iMammalia) and transported to veterinary authorities. Abundant species, such as red fox are useful for both general and targeted surveillance.</td><td colspan="2">ACTIVE surveillance of hunted/captured/shot specimen needed for early detection of changes of incidence, i.e., early epidemic detection. Also, active surveillance as routine is key to monitor already established endemic pathogens, their maintenance and fade out, and are particularly relevant for retrospective studies to elucidate past introduction/circulation of emergent zoonotic pathogens. The active component of national active surveillance programs must representatively cover the different regions and be organized as risk based to be cost effective and sensitive (see section on risks). Consider the contribution and inclusion of hunting grounds (public, private) and the network of protected areas (Natura 2000 network). They can contribute as sentinels of multi-host pathogens, depending on the characteristics of the ecological systems.</td><td>Passive surveillance recommended as complementary (pathogens of the list rarely develop symptoms or are fatal). Useful for early detection, changes of incidence (e.g., red fox as sentinel) and recent introduction of pathogens.</td></tr>
<tr><th colspan="2">Type of sample material to collect</th><th colspan="2">Diagnostic tests</th></tr>
<tr><td colspan="2">Blood/organ samples/swabs/ectoparasites from hunted animals might be sampled quite easily during hunting season or dead animals any time, faeces could be collected directly from individuals or by active searching. High quality blood for serum extraction can be taken from the endocranial venous sinuses or from the heart by puncture in hunted/dead animals. Blood, swabs and ectoparasites can be collected from live trapped hosts. Environmental sampling through molecular techniques is promising, both for host and pathogen presence. Environmental sampling through molecular techniques is promising, both for host and pathogen presence. Wild carnivores usually defecate in latrines, easily identifiable, which can be sampled for pathogen detection and host identification. The non-invasively collected faecal samples can provide information about the presence of viruses specific to the host and viruses derived from their prey as well (e.g., HEV). Techniques to preserve serum at room temperature (Blood Collected on Filter Paper) has been tested successfully but may require testing for specific pathogens and hosts.</td><td colspan="2">Some serological and pathogen detection tests available and validated in most common carnivores' species (e.g. red fox) or those with domestic counterpart (cats, wolves). However, multi-host test's reliability/performance needs to be tested in some specific host and pathogen dyads, as well as the sensitivity/specificity of PCR vs other tests (immunopathology, pathogen isolation). Environmental sampling through molecular techniques is promising.</td></tr>
</table>

3.3.4. Characteristics of **wild lagomorphs** relevant to early detection in disease surveillance

Table 20 shows the main characteristics relevant to zoonotic disease surveillance in European **wild lagomorphs.** Among wild lagomorphs, wild European rabbit (*Oryctolagus cuniculus*) and brown hare (*Lepus europaeus*) have the widest range and reach the highest local densities. This group present declining populations in most regions, but locally, European wild rabbits are considered pests. Presence data (atlases, citizen science) and hunting bags are available for several countries. European rabbits are gregarious and use common burrows (which may associate to presence of vectors such as ticks, fleas, or sandflies), hares are less social and do not use burrows. They are typical prey of meso-carnivores and may carry intermediate parasite forms. European wild rabbit is phylogenetically close to domestic rabbit, potentially sharing all pathogens. Their roles vary from intermediate hosts for host carnivore pathogens, to true maintenance hosts in nature and peri-urban areas (e.g., *Leishmania*). They can contribute as sentinels for multi-host pathogens (e.g., Q-fever). Similar to ungulates, lagomorphs may not play a direct role in the maintenance of certain vector borne pathogens, because, generally, the level of viraemia after natural infection is too low to infect vectors (e.g., the absence of the pathogens in the ticks collected from them is usual). Nevertheless, they play a role in the ecology of such vector borne pathogens, being a key host in granting survival and abundance of vector populations (amplifying effect).

The main characteristics relevant to surveillance in **European wild lagomorphs** aiming at early detection of zoonotic disease in wildlife are:

- Used environments and established interfaces: rabbits are common urban settlers and can form large colonies in the cities. In such environments, vector borne meditated interface may be relevant (e.g., ticks, fleas, sand flies). Hares are more used to frequent rural and natural habitats.
- Role in long-distance spread or cross-border spread in a setting comparable to the EU: Limited as hares and rabbits are very site faithful. Anthropogenic spread of pathogen and vectors through restocking, game meat transport and/or poor hygiene and biosecurity by hunters may happen.
- Role in spread or maintenance if pathogen was introduced by other host species: they are relevant for maintenance of multi-host pathogens (e.g., tularaemia in concomitance with rodents, Q-fever with wild or domestic ruminants).
- Risk factors relevant to designing general surveillance are:
    - Where direct and/or indirect contact with domestic lagomorphs;
    - Where high densities of lagomorphs;
    - Where pathogens have been reported in both in wild and domestic lagomorphs, as well as zoonotic cases in humans, often in peri-urban environment;
    - Where vectors for target pathogens are present;
    - Where they can play a role as sentinels for certain pathogens (e.g., *Coxiella burnetti*) (Sánchez et al. 2022);
    - At the borders of EU, with special attention to geographical borders of the continent: Eastern Europe, the Balkans, Southern Medi-terranean region;
    - Where highly diverse lagomorph communities occur;

  - Passively in sick animals detected by hunters, rangers, citizens (citizen science);
  - Where adequate definitive hosts (e.g., carnivore species) are present for heteroxenous parasites;
  - Where presence of other wildlife sharing the same pathogens (e.g., rodents and tularaemia; wild ruminants and Q-fever);
  - Where definitive hosts (e.g., carnivores) are present for heteroxenous parasites;
- Regarding the characteristics of disease surveillance for early detection in wild lagomorphs:
  - **Active surveillance** is feasible since most rabbits and hare species are hunted, and to a less extent, shot or captured for population control purposes. However, some species are rare, in general or locally, and hunting is restricted or forbidden.
    - ACTIVE surveillance of hunted/captured/shot specimen is needed for early detection of changes of incidence, i.e., early epidemic detection.
    - Also, active surveillance as routine is key to monitor already established endemic pathogens, their maintenance and fade out, and are particularly relevant for retrospective studies to elucidate past introduction/circulation of emergent zoonotic pathogens.
    - The active component of national active surveillance programs must representatively cover the different regions and species and organized as risk based to be cost-effective and sensitive (see section on risks). Consider the contribution and inclusion of hunting grounds (public, private) and the network of protected areas (Natura 2000 network). The role of hunters is essential.
  - **Passive surveillance** also feasible in most species for sick and naturally dead found animals, including road kills.
    - Passive surveillance is relevant for early detection of the introduction of the pathogen, the onset and duration of the period of increased risk; change in the geographic distribution/spread to new areas and increase in incidence, i.e., early epidemic detection.
    - Found dead could be reported via APP (e.g., *iMammalia*) and transported to Vet authorities.
- The type of sample material to collect include blood/organ samples/swabs/ectoparasites from hunted animals might be sampled quite easily during hunting season or dead animals any time, faeces could be collected directly from individuals or by active searching. High quality blood for serum extraction can be taken from the heart by puncture in hunted/dead animals. Blood, swabs and ectoparasites can be collected from live trapped hosts. Environmental sampling through molecular techniques is promising (e.g., Q-fever), both for host and pathogen presence. Techniques to preserve serum at room temperature (Blood Collected on Filter Paper) has been tested successfully but may require testing for specific pathogens and hosts.
- Diagnostic tests: Serological and pathogen detection tests available and validated in wild rabbit (normally already tested in domestic rabbit, a frequently used experiment animal). Test already used in several hare species, but their reliability still needs to be tested in hares, as well as the sensitivity/specificity of PCR *vs* other tests (immunopathology, pathogen isolation). Environmental sampling through molecular techniques is efficient (Abeykoon et al. 2021).

Table 20. The main characteristics of relevance for zoonotic disease surveillance in **wild lagomorphs** aiming at early detection of disease in wildlife.

<table>
<tr><th colspan="4">Hepatitis E, Q-fever</th></tr>
<tr><th colspan="4">Ecological characteristics relevant to epidemiology</th></tr>
<tr><th>Diversity and distribution</th><th>Current knowledge of populations in Europe</th><th colspan="2">Host ecology/epidemiology</th></tr>
<tr><td>The highest species diversity (out of the 8 species present) is found in the Iberian and the Italian peninsulas (4 or 5 species each). Wild European rabbit and brown hare have the widest range and reach the highest local densities. Pika species in cold, mountainous areas in Eastern Europe. Some local populations of exotic species introduced in the past.</td><td>Presence data (atlases, citizen science) and hunting bags are available for several countries. Abundance data are locally available and poor. In most regions declining populations, but locally, European wild rabbits are considered a pest.</td><td colspan="2">Rabbits are gregarious and use common burrows, hares are less social and do not use burrows. Vegetarian diet. Are typical prey of meso-carnivores. European wild rabbit is phylogenetically close to domestic rabbit, potentially sharing all pathogens. Their roles vary from intermediate hosts for carnivore pathogens, to true maintenance hosts in nature and peri-urban areas (e.g., Leishmania), and can contribute as sentinels. Similar to ungulates, lagomorphs do not seem to play a direct role in the maintenance of certain vector borne, because, generally, the level of viraemia after natural infection is too low to infect vectors (e.g., the absence of the pathogens in the ticks collected from them is usual). Nevertheless, they play a role in the ecology of such vector borne pathogens, being a key host in granting survival and abundance of vector populations (amplifying effect).</td></tr>
<tr><th colspan="4">Risks to be considered in disease surveillance design for early detection</th></tr>
<tr><th>Used environments and established interfaces</th><th>Role in long-distance spread / cross-border spread in a setting comparable to the EU</th><th>Role in spread or maintenance if pathogen was introduced by other host species</th><th>Risk factors for general surveillance</th></tr>
<tr><td>Rabbits are common urban settlers and can form large colonies in the cities. In such environments, vector borne mediated interface may be relevant (e.g., ticks, sand flies). Hares are more used to frequent rural and natural habitats.</td><td>Hares and rabbits are very site faithful. Mainly passive role. Anthropogenic spread through game meat and/or poor hygiene by hunters.</td><td>They are relevant for maintenance of multi-host pathogens (e.g., tularaemia in concomitance with rodents, or Q-fever with wild or domestic ruminants).</td><td>1) Where direct and/or indirect contact with domestic lagomorphs, 2) where high densities of lagomorphs, 3) where pathogens have been reported in both in wild or domestic lagomorphs, as well as zoonotic cases in human, often in peri-urban environment, 4) where vectors for target pathogens are present, 5) at the borders of EU, with special attention to geographical borders of the continent: eastern Europe, the Balkans, southern Mediterranean region, 6) where high lagomorph community composition, 7) passively in sick animals detected by hunters, rangers, citizens and citizen science, 8) where adequate definitive hosts (e.g. carnivore species) are present for heteroxenous parasites, 9) where presence of other wildlife sharing the same pathogens (e.g., rodents and tularaemia, ruminants and Q-fever).</td></tr>
</table>

Characteristics of surveillance for early detection		
Feasibility of surveillance	**Active Surveillance specific to priority diseases**	**Passive Surveillance specific to priority diseases**
Active monitoring is feasible since most rabbit and hare species are hunted, or to a less extent, shot or captured for population control purposes. However, some species are rare, in general or locally, and hunting is restricted or forbidden. Passive surveillance also feasible in most species for sick and naturally dead found animals, including road kills. Found dead could be reported via APP (e.g., *iMammalia*) and transported to Vet authorities.	ACTIVE surveillance of hunted/captured/shot specimen needed for early detection of changes of incidence, i.e., early epidemic detection. Also, active surveillance as routine is key to monitor already established endemic pathogens, their maintenance and fade out, and are particularly relevant for retrospective studies to elucidate past introduction/circulation of emergent zoonotic pathogens. The active component of national active surveillance programs must representatively cover the different regions and lagomorph species and be organized as risk based to be cost effective and sensitive (see section on risks). Consider the contribution and inclusion of hunting grounds (public, private) and the network of protected areas (Natura 2000 network).	PASSIVE surveillance is essential for early detection of the introduction of the pathogen, the onset and duration of the period of increased risk; change in the geographic distribution/spread to new areas and increase in incidence, i.e., early epidemic detection. The role of hunters is essential.

Type of sample material to collect	Diagnostic tests
Blood/organ samples/swabs/ectoparasites from hunted animals might be sampled easily during hunting season from dead animals any time, faeces could be collected directly from individuals or by active searching. High quality blood for serum extraction can be taken from the heart by puncture in hunted/dead animals. Blood, swabs and ectoparasites can be collected from live trapped hosts. Environmental sampling through molecular techniques is promising (e.g., Q-fever). Techniques to preserve serum at room temperature (Blood Collected on Filter Paper) has been tested successfully but may require testing for specific pathogens and hosts.	Serological and pathogen detection tests available and validated in wild rabbit (those of domestic rabbit, a frequently used experiment animal. Test already used in several hare species, but their reliability still needs to be tested in hares, as well as the sensitivity/specificity of PCR vs other tests (immunopathology, pathogen isolation). Environmental sampling through molecular techniques in common borrows (e.g., *Coxiella*).

3.3.5. Characteristics of **wild micromammals** relevant to early detection in disease surveillance

Table 21 shows the main characteristics relevant to zoonotic disease surveillance in European **wild micromammals.** This functional group is the most diverse in Europe, including 63 rodents (Order Rodentia) and 36 insectivores (Order Eulipotyphla, previously Insectivora, 23 of which are shrews). Some species are common and widely distributed, while others have restricted range or local populations, and a few are exotic (such as the American grey squirrel). Some rodent species are actually medium sized (e.g., some squirrels, porcupine, marmots, beaver or exotic coypu and nutria rat). Occurrence data available for most species across Europe (atlases, citizen science, EU agencies); abundance data only available locally, mostly collected for research purposes. They present diverse social behaviours and ecologies; from solitary to gregarious (sometimes seasonally); from semi-aquatic, terrestrial, to arboreal; many species use burrows (temporarily or permanently); mostly herbivorous diet but may take animal matter. They normally are relevant prey species, and intermediate hosts for different pathogens. Some rodents (e.g., voles) present marked population fluctuations and disease outbreaks (e.g., tularaemia) as a function of several factors, such as tree-masting years (result in abundance increase), with spill over to other species (Herrero-Cófreces et al. 2021). They play a direct role in the maintenance of several vector borne and/or an amplifying effect by granting survival and abundance of vector populations (e.g., ticks).

The **main risks associated to wild micromammals** to be considered in disease surveillance design for early detection are:

- Used environments and established interfaces: Full range of natural habitats and urbanisation gradient (from settlements to city centres); interfaces include farms, city parks, natural areas used for leisure activities; potential contact mainly indirect through the environment.
- Role in long-distance spread or cross-border spread in a setting comparable to the EU: Sedentary or with limited movement capacity; spreading potential on at local scales; irrelevant for cross-border spread, except when moved through anthropogenic means.
- Role in spread or maintenance if pathogen was introduced by other host species: important role as a maintenance host for several pathogens shared by the micromammal community, and other mammals (e.g., tularaemia and lagomorphs, possibly Q-fever and ruminants and lagomorphs) interact with several species, habitats, and humans, often mediated by vectors (mainly ticks).
- Risk factors relevant to designing general surveillance are:
    - Where direct and indirect contact with livestock, in farms and peri-urban areas;
    - Where high densities of micromammals;
    - Where pathogens, morbidity or mortality has been reported in micromammals, as well as zoonotic cases in human (e.g., TBE in central and East Europe);
    - When and where unusual or expected (cyclic) population explosion (e.g., population fluctuations in voles) or pests in anthropized farmland;
    - Where vectors for target pathogens are present;
    - Where high diverse micromammal community compositions;
    - Passively in dead animals detected by hunters, rangers and by citizen science;
    - Where definitive hosts (e.g., carnivore species) are present for heteroxenous parasites;

- Where presence of other wildlife sharing the same pathogens (e.g., lagomorphs and ruminants);
- Where direct and indirect contact with livestock, pets, humans; outdoor recreational activities (e.g., mushroom picking); land-use changes; urban sprawl; tree-masting years (result in abundance increase);
- Abundant and widely, ecologically opportunistic distributed species are relevant as sentinels;
- Some groups/spp. are more susceptible or useful as indicator for certain pathogens.

- Regarding the characteristics of disease surveillance for early detection in wild micromammals, the feasibility of sampling is variable depending on their abundance, diversity, and legal status.
  - **Active surveillance** in micromammal species that are not hunted is difficult (except some medium sized rodents), unless they are captured on purpose, or they are subject to control as pest species. Some species are protected and require trapping and handling, which should be performed by trained personnel.
    - Thousands of micromammals (incl. protected spp.) are captured in Europe every year in the frame of research activities and population control (e.g., urban, peri-urban and farm pest control, often associated to population booms, cyclic or not, e.g., voles). This could be a relevant source of samples for active surveillance, but also for passive surveillance.
    - Active surveillance of captured specimens needed for early detection of changes of incidence, i.e., early epidemic detection, but also key to monitor already established endemic pathogens, their maintenance and fade out, and are particularly relevant for retrospective studies to elucidate past introduction/circulation of emergent zoonotic pathogens.
    - The active component of national active surveillance programs must representatively cover the different regions, species, host communities, landscape, levels of anthropizing, i.e., be organized as risk based to be cost effective and sensitive (see section on risks). Consider the contribution and inclusion the network of protected areas (Natura 2000 network), and the existence of rodent control programs. Environmental techniques to detect pathogens and micromammal host community are promising.
  - **Passive surveillance** is possible when unusual or cyclic population fluctuations and associated mortalities are detected, but normally, access to carcasses is difficult. It is recommended as enhanced passive surveillance, which can be compulsory under specific disease emergence circumstances or regions (sick/dead micromammals are difficult to find in normal contexts but easier when unusual or cyclic mortality events).
    - However, passive surveillance in micromammals is normally impractical as the clinical course of listed diseases is asymptomatic, and carcasses are difficult to find. It may complement early detection by active surveillance.
    - Passive surveillance is useful as a source of (limited number) samples even when no morbidity or mortality are detected. Passive surveillance can be coupled with population booms and mortality associated to diseases (e.g., tularaemia).
- The type of sample to collect include blood/organ samples/swabs/ectoparasites might be sampled from dead animals any time, faeces could be collected directly from individuals

or in some cases, by active searching. Limited amount of blood, swabs and ectoparasites can be collected from live trapped hosts. Environmental sampling through molecular techniques is promising, both for hosts and pathogens presence. Techniques to preserve serum at room temperature (Blood Collected on Filter Paper) has been tested successfully but may require testing for specific pathogens and hosts.

- Diagnostic tests: Serological and pathogen detection tests available and validated in mice (those of domestic mouse, a common laboratory animals), but usefulness need testing for other species, as well as the sensitivity/specificity of PCR *vs* other tests (immunopathology, pathogen isolation). Environmental sampling through molecular techniques is promising, also as bar coding for determining host communities (Galán et al. 2021).

Table 21. The main characteristics of relevance for zoonotic disease surveillance in **wild micromammals** aiming at early detection of disease in wildlife.

Tick-Borne-Encephalitis, Echinococcosis (*E. multilocularis*), Hepatitis E, Lyme Borreliosis, Q-fever, Rift Valley Fever			
Ecological characteristics relevant to epidemiology			
Diversity and distribution	**Current knowledge of populations in Europe**	**Host ecology/epidemiology**	
The most diverse group including 63 rodents (Order Rodentia) and 36 insectivores (Order Eulipotyphla, 23 of which are shrews); some species common and widely distributed (mice, voles, rats, squirrels); others have restricted range or local populations (dormice, shrews); some species threatened, a few are exotics (American grey squirrel). Several orders of small mammals, although in the same taxonomic group (rodents) some species are medium sized (e.g., squirrels, porcupine, marmots, or exotic coypu).	Occurrence data available for most species across Europe (atlases, citizen science, EU agencies); abundance data only available locally, mostly collected for research purposes	Among the rodents, two groups are of particular relevance. Peri-domestic mice and rats, for instance, are important bridge hosts regarding zoonotic bacterial pathogens such as *Salmonella* or *Leptospira*, among others or good intermediate hosts for *Toxoplasma gondii* or *Neospora caninum* with important effects on the human health in the first case and on livestock abortion storms in the second. Voles and other rodents sometimes are important in the cycle of emerging tick born pathogen such as *Borrelia burgdorferi*, tick borne encephalitis or zoonotic *Babesia*. Diverse social behaviours and ecologies; from solitary to gregarious (sometimes seasonally); from semi-aquatic, terrestrial, to arboreal; many species use borrows (temporarily or permanently); herbivorous diet but may take animal matter. Relevant prey species, and intermediate hosts for different pathogens. Some present explosive population dynamics and disease outbreaks (e.g., tularaemia) as a function of several factors, such as tree-masting years (result in abundance increase), with spill over to other species. They play a direct role in the maintenance of several vector borne (e.g., ticks) and/or an amplifying effect by granting survival and abundance of vector populations.	
Risks to be considered in disease surveillance design for early detection			
Used environments and established interfaces	**Role in long-distance spread / cross-border spread in a setting comparable to the EU**	**Role in spread or maintenance if pathogen was introduced by other host species**	**Risk factors for general surveillance**
Full range of natural habitats and urbanisation gradient (from settlements to city centres); interfaces include farms, city parks, natural areas used for leisure activities; potential contact mainly indirect through the environment.	Sedentary or with limited movement capacity; spreading potential at local scales; irrelevant for natural cross-border spread.	Important role as a maintenance host for several pathogens shared by the micromammal community, also with lagomorphs and rabbits. Micromammals interact with several species, habitats, and humans, often mediated by vectors (mainly ticks).	1) Where direct and indirect contact with livestock, in farms and peri-urban areas, 2) where high densities of micromammals, 3) where pathogens, morbidity or mortality has been reported in micromammals, as well as zoonotic cases in human, 4) when and where unusual or expected (cyclic) population booms (e.g. voles), 5) where vectors for target pathogens are present, 6) at the borders of EU, 7) where high micromammal community compositions, 8) passively in dead animals detected by hunters, rangers an citizen science, 9) where definitive hosts (e.g. carnivore species) are present for heteroxenous parasites, 10) where presence of other wildlife sharing the same pathogens (e.g. lagomorphs and tularaemia, ruminants and Q-fever), 11) where direct and indirect contact with livestock, pets, humans; outdoor recreational activities (e.g. mushroom picking); land-use changes; urban sprawl; tree-masting years (result in abundance increase), 12) abundant and widely, ecologically opportunistic distributed species are relevant as sentinels, taking advantage of population control campaigns, 13) some groups/species are more susceptible or useful as indicator for certain pathogens.
Characteristics of surveillance for early detection			

Feasibility of surveillance	Active Surveillance specific to priority diseases	Passive Surveillance specific to priority diseases
Their abundance, diversity and legal status is variable. Active surveillance in micromammal species that are not hunted is difficult (except some medium sized rodents), unless they are captured on purpose, or they are subject to control as pest species. Some species are protected and require trapping and handling, which should be performed by trained personnel. Passive surveillance possible when unusual or cyclic mortalities are detected, but normally, access to carcasses is difficult.	ACTIVE surveillance of captured specimens needed for early detection of changes of incidence, i.e., early epidemic detection, but also key to monitor already established endemic pathogens, their maintenance and fade out, and are particularly relevant for retrospective studies to elucidate past introduction/circulation of emergent zoonotic pathogens. Relevant for early detection of pathogen introduction (TBE). species, host communities, landscape, levels of anthropizing, i.e., be organized as risk based to be cost effective and sensitive (see section on risks). Consider the contribution and inclusion the network of protected areas (Natura 2000 network), and rodent control programs. Active surveillance, which can also be developed in the frame of research activities, population control (e.g., urban, peri-urban and farm pest control, often associated to population booms, cyclic or not, e.g., voles). Environmental techniques to detect pathogens (e.g., at micromammal burrows) can be used in active surveillance.	PASSIVE surveillance in micromammals is normally impractical for the range of pathogens as normally the clinical course of disease is asymptomatic, and carcasses are difficult to find. However, it may complement early detection by active surveillance. Passive surveillance is useful as a source of samples even when no morbidity or mortality are detected. Passive surveillance can be coupled with population booms and mortality associated to diseases not in the list (tularaemia).

Type of sample material to collect	Diagnostic tests
Blood/organ samples/swabs/ectoparasites might be sampled from dead animals any time, faeces could be collected directly from individuals or in some cases, by active searching in borrows. Limited amount of blood, swabs and ectoparasites can be collected from live trapped hosts. Environmental sampling through molecular techniques is promising, both for hosts and pathogens presence. Techniques to preserve serum at room temperature (Blood Collected on Filter Paper) has been tested successfully but may require testing for specific pathogens and hosts.	Serological and pathogen detection tests available and validated in mice (those of domestic mouse, a common laboratory animal), but usefulness need testing for other species, as well as the sensitivity/specificity of PCR vs other tests (immunopathology, pathogen isolation). Environmental sampling through molecular techniques is promising.

3.3.6. Characteristics of **chiropterans** relevant to early detection in disease surveillance

Table 22 shows the main characteristics relevant to zoonotic disease surveillance in **chiropterans** (Order Chiroptera). Bats constitute the second most diverse mammal group in Europe. Overall, their population trends seem to be increasing or stable in number, but more monitoring effort is required to confirm this. Bats harbour the most virulent zoonotic viruses even when compared to birds. Bats possess several characteristics that might make them effective reservoirs for other pathogens: high species diversity, long life span, capacity to travel long distances, dense aggregations, social behaviour, and a unique immunology which is hypothesized to be due to molecular adaptations that support the physiology of flight. Some species present long-term, long-distance migrations. The play a key ecological role with impact on vector borne disease epidemiology since they contribute to vector control (mainly mosquitoes), promoting cascading effects (reduction of vectors and disease spread, also zoonosis).

The **main risks associated to chiropterans** to be considered in disease surveillance design for early detection are:

- Used environments and established interfaces: bats occupy a variety of environments, either natural such as plants, caves, rocks or even manmade, such buildings and bridges. They can therefore be present in urban, peri-urban, farmland or natural areas and establish interfaces with humans and domestic animals.
- Role in long-distance spread or cross-border spread in a setting comparable to the EU: some species are capable of performing large migrations that could result in a cross-border spread. Many European species of bats migrate long distances. Some are known to migrate over more than 1,000 km. There is a need for precise assessment of migration routes, including possible movements between Africa and Southern Europe.
- Role in spread or maintenance if pathogen was introduced by other host species: Bats have a gregarious behaviour that leads to a concentration of many individuals in a small space (roosts) and they are a highly mobile animal, which increases the change that they could act as reservoir or vector for several pathogens, spread and maintain them. Unknow epidemiological role for several zoonotic pathogens which has been detected in bats.
- Specific risk factors relevant to design general surveillance are:
    - Where direct and indirect contact with livestock is suspected, in farms and peri-urban areas;
    - Where high aggregation of bats in colonies, particularly mixed colonies (different species) or colonies associated with human habitation;
    - Where pathogens, morbidity or mortality have been reported in bats, as well as zoonotic cases in human;
    - Where vectors for target pathogens are present;
    - At the borders of EU and where migration from Africa (Mediterranean basin);
    - Passively in dead animals detected by hunters, rangers, citizen science, rescue centres, aerogenerators;
    - Where presence of other wildlife sharing the same pathogens;

    - Areas susceptible to rapid environmental change could be most at risk and it is more likely that a spill over event occurs in bat populations nearest to human settlements or domestic animals;
    - Preferably target some species capable of performing large migrations that could result in a cross-border spread (e.g., *Nyctalus* and *Pipistrelus*);
    - Some species are more susceptible or useful as indicator for certain pathogens (e.g., coronaviruses and horseshoe-rhinolophid bats).
- Regarding the characteristics of disease surveillance for early detection in bats, they are not hunted, and all species are protected.
    - **Active surveillance:** Bats represent a wide diversity of species which are not hunted.
        - Based on sampling live animals, which will require capture and trained personnel with mist nets and harp traps. Some species are more difficult to capture (e.g., forestall ones) and therefore their sample size maybe too small to detect pathogens circulating at low prevalence (Hayman et al. 2012). Longitudinal data from migrating species is also very difficult to gather.
        - Environmental samples such as urine and faeces can be an option. Wildlife rescue centres might also be a good source for passive surveillance.
        - Active surveillance of captured specimens needed for early detection of changes of incidence, i.e., early epidemic detection, but also key to monitor already established endemic pathogens, their maintenance and fade out.
        - Active surveillance is particularly relevant for retrospective studies to elucidate past introduction/circulation of zoonotic pathogens (bats are long lived).
        - Active surveillance can be developed in the frame of research activities.
        - The active component of national active surveillance programs must representatively cover the different regions, bat communities and ecological adaptions (cavern, forestall), and be organized as risk based to be cost effective and sensitive. Consider the contribution and inclusion of the network of protected areas (Natura 2000 network) and stakeholders (e.g., Eurobats, https://www.eurobats.org/).
    - **Passive surveillance**: Although bats are not hunted, they may be found sick and passed to human carers, or dead (passive surveillance is possible).
        - Recommended passive (enhanced), but sick/dead bats are difficult to find in normal contexts. Pay special attention to unusual mortalities in colonies, and use rescue centres, zoos, fields of aerogenerators, etc.
        - Passive surveillance in bats is normally impractical for these range of pathogen as normally the clinical course is asymptomatic
- The type of sample material to collect: individual samples during capture include blood, ectoparasites, oral, urogenital, and anal swabs. Pooled samples: environmental samples such as urine and faeces can be collect-ed by "under-roost sheet sampling" (e.g., which uses plastic sheets to collect pooled samples of bat excreta (Bourgarel et al. 2018). In addition, tissues ca be collected during necropsy. Techniques to preserve serum at room temperature (blood collected on filter paper) has been tested successfully but may

require testing for specific pathogens and hosts. Environmental sampling through molecular techniques is promising, both for hosts and pathogens presence.

- Diagnostic tests: multi-host serological and pathogen detection tests already used in several bat species in the literature, but their reliability needs to be tested in other species, as well as the sensitivity/specificity of PCR *vs* other tests (immunopathology, pathogen isolation). Environmental sampling through molecular techniques is promising.

Table 22. The main characteristics of relevance for zoonotic disease surveillance in **chiropterans** (bats) aiming at early detection of disease in wildlife.

Crimean Congo Haemorrhagic Fever, Hepatitis E, Q-fever, Rift Valley Fever			
Ecological characteristics relevant to epidemiology			
Diversity and distribution	**Current knowledge of populations in Europe**	**Host ecology/epidemiology**	
Bats constitute the second most diverse mammal group in Europe (45 species of insect eating bats in the EU, belonging to 5 families and 12 genera). The number of species increases towards southern latitude.	After great declines in the second half of the 20th century and with at least 14 spp classified as threatened by the IUCN red list, most of them seem to be increasing or stable in number. However, there is a need to increase available evidence of population trends and their drivers change utilising new monitoring tools and statistical methods. Since bats are predators, bat density is expected to be linked closely to the abundance of key invertebrate prey groups.	Bats possess several characteristics that might make them effective reservoirs for other pathogens: high species diversity, long life span, capacity to travel long distances, dense aggregations, social behaviour, and a unique immunology. Predominantly nocturnal, their daily movement distances vary greatly (from 15 to more than 80km) and some species are also known for long-term, long-distance migrations. Bats hibernate in winter. The play a key ecological role with impact on vector borne disease epidemiology since they contribute to vector control (mainly mosquitoes), promoting cascading effects (reduction of vectors and disease spread, incl. zoonosis).	
Risks to be considered in disease surveillance design for early detection			
Used environments and established interfaces	**Role in long-distance spread / cross-border spread in a setting comparable to the EU**	**Role in spread or maintenance if pathogen was introduced by other host species**	**Risk factors for general surveillance**
Bats occupy a variety of environments, either natural such as plants, caves, rocks or even manmade, such as tombs, buildings, bridges. They can therefore be present in urban, peri-urban, farmland or natural areas and establish interfaces with humans and domestic animals.	Some species are capable of performing large migrations that could result in a cross-border spread (e.g., *Nyctalus* and *Pipistrelus* genus). Many European species of bats migrate long distances. Some are known to migrate over more than 1,000 km. Precise assessment of migration routes, including possible movements between Africa and Southern Europe.	Bats have a gregarious behaviour that leads to a concentration of many individuals in a small space (roosts) and they are a highly mobile animal, which increases the change that they could act as reservoir or vector for several pathogens, spread and maintain them. Unknow epidemiological role for several zoonotic pathogens which has been detected in bats.	1) Where direct and indirect contact with livestock is suspected, in farms and peri-urban areas, 2) where high aggregation of bats in colonies, particularly mixed colonies (different species), 3) where pathogens, morbidity or mortality have been reported in bats, as well as zoonotic cases in human, 4) where vectors for target pathogens are present, 5) at the borders of EU and southern regions, 6) passively in dead animals detected by hunters, rangers, citizen science, rescue centres, aerogenerators, 7) where presence of other wildlife sharing the same pathogens, 8) areas susceptible to rapid environmental change could be most at risk and it is more likely that a spill over event occurs in bat populations nearest to human settlements or domestic animals, 9) target preferably in some species capable of performing large migrations that could result in a cross-border spread (e.g. *Nyctalus* and *Pipistrelus*), 10) Some species are more susceptible or useful as indicator for certain pathogens (e.g., coronaviruses and horseshoe-rhinolophid bats).

<table>
<tr><th colspan="6">Characteristics of surveillance for early detection</th></tr>
<tr><th colspan="2">Feasibility of surveillance</th><th colspan="2">Active Surveillance specific to priority diseases</th><th colspan="2">Passive Surveillance specific to priority diseases</th></tr>
<tr><td colspan="2">They are not hunted and all species are protected. Active surveillance based on sampling live animals requires capture and trained personnel with mist nets and harp traps. Some species are more difficult to capture (e.g., forestall ones) and therefore their sample size maybe too small to detect pathogens circulating at low prevalence (Hayman et al., 2012). Longitudinal data from migrating species is also very difficult to gather (it would be interesting as bats are long lived). Therefore, environmental samples such as urine and faeces can be an option. Wildlife rescue centres might also be a good tool for passive surveillance.</td><td colspan="2">Active surveillance of captured specimens needed for early detection of changes of incidence, i.e., early epidemic detection, but also key to monitor already established endemic pathogens, their maintenance and fade out. Active surveillance is particularly relevant for retrospective studies to elucidate past introduction/circulation of emergent zoonotic pathogens. The active component of national active surveillance programs must representatively cover the different regions and be organized as risk based to be cost effective and sensitive (see section on risks). Consider the contribution and inclusion of the network of protected areas (Natura 2000 network) and stakeholders (e.g., Eurobats).</td><td colspan="2">PASSIVE surveillance in bats is normally impractical for these range of pathogen as normally the clinical course is asymptomatic, and carcasses are difficult to find. However, it may complement early detection by active surveillance (relevance of rescue centres, zoos, fields of aerogenerators). Pay special attention to unusual mortalities in colonies.</td></tr>
<tr><th colspan="3">Type of sample material to collect</th><th colspan="3">Diagnostic tests</th></tr>
<tr><td colspan="3">Individual samples during capture include blood, ectoparasites, oral, urogenital, and anal swabs. Pooled samples: environmental samples such as urine and faeces can be collected by "under-roost sheet sampling" (e.g., which uses plastic sheets to collect pooled samples of bat excreta. In addition, tissues ca be collected during necropsy. Techniques to preserve serum at room temperature (Blood Collected on Filter Paper) has been tested successfully but may require testing for specific pathogens and hosts. Environmental sampling through molecular techniques is promising, both for hosts and pathogens presence.</td><td colspan="3">Multi-host serological and pathogen detection tests already used in several bat species in the literature, but their reliability needs to be tested in other species, as well as the sensitivity/specificity of PCR vs other tests (immunopathology, pathogen isolation). Environmental sampling through molecular techniques is promising.</td></tr>
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3.3.7. Characteristics of **birds** relevant to early detection in disease surveillance

Table 23 (sections I, II and III) shows the main characteristics relevant to zoonotic disease surveillance in European **birds** (Clase Aves). Europe is home to more than 540 regularly occurring wild bird species, across 73 families. Functionally, ecologically and from the epidemiological point of view, they can be divided in:

- **Waterbirds**, which refers to a large group of migratory birds associated to wetlands distributed all over Europe, occupying different niches, most having seasonal distribution patterns as they are migratory. As indicative, waders, gulls, auks, ducks, geese, swans, and herons account for approximately 30% of bird species present in Europe.
- **Other migratory species**, includes a diversity of species (including raptors and passerine birds), scattered throughout all of Europe, some breed in the continent, others might just use it as a stopover along their flyways. Passerine (many of which are migratory), account for about 40% of bird biodiversity in Europe.
- **Non migratory birds**, include a group of very diversified species. Among others, include the Order Galliformes (partridges, pheasants, and grouses), relevant as game and game farm species.

Overall, bird populations across the EU have declined by nearly 20% (600 million breeding birds lost) since 1980, with big declines among birds that breed in farmlands and grasslands, as well as long-distance migratory birds. In more detail, waterbirds show a long-term fluctuating trend, almost half of all waterbirds had bad or poor status. Among raptors, over 50 % have a good population status and many with a poor or bad status are improving. Member States report on a subset of wintering taxa, most of which are waterbirds, and several monitoring schemes occur throughout all of Europe (https://pecbms.info/country/).

Water birds and other migratory birds gather together in large groups during migration/wintering periods and several species are known to occur at the same time, favouring direct and indirect disease spread. Migrations implies a continuous change in local avian community composition over Europe (https://eurobirdportal.org/). Anseriformes and passerines are phylogenetically close to domestic counterparts, which is epidemiologically relevant for sharing pathogens. Weather and climate shapes arrivals of migratory birds in Europe. Many northern and eastern European species of birds have pronounced migratory tendencies (temporally overlapping with birds that may arrive from Asia). The populations of several passerine species are usually sedentary in western Europe; they are usually migratory, however, in northern Europe, where their flights resemble a short migration. Starlings are sedentary in western Europe, where large numbers gather from eastern Europe. Large flocks also pass the winter in North Africa. Some birds are nomadic in winter, others spend the colder months in the southwestern part of the continent or in the Mediterranean region. Many migrant populations migrate to Africa south of the Sahara. The geographical conditions determine several main routes, winter and spring passes, and spatio-temporal concentrations of birds. Ducks, geese, and swans winter partly in western Europe and partly in tropical Africa. Wading birds (shorebirds) are typical migrants, most of them nesting in tundra of the Arctic region and wintering along the seacoasts from western Europe to South Africa.

The **main risks associated to migratory birds** to be considered in disease surveillance design for early detection are:

- Used environments and established interfaces:

  - Waterbirds: Present mostly in natural areas, but also in urban parks and ponds in farmlands. May establish contact with domestic waterfowl and humans who frequent their habitats (naturalist, ornithologists). Their habitats, water points, are typical aggregations for wildlife. Relevant to also focus on Charadriiformes, an Order (shorebirds) which may bring waterfowl and themselves into close proximity to poultry and humans.
  - Other migratory birds: Might be present in urban-peri-urban, farmland or natural areas, and can enter in close contact with humans and domestic animals.
- Role in long-distance spread or cross-border spread in a setting comparable to the EU:
  - Waterbirds: They travel across national and international borders and might act both as a biological and/or as a mechanical carrier spreading diseases across their migration routes. Anthropogenic spread through game meat and/or poor hygiene during hunting may occur for huntable waterfowl.
  - Other migratory birds: Might be present in urban-peri-urban, farmland or natural areas and can enter in close contact with humans and domestic animals. Of particular relevance are wild Galliformes as they contact and breed with released gamebirds, such as partridges and pheasants.
- Role in spread or maintenance if pathogen was introduced by other host species:
  - Waterbirds: they have a wide range of movement, and they concentrate in wetlands which is an aggregation point for several species that might facilitate spreading and spill over of pathogens. The stress of migration can lead to immunosuppression which increases disease susceptibility.
  - Other migratory birds: their periodic movements make them capable of carrying pathogens along their migration routes. Similarly, the stress of migration can lead to immunosuppression which increases disease susceptibility. Can play a role as bridge host between farms, between other wild birds and poultry, as well as humans.
- Specific risk factors relevant to design surveillance are:
  - Risk areas such as wetlands (marine, coastal and freshwater wetlands incl. lakes, rivers, bogs and marshes) and breeding grounds, stopover areas, where wintering periods lead to intermingling of different species, where high aggregation and densities occurs over their migratory routes (also landfills), at the borders of EU;
  - Special attention to cross-border regions and where different migration paths overlap, which is more relevant when outbreaks have been recently detected in neighbour countries and geographical borders of the continent on migration routes: the Balkans, southern Mediterranean regions (e.g., Gibraltar stretch), pre-migration concentrations in northern latitudes (e.g., Scandinavia, the North Sea), many wetland areas border agricultural land, and most are near transport infrastructure;
  - Where high waterbird community compositions (see Natura 2000 network, a large proportion of wetlands of international importance has been designated as Ramsar sites);
  - Specific taxa, apart from waterfowls, as above commented, it is remarkable to consider Charadriiformes Order (shorebirds, such as gulls or cattle egret) at all range of habitats and birds aggregation sites because they may bring waterfowl, other aquatic birds and themselves into close proximity to poultry and humans;

  - Where direct and indirect contact with poultry, gamebirds and livestock occur (outdoor or extensive more relevant);
  - Where pathogens have previously been reported both in waterbirds, gamebirds and poultry, or at least in one compartment, as well as zoonotic cases in humans;
  - Passively in sick animals detected by hunters, rangers, citizen, citizen science, rescue centres;
  - Where there is direct contact with hunters and consumers of meat (e.g., Anseriformes, pigeons, thrushes)
  - Where gamebird release, including Anseriformes and Galliformes, is practiced.
  - For certain pathogens winter is the best season (e.g., HPAI) or certain areas, for example in central and Southern Europe during extreme cold waves affecting norther latitudes followed by unusual migrations;
  - Adapt to automated risk maps (migrations of birds, https://eurobirdportal.org/);
  - Active sentinel surveillance (either active or passive) on species such as mallard ducks placed in pens in natural water bodies, allowing continuous direct contact with wild water birds, or zoo birds;
  - Some species are more susceptible or useful as indicator for certain pathogens.

Regarding non migratory birds, different species (e.g., pigeons, storks) have become sedentary in urban and peri-urban habitats. The diverse range of species shows diverse movement patterns, social behaviours, and diets. Among this functional group, Galliformes and Passerines are phylogenetically close to their domestic counterparts, including game and songbirds.

The **main risks associated to non-migratory birds** to be considered in disease surveillance design for early detection are:

- Used environments and established interfaces:
  - Non migratory birds might be present in urban-peri-urban, farmland or natural areas and can enter in close contact with humans and domestic animals;
  - Of particular relevance are wild Galliformes as they contact and breed with released gamebirds, such as partridges and pheasants, and may bring wild birds into close proximity to poultry.
  - Released gamebirds may act to concentrate infection permitting spread to other species.
- Role in long-distance spread or cross-border spread in a setting comparable to the EU:
  - As their range of movement is more restricted, their role in long distance spread should not be so significant;
  - Anthropogenic spread through game meat (e.g., partridges) and/or poor biosecurity by hunters.
- Role in spread or maintenance if pathogen was introduced by other host species:
  - Easy of movement and especially urban birds enter in close contact with humans and domestic animals, mainly poultry;
  - Others, such as storks have adapted to feed on landfill/rubbish dumps. They may play a role as bridge hosts between farms, or between migratory birds and poultry, as well as humans.

- Specific risk factors relevant to design general surveillance are:
    - Overwintering colonies and landfills leading to intermingling of different species, and high aggregation and densities occur;
    - Pest/exotic species in urban/peri urban areas such as pigeons subject to population control;
    - Where high diversity of species meets (see Natura 2000 network) and coincide with migratory birds (wetlands);
    - In urban parks where many bird species have become sedentary;
    - Gamebirds after release and hunted;
    - Where direct and indirect contact with poultry and livestock occurs (outdoor or extensive more relevant);
    - Where pathogens have previously been reported in birds, including gamebirds, and poultry, or at least in one compartment, as well as zoonotic cases in human;
    - At the borders of EU, with special attention to geographical borders of the continent: eastern Europe, the Balkans, southern Mediterranean region. Pathogen specific;
    - Where vectors for target pathogens (vector borne) are present;
    - Passively in sick animals detected by hunters, rangers, citizen, citizen science, rescue centres, especially when unusual mortality/morbidity occurs;
    - Where there is direct contact with hunters and consumers of meat, of particular relevance for Galliformes;
    - Interface: direct/indirect contact with pets, humans: outdoor recreational activities, farms and peri-urban areas;
    - Adapt to automated risk maps (migrations of birds, https://eurobirdportal.org/) for pathogens which may be brought by migratory species;
    - Songbirds as sentinels, e.g., at feeders.
- Regarding the characteristics of disease surveillance for early detection in **ALL BIRDS**, feasibility of surveillance is complicated because birds are mobile flying animals. Migratory species might act as carriers of pathogens from other points of the globe. Birds that frequent aggregation points, or urban settlements and might act as bridge species can serve as a sentinel for pathogen detection. Several waterbirds (mainly Anseriformes) and other migratory (e.g., thrushes and pigeons) or non-migratory (e.g., Galliformes) species are hunted or subject to population control (gulls, cormorants), so active surveillance is feasible. Active surveillance for protected species requires sampling live birds, which requires their capture through special techniques such as corral trap, mist nest, drop traps, night-lighting, baited traps, funnel traps, dive-in traps, or cannon nets. Handling and sampling of animals should be performed by trained personnel, such as during ringing programs by ornithologists. Environmental samples such as excreta or water can also be helpful for waterbirds. Passive surveillance can be accomplished through the detection and collection of dead animals and sampling individuals that enter wildlife rehabilitation centres. Found dead or sick could be reported via citizen science APPs, although they need to develop functionalities to be automatically communicated (and later transported) to veterinary authorities.
    - ACTIVE surveillance of hunted/captured/shot specimen needed for early detection of changes of incidence, i.e., early epidemic detection.

        - Active surveillance as routine is key to monitor already established endemic pathogens, their maintenance and fade out, and are particularly relevant for retrospective studies to elucidate past introduction/circulation of emergent zoonotic pathogens (this includes sentinels).
        - Active surveillance must be organized as risk based to be cost effective and sensitive (see section on risks), representatively covering the different regions and diversity of species (according to the pathogen of interests), to be cost effective and sensitive enough.
        - Lists of priority host specific for specific pathogen (e.g., active surveillance of HPAI mainly in waterbirds as indicated by EFSA, or for West Nile in urban, peri urban, farm environments) need to be developed for each pathogen.
        - Consider the contribution and inclusion of hunting grounds (public/private) and the network of protected areas (Natura 2000 network) and stakeholders (bird monitoring intl. and national organizations). The involvement of hunters is important.
    - PASSIVE as a source of samples even when no morbidity or mortality is detected.
        - Passive surveillance is essential for early detection of the introduction of the pathogen, the onset and duration of the period of increased risk; change in the geographic distribution/spread to new areas and increase in incidence, i.e., early epidemic detection.
        - Found dead or sick could be reported via citizen science APPs, although they need to develop functionalities to be automatically communicated (and later transported) to veterinary authorities. The involvement of hunters is important also for passive surveillance.
- The type of sample material to collect: Individual blood/organ samples/oral and cloacal swabs/ectoparasite samples from birds might be sampled quite easily during hunting season or dead animals any time, faeces could be collected directly from individuals or by active searching. High quality blood for serum extraction can be taken by puncture live birds, as well as swabs, faecal material and ectoparasites. Environmental sampling through molecular techniques can be used both for host and pathogen presence (e.g., HPAI). Techniques to preserve serum at room temperature (blood collected on filter paper) has been tested successfully but may require testing for specific pathogens and hosts.
- Diagnostic tests: multi-host serological and pathogen detection tests already used in domestic and many wild bird species in the literature, but their reliability needs to be tested in other species, as well as the sensitivity/specificity of PCR vs other tests (immunopathology, pathogen isolation). Environmental sampling through molecular techniques is already in use (Hood et al. 2010) and its development is promising, and its reliability and practical use need to be evaluated.

Table 23. The main characteristics of relevance for zoonotic disease surveillance in **wild birds** aiming at early detection of disease in wildlife.

Highly Pathogenic Avian Influenza, West Nile Disease, Crimean Congo Haemorrhagic Fever (tick sampling on migratory birds), **Q-fever**			
I	Ecological characteristics relevant to epidemiology		
	Diversity and distribution	**Current knowledge of populations in Europe (denominator data)**	**Host ecology/epidemiology**
Water-birds	Europe is home to more than 540 regularly occurring wild bird species, across 73 families (including other than waterbirds). Waterbirds refers to a large group of birds associated to wetlands distributed all over Europe, occupying different niches, most having seasonal distribution patterns as they are migratory. As indicative, waders, gulls, auks, ducks, geese, swans, and herons account for approximately 30% of bird species present in Europe.	Bird populations across the EU have declined by nearly 20% since 1980, with big declines among birds that breed in farmlands and grasslands, as well as long-distance migratory birds. The declines amount to about 600 million breeding birds lost in Europe over the past four decades. Many waterbirds' populations are declining with almost half of them having a poor or bad status. Overall, waterbirds show a long-term fluctuating trend with an increase until the mid-1990s, followed by a decline until 2010 and subsequent recovery in more recent years. Almost half of all waterbirds had bad or poor status. However, based on the wintering data, more species are declining than increasing in each of the species' groups and the positive tendency in the multi-species indices are driven by only a few rapidly increasing species. Among raptors, over 50 % have a good population status and many with a poor or bad status are improving. However, over 50 % of falcons and harriers have a bad status. Species associated to farm/agricultural present (either migratory or not) present decreasing trends, however, within the EU, forest and shrubland habitats seem to fare better, with 60% of species having stable or increasing populations. Several monitoring schemes occur throughout all of Europe (https://pecbms.info/country/). Member States report on a subset of wintering taxa, called key wintering species, most of which are waterbirds. The European Association of Wetlands International follows trends of the wintering waterbird species in the EU, includes multi-species trends for groups of species according to which of the annex a species is listed on the Birds Directive and the trends for individual species. European bird portal (EBD) attempts to create a common repository holding data from different existing monitoring systems.	Gather together in large groups during migration/wintering periods and several species are known to occur at the same time. Waterbirds gather in large flocks on water bodies, favouring direct and indirect disease spread. Migrations implies a change in avian community composition. Anseriformes and passerines are phylogenetically close to domestic counterparts. While birds harbour several zoonotic viruses that are virulent in humans such as Highly Pathogenic Avian Influenza (HPAI), West Nile, and Equine Encephalitis viruses, only some avian species are tolerant of these infections (applies also to non-migratory birds).
Other migratory birds	Includes a diversity of species (including raptors, passerine birds), scattered throughout all of Europe, some breed in the continent, others might just use it as a stop-over along their flyways. Passerine (many of which are migratory), account for about 40% of bird biodiversity in Europe.		
Non migratory including farmed (released) game-birds	A group of very diversified species. Among others, include the Order Galliformes (partridges, pheasants, and grouse), relevant as game farm species.		Different species (e.g., pigeons, storks) have become sedentary in urban and peri-urban habitats. Easy of movement, diverse social behaviours, and diets. Galliformes and passerines are phylogenetically close to their domestic counterparts, including game and songbirds.

II	Risks to be considered in disease surveillance design			
	Used environments and established interfaces	Role in long-distance spread / cross-border spread in a setting comparable to the EU	Role in spread or maintenance if pathogen was introduced by other host species	Risk factors for general surveillance
Waterbirds	Present mostly in natural areas, but also in Parks and ponds in farmlands. May establish contact with domestic waterfowl and humans who frequent their habitats (naturalist, ornithologists). Their habitats are water points, typical aggregation for wildlife. Relevant focus on Charadriiformes Order (shorebirds) which may bring waterfowl and themselves into close proximity to poultry and humans.	They travel across national and international borders and might act both as a biological and/or as a mechanical carrier spreading diseases across their migration routes. Anthropogenic spread through game meat and/or poor hygiene/biosecurity by hunters for huntable waterfowl.	Waterbirds have a wide range of movement, and they concentrate in wetlands which is an aggregation point for several species that might facilitate spreading and spill over of pathogens. The stress of migration can lead to immunosuppression which increases disease susceptibility.	1) Risk areas such as wetlands (marine, coastal and freshwater wetlands incl. lakes, rivers, bogs and marshes) and breeding grounds, stopover areas, where wintering periods lead to intermingling of different species, where high aggregation and densities occurs over their migratory routes (also landfills), at the borders of EU, with special attention cross-border regions and where different migration paths overlaps, which is more relevant when outbreaks have been recently detected in neighbour countries and geographical borders of the continent on migration routes: the Balkans, southern Mediterranean regions (e.g. Gibraltar stretch), pre-migration concentrations in northern latitudes (e.g. Scandinavia, the north sea), many wetland areas border agricultural land and most are near transport infrastructure, 2) where high waterbird community compositions (see Natura 2000 network, a large proportion of wetlands of international importance has been designated as Ramsar sites), 3) as for specific taxa, it is remarkable that consider Charadriiformes Order (shorebirds, such as gulls or cattle egret) at all range of habitats and birds aggregation sites because they may bring waterfowl, other aquatic birds and themselves into close proximity to poultry and humans, 4) where direct and indirect contact with poultry, gamebirds and livestock occurs (outdoor or extensive more relevant), 5) where pathogens has previously been reported in both in waterbirds, gamebirds and poultry, or at least in one compartment, as well as zoonotic cases in humans, 6) passively in sick animals detected by hunters, rangers, citizen, citizen science, rescue centres, 7) where there is direct contact with hunters and consumers of meat (e.g. Anseriformes, pigeons, trushes), 8) where gamebird release, including Anseriformes and Galliformes, is practiced, 9) for certain pathogens winter is the best season (e.g. HPAI) or certain areas, for example in central and Southern Europe during extreme cold waves affecting norther latitudes followed by unusual migrations, 10) adapt to automated risk maps (migrations of birds, see Euro Bird Portal BP), 11) active sentinel surveillance either active or passive) on species such as mallard ducks placed in pens in natural water bodies, allowing continuous direct contact with wild water birds, or zoo birds, 12) some species are more susceptible or useful as indicator for certain pathogens.
Other migratory birds	Might be present in urban-peri-urban, farmland or natural areas and can enter in close contact with humans and domestic animals (9)	They travel across national and international borders and might act both as a biological and/or as a mechanical carrier spreading diseases across their migration routes. anthropogenic spread through game meat (e.g., pigeons) and/or poor hygiene by hunters.	Their periodic movements make them capable of carrying pathogens along their migration routes. The stress of migration can lead to immunosuppression which increases disease susceptibility (9). Can play a role as bridge host between farms, between other wild birds and poultry, as well as humans.	

Non migratory including farmed (released) gamebirds	Might be present in urban-peri-urban, farmland or natural areas and can enter in close contact with humans and domestic animals. Of particular relevance are wild Galliformes as they contact and breed with released gamebirds, such as partridges and pheasants. Relevant focus on Galliformes Order (partridges, pheasants, grouses, turkeys) which may bring wild birds into close proximity to poultry	As their range of movement is more restricted, their role in long distance spread should not be so significant. Anthropogenic spread through game meat (e.g., partridges) and/or poor hygiene/biosecurity during hunting.	Easy of movement and especially urban birds enter in close contact with humans and domestic animals, mainly poultry. Others, such as storks have adapted to feed on rubbish dumps. May play a role as bridge hosts between farms, or between migratory birds and poultry, as well as humans.	1) Overwintering colonies and landfills leading to intermingling of different species, and high aggregation and densities occurs, 2) pest /exotic species in urban/peri urban areas such as pigeons subject to population control, 3) where high diversity of species meet (see Natura 2000 network) and coincide with migratory birds (wetlands), 4) in urban parks where many bird species have become sedentary, 5) gamebirds after release and hunted, 6) where direct and indirect contact with poultry and livestock occurs (outdoor or extensive more relevant), 7) where pathogens has previously been reported in both in birds, including gamebirds, and poultry, or at least in one compartment, as well as zoonotic cases in human, 8) At the borders of EU, with special attention to geographical borders of the continent: eastern Europe, the Balkans, southern Mediterranean region. Pathogen specific, 9) Vectors for target pathogens (vector borne) are present, 10) passively in sick animals detected by hunters, rangers, citizen, citizen science, rescue centres, especially when unusual mortality/morbidity occurs, 11) where there is direct contact with hunters and consumers of meat, of particular relevance for Galliformes, 12) interfaces where direct/indirect contact with pets, humans occur: outdoor recreational activities, farms and peri-urban areas; 14) adapt to automated risk maps (migrations of birds, see Euro Bird Portal BP) for pathogens which may be brought by migratory species, 15) song birds as sentinels, e.g., at feeders.

	Characteristics of surveillance for early detection		
III	**Feasibility of surveillance**	**Active Surveillance specific to priority diseases**	**Passive Surveillance specific to priority diseases**
All	Surveillance is complicated because birds are mobile flying animals. Migratory species might act as carriers of pathogens from other points of the globe. Birds that frequent aggregation points, or urban settlements and might act as bridge species can serve as a sentinel for pathogen detection. Several waterbirds (mainly Anseriformes) and other migratory (e.g., thrushes and pigeons) or non-migratory (e.g., Galliformes) species are hunted or subject to population control (gulls, cormorants), so active surveillance is feasible. Active surveillance for protected species requires sampling live birds, which requires their capture through special techniques such as corral trap, mist nest, drop traps, night-lighting, baited traps, funnel traps, dive-in traps, or cannon nets. Handling and sampling of animals should be performed by trained personnel, such as during ringing programs by ornithologists. Environmental samples such as excreta or water can also be helpful for waterbirds. Passive surveillance can be accomplished through the detection and collection of dead animals and sampling individuals that enter wildlife rehabilitation centres. Found dead or sick could be reported via citizen science APPs, although they need to develop functionalities to be automatically communicated (and later transported) to veterinary authorities.	ACTIVE surveillance of hunted/captured/shot specimen needed for early detection of changes of incidence, i.e., early epidemic detection. Also, active surveillance as routine is key to monitor already established endemic pathogens, their maintenance and fade out, and are particularly relevant for retrospective studies to elucidate past introduction/circulation of emergent zoonotic pathogens (this includes sentinels). Active surveillance must be organized as risk based to be cost effective and sensitive (see section on risks). The active component of national active surveillance programs must representatively cover the different regions and be organized as risk based to be cost effective and sensitive (see section on risks). Consider the contribution and inclusion of hunting grounds (public, private) and the network of protected areas (Natura 2000 network) and stakeholders (bird monitoring intl. and national organizations). Lists of priority host specific for specific pathogen (e.g., active surveillance of HPAI mainly in waterbirds as indicated by EFSA, or for West Nile in urban, peri urban, farm environments) need to be developed for each pathogen. Hunters involvement is essential for surveillance.	PASSIVE as a source of samples even when no morbidity or mortality are detected. Passive surveillance is essential for early detection of the introduction of the pathogen, the onset and duration of the period of increased risk; change in the geographic distribution/spread to new areas and increase in incidence, i.e., early epidemic detection. Lists of priority host specific for specific pathogen

	Type of sample material to collect	Diagnostic tests
All	Individual blood/organ samples/oral and cloacal swabs/ectoparasite samples from hunted animals might be sampled quite easily during hunting season or dead animals any time, faeces could be collected directly from individuals or by active searching. High quality blood for serum extraction can be taken by puncture live birds, as well as swabs, faecal material and ectoparasites. Environmental sampling through molecular techniques is promising, both for host and pathogen presence. Techniques to preserve serum at room temperature (Blood Collected on Filter Paper) has been tested successfully but may require testing for specific pathogens and hosts.	Multi-host serological and pathogen detection tests already used in domestic and many wild bird species in the literature, but their reliability needs to be tested in other species, as well as the sensitivity/specificity of PCR vs other tests (immunopathology, pathogen isolation). Environmental sampling through molecular techniques is promising.

3.3.8. Risk factors for early detection across host groups

Considering the selected pathogens (see tables above), Figure 7 represent risks as a function of the number of host groups for which they were selected as relevant. The summary we present in this section is valuable to plan and develop surveillance components able to address general surveillance for as many hosts as possible (out of the 11 selected in the list) while targeting specifically the priority pathogens. As commented above, it is not practical to have only targeted (specific, usually based on active surveillance) surveillance programs for every disease or pathogen and a combination with general surveillance (which usually relies more on passive surveillance) is the best approach. An approach strategically incorporating risk-based surveillance applicable to several pathogens is cost/effective.

A total of 5 different risk factors applied commonly to all listed priority pathogens, namely:

- Where? At the borders of EU, with special attention to geographical borders of the continent: eastern Europe, the Balkans, southern Mediterranean region. Pathogen specific;
- Pathogens, morbidity and/or mortality has been reported in livestock and/or wildlife (listed groups, also i other groups sharing the same pathogens), as well as zoonotic cases in humans. If endemic, when unusual episodes occurs;
- Interface: Farming direct/indirect contact with livestock/poultry (inc. gamebird) (outdoor or extensive more relevant);
- Vectors for target pathogens (vector borne) are present;
- High diversity of hosts (groups and species). But dilution effect (opposite situation: pests).

Two risk factors were considered relevant for 6 host groups:

- High host density and/or intensive game management of listed groups including artificial feeding and game release (birds: include Anseriformes & Galliformes);
- Interface: Direct contact with hunters and consumers of meat, regions where meat is commercialized.

However, for these risks that are relevant across a large number of different host groups, the specific region, site, or context where implementing risk-based surveillance vary according to the pathogen. For instance, geographically target areas for TBE for early detection of introduction as different to Lyme or HPAI, or target animal interfaces depend on specific host.

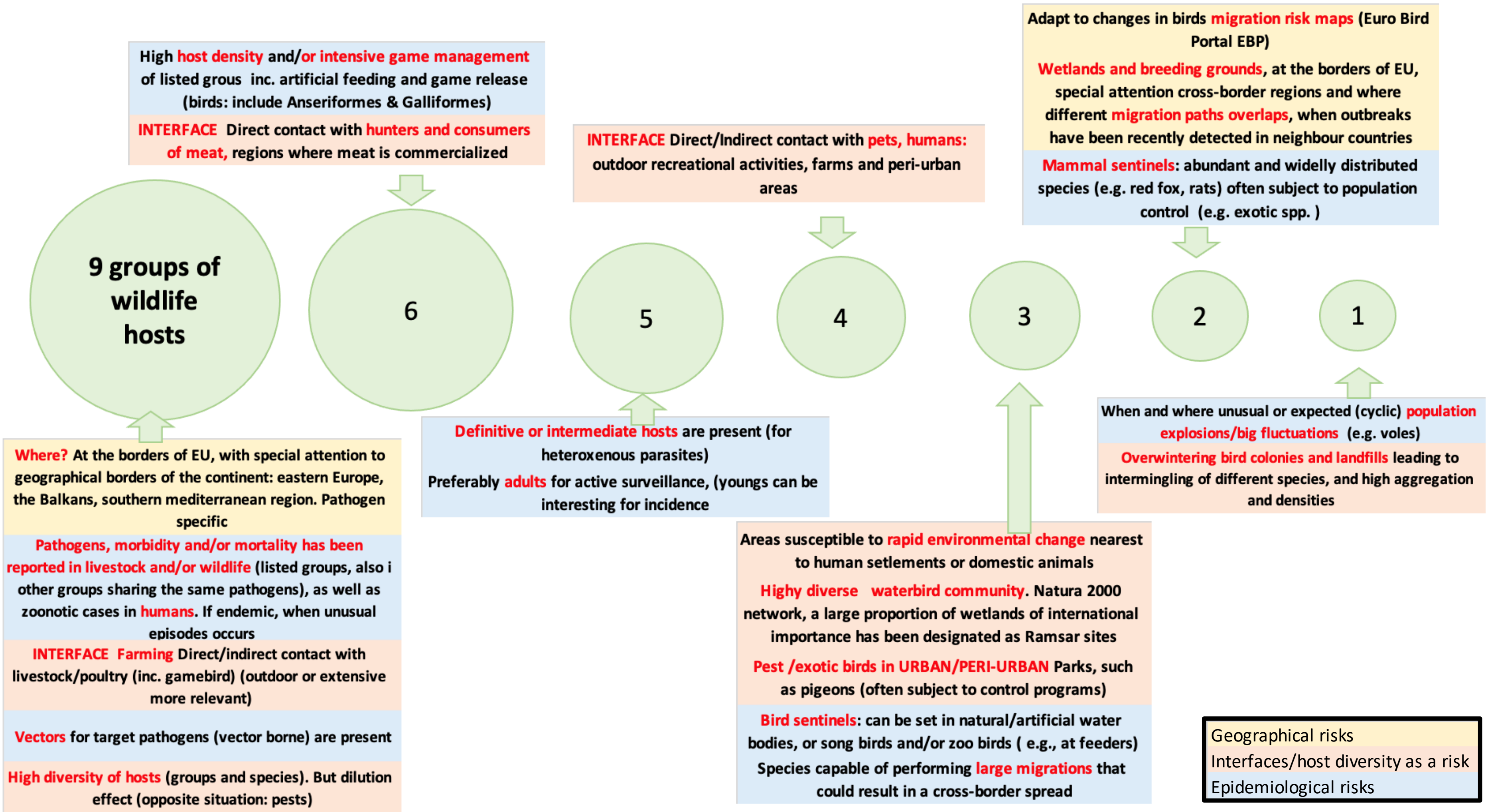

Figure 7. The risk factors for early detection in disease surveillance (cost-effective risk-based design) associated to the number of different host groups to which they apply. See legend of types of factors on top right.

3.4. Risks relevant to early detection for the selected pathogens in the main wild host groups

Tables 24-31 show the relevance of risk factors for early detection surveillance for all pathogens, separately for each host group. The host group role as reservoir and/or sentinel species is also indicated in the heading row.

Regarding wild ruminants, they are relevant to the highest number of pathogens (at least 6: TBE, *E. granulosus*, CCHF, HEV, Q-fever and RVF), with different epidemiological roles, or as sentinels. The risks that scored highest were:

- Pathogens, morbidity and/or mortality has been reported in livestock and/or wildlife, and humans (unusual episodes).
- Interface: Direct/Indirect contact with pets, humans: outdoor recreational activities, farms, peri-urban areas & parks
- Interface: Farming direct/indirect contact with livestock (outdoor or extensive more relevant).

As regards to wild boar (relevant to 3 pathogens), wild carnivores and wild lagomorphs (relevant to only Q-fever), and micromammals 2 variables ranking the highest were coincident with wild ruminants:

- Pathogens, morbidity and/or mortality has been reported in livestock and/or wildlife, and humans)
- Farming Direct/indirect contact with livestock (outdoor or extensive more relevant).

These results indicate that their presence at the interface with farms, and subsequent contacts with livestock is particularly relevant for these groups of species, which account for most pathogens included in the list as potential reservoir and/or as host of value for surveillance. Definitively, farmlands should be incorporated to sampling design strategy for wildlife in relation to most pathogens (this risk also ranked high for other hosts, such as micromammals and birds).

The risk "Pathogens, morbidity and/or mortality has been reported in livestock and/or wildlife, and humans (unusual episodes)" ranked the highest for all groups of wildlife host, which indicates the importance of mapping disease in any host for risk assessment and development of surveillance (planning and sampling). Most pathogens here listed are communicable, however the disaggregation of data sources, lack of harmonization and interrupted data flow for these data, and unpublished research, makes difficult to generate "live" maps on the distribution of pathogens over Europe.

The same problem applies to the second most ranked risk: "Farming Direct/indirect contact with livestock (outdoor or extensive more relevant)". As previously shown by *ENETWILD* (*ENETWILD* consortium et al. 2020, 2021), due to insufficient data it is currently not possible to produce good resolution maps of the spatial distribution of the wildlife/livestock interfaces in Europe but can only be done in some countries and for some species. If not at European level, different countries should develop these maps as a basis for designing future surveillance of zoonotic pathogens at the wildlife/livestock interfaces, paying special attention to outdoor livestock production (there is a need to standardize the nomenclature of different types of production systems over Europe).

The risk "Interface: direct/Indirect contact with pets, humans: outdoor recreational activities, farms, peri-urban areas & Parks", scored high for all groups, particularly for ruminants, carnivores, lagomorphs and micromammals, and this was mainly due to the risks associated to contact with vectors that are maintained by these host, such as ticks and mosquitoes. In other cases, this risk is also relevant due to direct or indirect transmission, such as birds and HPAI, or the presence of parasitic stages in the environment (e.g., *E. multilocularis* associated to peri-urban rodents and fox). Definitively, this type of environment is a clear target for disease surveillance in most wildlife groups and should be considering during planning phase. The different stakeholders involved in surveillance in this interface (local veterinary services, rescue centres) and citizens should play a coordinated role. In general, about the wildlife/human interface, we note that zoonotic viruses have been associated with some of the highest death burdens induced by viral zoonosis despite lacking forward transmission in human populations. This is likely because these viruses spill over to humans from animal host populations that live amongst human communities (e.g., hantavirus spills over from rodents that may inhabit agricultural fields).

The relevance of the risk "when vectors are present" is variable according to the host group. This is very much determined by the nature of pathogens included in the list for each host group (i.e., the host is relevant for). For example, no vectors are involved in the pathogens for which wild boar play a relevant role in surveillance (Swine Influenza, *E. granulosus*, Hepatitis E), but the role of wild boar is not completely ruled out, for example, for diseases where they could play a role as amplifying host of vectors (e.g., TBE). The relevance of this risk for surveillance planning and implementation indicates that efforts are needed to map at the finest possible resolution at large biogeographical scales where hosts and vectors distribution overlap, and to determine at local level, the habitat, land uses and features where both vectors and host sampling, in concomitance, is recommended. This approach is also needed to elucidate where and when host and/or vector sampling for determining the presence of vectors borne pathogens is more effective for early detection purposes, and in which conditions and for which pathogens and vectors, which may vary. This information will provide a solid background for sustainable vector borne zoonotic disease surveillance in the future.

Two risks normally scored high for most host groups:

- High diversity of hosts (groups and species);
- High host density and/or intensive game management, inc. artificial feeding and game release (ungulates, birds).

Changes in host species diversity have been described as important factors influencing transmission risk of infectious diseases. A high diversity of hosts may offer an opportunity for a wide range of pathogens to persist in host communities. However, well preserved host communities in healthy environment may prevent rather than become a risk. This is relatively well studied in large natural areas of the planet, such as highly biodiversity points. The extent to which this applies to Europe, highly impacted by human activities, is unknown for most pathogens and host communities. Therefore, a gradient of host biodiversity situations should be considered in surveillance planning in an adaptive way, learning continuously which host communities should be targeted for early detection: highly biodiverse *versus* simplified host communities where one or a few species predominate, often as pest (e.g., rodents, such as peri-urban). More diverse assemblages would support a greater fraction of low-competence hosts, and therefore biodiversity losses may have the potential to increase disease ("dilution effect") (Johnson et al. 2013). However, the principle underlying this phenomenon remains unknown. Some studies have claimed that healthy ecosystems may be richer in parasite diversity and biodiversity, even on a global scale, with this being associated with increased risk of zoonotic pathogens; while others argue that preserving intact ecosystems and their endemic biodiversity should generally reduce the prevalence of infectious diseases (Hudson et al. 2006, Jones et al. 2008, Salkeld et al. 2013). To establish whether disease emergence, maintenance and risk of transmission maybe determined by particular host community assemblages, specific but diverse examples are required. The EOW (https://wildlifeobservatory.org/), involving a wide range of scenarios (potentially including or coordinated with the Natura 2000 network of protected areas) offers a possibility to work on this matter with a European perspective, since variable well distributed host communities over Europe are included. The outcome of host richness changes for infectious disease risk depends on a community´s ability to support infection–community competence. We have shown that certain risks are more relevant to certain pathogens and/or groups of hosts, and that the selection of where and how to develop sampling can be quite specific (target surveillance). However, because outcome of host richness changes for infectious disease risk depends on a community´s ability to support infection (community competence), the European future zoonotic surveillance should establish as a complementary approach a network of study areas with different host community assemblages (both wildlife and domestic) and environmental conditions to develop jointly the surveillance of the 11 selected pathogens. This will provide valuable lessons for an adaptive surveillance model at European level, continuously improving. It also would provide a priceless network of study areas under continuous monitoring for early detection of pathogen.

Regarding the risk "high host density and/or intensive game management", this includes situations where wildlife is abundant (can often be referred to as overabundant, Gortázar et al 2006, Carpio et al. 2021). This often associates with intensive management practices such as artificial feeding and game release (for a wide range of species, ranging from ungulates to birds -Anseriformes, Galliformes-, lagomorphs in certain cases). Overabundance of certain species tends to associate (or causes) with simplified ecosystems and low diversity of hosts. This may imply that some pathogens become widespread in the community and the risk for transmission to humans increases in such simplified host communities, as expected by the "dilution effect" (see above). These communities may also be more susceptible for introduced pathogens to establish. Since overabundant situation are mainly driven by human activity, often directly promoting high densities of wildlife (either directly or indirectly, often by releasing farmed animals, in fenced or open areas), special attention should be paid to local/regional management schemes. Therefore, both abundance of wild species and their management should be mapped over Europe. The effort done by the *ENETWILD* project during the last 5 years (basically starting from scratch, since standards for data collection had to be developed from the very beginning, for the network of data providers) shows that it is possible to have sufficiently precise data on wildlife abundance at a continental level (progressively improving for certain species). Other European initiatives focusing on specific taxa, such as international organizations collecting data on abundance and distribution of birds, provide similar evidence (e.g., data on bird abundance and migrations, useful for HPAI surveillance, see EFSA, 2021, collaboration with EBP, https://eurobirdportal.org/). Therefore, we are currently capable of incorporating wildlife abundance into disease surveillance planning. Regarding prevalent management systems in Europe, natural areas are well defined (Natura 2000 network) and regions, or specific management areas where intensive hunting management are performed are already known by administrations. However, the latter information is not available at the European level. Some national administrations, and for certain pathogens, organize disease control programs in wildlife as a function of the risks associated to wildlife management (e.g., animal tuberculosis in Spain, PATUBES program: https://www.mapa.gob.es/es/ganaderia/temas/sanidad-animal-higiene-ganadera/patubes2017_3_tcm30-378321.pdf). This approach should be extended to other zoonotic pathogens and host communities, but the previous step is collecting in a harmonized way the information on wildlife management schemes at the European level (game release, fenced areas, artificial feeding practices). Population abundance parameters could be a surrogate indicator of that management.

Some relevant considerations relevant to specific groups of hosts are:

- Regarding wild boar, "the interface: direct contact with hunters and consumers of meat, regions where meat is commercialized" was very relevant as a risk to be considered during surveillance activities of zoonosis. The wild boar is a species widely distributed, hunted, and consumed all over Europe, and therefore areas where zoonotic cases are detected associated with wild boar meat consumption habits should be prioritized (Rivero-Juarez et al. 2017). The spatial pattern of zoonotic cases associated with wild boar should be analysed. Pathogens such as HEV should be listed in European reports on zoonosis, and the possible role of wildlife as a source of zoonotic cases should be detailed. This can be the basis for prioritizing surveillance strategies in wildlife, which not only applies to the case of wild boar, but to all wildlife.
- Wild carnivores have potential as sentinels, and because of taxonomic and epidemiological reasons the risk factor "interaction with pets and humans outside" should be considered in risk-based sampling.
- Wild lagomorphs and micromammals respective interfaces are relevant as a risk ("where direct or indirect contact with pets, humans, where outdoor recreational activities occur, in farms and peri-urban areas and parks"). For instance, in the case of *Leishmania* (not listed as priority) for rabbits and hares, but also for other vector borne pathogens in the case of rodents, or for indirectly transmitted pathogens such as Q-fever in both rabbits and rodents. Micromammals may play a relevant role as sentinels, such as abundant and widely distributed species (e.g., rats) often subject to population control.
- Regarding wild birds, "wetlands, breeding ground, EU borders, migration paths overlaps, recent outbreaks in neighbour countries" are essential risks to be considered in surveillance sampling design, as a consequence from that many of them are migratory, and other which do not migrate join migratory birds in these areas and may play role as bridge hosts.

Table 24. Ranking for risk factors for early detection associated to **ruminant** specific pathogens. The roles of ruminant as primary maintenance reservoirs (dark green), secondary reservoirs (light green) or not susceptible to infection (white) is indicated, as well as the potential role as sentinel species (dotted cells).

Risk factors of relevance for surveillance design and targeting	TBE	E. granulosus	CCHF	Hepatitis E	Q-fever	Rift Valley Fever	Score
Pathogens, morbidity and/or mortality has been reported in livestock and/or wildlife, and humans (unusual episodes)	3	3	3	2	3	3	17
INTERFACE Direct/Indirect contact with pets, humans: outdoor recreational activities, farms, peri-urban areas & Parks	3	1	3	1	3	3	14
INTERFACE Farming Direct/indirect contact with livestock (outdoor or extensive more relevant)	1	3	1	3	3	1	12
Vectors for target pathogens (vector borne) are present (macroscale and microhabitat)	3	0	3	0	2	3	11
Where? At the borders of EU: eastern Europe, the Balkans, southern mediterranean region. Pathogen specific	1	1	3	1	1	3	10
High diversity of hosts (groups and species) (e.g. Natura 2000 network)	1	1	1	3	2	1	9
High host density and/or intensive game management inc. artificial feeding & game release	1	2	1	3	1	1	9
Mammal sentinels: abundant and widelly distributed species (e.g.red & roe deer) often subject to population control	1	2	3	1	2	¿?	9
Areas susceptible to rapid environmental change nearest to human setlements or domestic animals	2	1	2	1	1	1	8
INTERFACE Direct contact with hunters and consumers of meat, regions where meat is commercialized	0	3	0	3	1	0	7
When and where unusual or expected (cyclic) population explosion of reservoirs (e.g. voles)	3	0	0	0	2	1	6
Species capable of performing large migrations that could result in a cross-border spread (birds)	0	0	2	0	0	3	5
Highy diverse waterbird community. Natura 2000 network,Ramsar sites	1	0	2	1	0	0	4
Pest & exotic birds in URBAN/PERI-URBAN Parks (often subject to control programs)	1	0	2	1	0	0	4
Overwintering bird colonies and landfills leading to intermingling of different species, high aggregation & densities	0	0	0	3	0	0	3
Definitive or intermediate hosts are present (for heteroxenous parasites)	0	3	0	0	0	0	3
Wetlands, breeding ground, EU borders, migration paths overlaps, recent outbreaks in neighbour countries	0	0	1	0	0	0	1
Adapt to changes in birds migration risk maps (Euro Bird Portal EBP)	0	0	1	0	0	0	1

Geographical risks
Interfaces/host diversity as a risk
Epidemiological risks

Score for risks		
	0	No relevant at all or N.A.
	1	Optional/not too relevant
	2	Recommended
	3	Highly recommended

Table 25. Ranking for risk factors for early detection associated to **wild boar** specific pathogens. The role of wild boar as primary maintenance reservoirs (dark green), secondary reservoirs (light green) or not susceptible to infection (white) is indicated, as well as the potential role as sentinel species (dotted cells).

Risk factors of relevance for surveillance design and targeting	Swine Influenza	E. granulosus	Hepatitis E	Score
INTERFACE Farming Direct/indirect contact with livestock (outdoor or extensive more relevant)	3	3	3	9
INTERFACE Direct contact with hunters and consumers of meat, regions where meat is commercialized	3	3	3	9
Pathogens, morbidity and/or mortality has been reported in livestock and/or wildlife, and humans (unusual episodes)	3	3	2	8
High host density and/or intensive game management inc. artificial feeding & game release	3	2	3	8
High diversity of hosts (groups and species) (e.g. Natura 2000 network)	3	1	3	7
Mammal sentinels: abundant and widelly distributed species (e.g.red & roe deer) often subject to population control	3	2	1	6
Overwintering bird colonies and landfills leading to intermingling of different species, high aggregation & densities	2	0	3	5
Highy diverse waterbird community. Natura 2000 network,Ramsar sites	3	0	1	4
Pest & exotic birds in URBAN/PERI-URBAN Parks (often subject to control programs)	3	0	1	4
INTERFACE Direct/Indirect contact with pets, humans: outdoor recreational activities, farms, peri-urban areas & Parks	1	1	1	3
Where? At the borders of EU: eastern Europe, the Balkans, southern mediterranean region. Pathogen specific	1	1	1	3
Areas susceptible to rapid environmental change nearest to human setlements or domestic animals	1	1	1	3
Wetlands, breeding ground, EU borders, migration paths overlaps, recent outbreaks in neighbour countries	3	0	0	3
Adapt to changes in birds migration risk maps (Euro Bird Portal EBP)	3	0	0	3
Definitive or intermediate hosts are present (for heteroxenous parasites)	0	3	0	3
Bird sentinels: can be set in natural/artificial water bodies, or song birds and/or zoo birds (e.g., at feeders)	2	0	0	2

Geographical risks	
Interfaces/host diversity as a risk	
Epidemiological risks	

Score for risks		
	0	No relevant at all or N.A.
	1	Optional/not too relevant
	2	Recommended
	3	Highly recommended

Table 26. Ranking for risk factors for early detection associated to wild **carnivore** specific pathogens. The roles of carnivores as primary maintenance reservoirs (dark green), secondary reservoirs (light green) or not susceptible to infection (white) is indicated, as well as the potential role as sentinel species (dotted cells).

Risk factors of relevance for surveillance design and targeting	Swine Influenza	*E. granulosus*	*E. multilocularis*	Lyme Borreliosis	Q-fever	Score
Pathogens, morbidity and/or mortality has been reported in livestock and/or wildlife, and humans (unusual episodes)	3	3	3	3	3	15
INTERFACE Farming Direct/indirect contact with livestock/poultry (inc. gamebird, outdoor more relevant)	3	3	2	1	3	12
Mammal sentinels: abundant and widelly distributed species often subject to population control	3	2	2	3	2	12
INTERFACE Direct/Indirect contact with pets, humans: outdoor recreational activities, farms and peri-urban areas & Parks	1	1	2	3	3	10
High diversity of hosts (groups and species) (dilution effect vs pests) (e.g. Natura 2000 network)	3	1	1	2	2	9
High host density and/or intensive game management, inc. artificial feeding and game release (ungulates, birds)	3	2	1	1	1	8
INTERFACE Direct contact with hunters and consumers of meat, regions where meat is commercialized	3	3	1	0	1	8
Areas susceptible to rapid environmental change nearest to human setlements or domestic animals	1	1	2	2	1	7
When and where unusual or expected (cyclic) population explosion (e.g. voles)	0	0	1	3	2	6
Definitive or intermediate hosts are present (for heteroxenous parasites)	0	3	3	0	0	6
Where? At the borders of EU: eastern Europe, the Balkans, southern mediterranean region. Pathogen specific	1	1	1	1	1	5
Vectors for target pathogens (vector borne) are present (macroscale and microhabitat)	0	0	0	2	2	4
Highy diverse waterbird community. Natura 2000 network, Ramsar sites	3	0	0	0	0	3
Pest & exotic birds in URBAN/PERI-URBAN Parks, such as pigeons, ducks (often subject to control)	3	0	0	0	0	3
Overwintering bird colonies and landfills leading to intermingling of different species, and high aggregation and densities	2	0	1	0	0	3
Wetlands, breeding ground, EU borders, migration paths overlaps, recent outbreaks in neighbour countries	3	0	0	0	0	3
Adapt to changes in birds migration risk maps (Euro Bird Portal EBP)	3	0	0	0	0	3
Bird sentinels: can be set in natural/artificial water bodies, or song birds and/or zoo birds (e.g., at feeders)	2	0	0	0	0	2

Geographical risks	
Interfaces/host diversity as a risk	
Epidemiological risks	

Score for risks

0	No relevant at all or N.A.
1	Optional/not too relevant
2	Recommended
3	Highly recommended

Table 27. Ranking for risk factors for early detection associated to **wild lagomorph** specific pathogens. The roles of wild lagomorphs as primary maintenance reservoirs (dark green), secondary reservoirs (light green) or not susceptible to infection (white) is indicated, as well as the potential role as sentinel species (dotted cells).

Risk factors of relevance for surveillance design and targeting	Q-fever	Score
Pathogens, morbidity and/or mortality has been reported in livestock and/or wildlife, and humans (unusual episodes)	3	3
INTERFACE Farming Direct/indirect contact with livestock (outdoor more relevant)	3	3
INTERFACE Direct/Indirect contact with pets, humans: outdoor recreational activities, farms and peri-urban areas & Parks	3	3
High diversity of hosts (groups and species) (dilution effectvs pests) (e.g. Natura 2000 network)	2	2
Mammal sentinels: abundant and widely distributed species (e.g. rabbits) often subject to control	2	2
Vectors for target pathogens (vector borne) are presen (macroscale and microhabitat)	2	2
When and where unusual or expected (cyclic) population explosion (e.g. rabbits, other hosts such as voles)	2	2
High host density and/or intensive game management, inc. artificial feeding and game release (ungulates, birds)	1	1
Where? At the borders of EU: eastern Europe, the Balkans, southern mediterranean region. Pathogen specific	1	1
Areas susceptible to rapid environmental change nearest to human setlements or domestic animals	1	1
INTERFACE Direct contact with hunters and consumers of meat, regions where meat is commercialized	1	1

Geographical risks
Interfaces/host diversity as a risk
Epidemiological risks

Score for risks

0	No relevant at all or N.A.
1	Optional/not too relevant
2	Recommended
3	Highly recommended

Table 28. Ranking for risk factors for early detection associated to **wild lagomorphs** specific pathogens. The roles of micromammals as primary maintenance reservoirs (dark green), secondary reservoirs (light green) or not susceptible to infection (white) is indicated, as well as the potential role as sentinel species (dotted cells).

Risk factors of relevance for surveillance design and targeting	Q-fever	Score
Pathogens, morbidity and/or mortality has been reported in livestock and/or wildlife, and humans (unusual episodes)	3	3
INTERFACE Farming Direct/indirect contact with livestock (outdoor more relevant)	3	3
INTERFACE Direct/Indirect contact with pets, humans: outdoor recreational activities, farms and peri-urban areas & Parks	3	3
Preferably adults for active surveillance, (youngs can be interesting for incidence	2	2
High diversity of hosts (groups and species) (dilution effectvs pests) (e.g. Natura 2000 network)	2	2
Mammal sentinels: abundant and widely distributed species (e.g. rabbits) often subject to control	2	2
Vectors for target pathogens (vector borne) are presen (macroscale and microhabitat)	2	2
When and where unusual or expected (cyclic) population explosion (e.g. rabbits, other hosts such as voles)	2	2
High host density and/or intensive game management, inc. artificial feeding and game release (ungulates, birds)	1	1
Where? At the borders of EU: eastern Europe, the Balkans, southern mediterranean region. Pathogen specific	1	1
Areas susceptible to rapid environmental change nearest to human setlements or domestic animals	1	1
INTERFACE Direct contact with hunters and consumers of meat, regions where meat is commercialized	1	1

Geographical risks
Interfaces/host diversity as a risk
Epidemiological risks

Score for risks

0	No relevant at all or N.A.
1	Optional/not too relevant
2	Recommended
3	Highly recommended

Table 29. Ranking for risk factors for early detection associated to **micromammals** specific pathogens. The roles of bats as primary maintenance reservoirs (dark green), secondary reservoirs (light green) or not susceptible to infection (white) is indicated, as well as the potential role as sentinel species (dotted)

Risk factors of relevance for surveillance design and targeting	TBE	*E. multilocularis*	*Lyme Borreliosis*	*Q-fever*	Score
Pathogens, morbidity and/or mortality has been reported in livestock and/or wildlife, and humans (unusual episodes)	3	3	3	3	12
INTERFACE Direct/Indirect contact with pets, humans: outdoor recreational activities, farms and peri-urban areas & Parks	3	2	3	3	11
When and where unusual or expected (cyclic) population explosion (e.g. voles)	3	1	3	2	9
Mammal sentinels: abundant and widelly distributed species (e.g.rats) often subject to population control	1	2	3	2	8
INTERFACE Farming Direct/indirect contact with livestock (outdoor more relevant)	1	2	1	3	7
Vectors for target pathogens (vector borne) are presen (macroscale and microhabitat)	3	0	2	2	7
Areas susceptible to rapid environmental change nearest to human setlements or domestic animals	2	2	2	1	7
High diversity of hosts (groups and species) (ddilution effect vs pests) (e.g. Natura 2000 network)	1	1	2	2	6
High host density and/or intensive game management of listed grous inc. artificial feeding and game release (e.g. ungulatess)	1	1	1	1	4
Where? At the borders of EU: eastern Europe, the Balkans, southern mediterranean region. Pathogen specific	1	1	1	1	4
Definitive or intermediate hosts are present (for heteroxenous parasites)	0	3	0	0	3
INTERFACE Direct contact with hunters and consumers of meat, regions where meat is commercialized	0	1	0	1	2
Highy diverse waterbird community. Natura 2000 network, Ramsar sites	1	0	0	0	1
Pest & exotic birds in URBAN/PERI-URBAN Parks, such as pigeons (often subject to control programs)	1	0	0	0	1
Overwintering bird colonies and landfills leading to intermingling of different species, and high aggregation and densities	0	1	0	0	1

Geographical risks
Interfaces/host diversity as a risk
Epidemiological risks

Score for risks		
	0	No relevant at all or N.A.
	1	Optional/not too relevant
	2	Recommended
	3	Highly recommended

Table 30. Ranking for risk factors for early detection associated to **waterbirds** specific pathogens. The roles of waterbirds as primary maintenance reservoirs (dark green), secondary reservoirs (light green) or not susceptible to infection (white) is indicated, as well as the potential role as sentinel species (dotted cells)

Risk factors of relevance for surveillance design and targeting	HPAI	Swine Influenza	West Nile Disease	Score
Pathogens, morbidity and/or mortality has been reported in livestock and/or wildlife, and humans (unusual episodes)	3	3	3	9
INTERFACE Farming Direct/indirect contact with livestock/poultry (inc. gamebird) (outdoor or extensive more relevant)	3	3	3	9
Wetlands, breeding ground, EU borders, migration paths overlaps, recent outbreaks in neighbour countries	3	3	3	9
Highy diverse waterbird community. Natura 2000 network, Ramsar sites	3	3	2	8
Pest & exotic birds in URBAN/PERI-URBAN Parks, such as pigeons (often subject to control programs)	3	3	2	8
Bird sentinels: can be set in natural/artificial water bodies, or song birds and/or zoo birds (e.g., at feeders)	3	2	3	8
Adapt to changes in birds migration risk maps (Euro Bird Portal EBP)	3	3	2	8
High diversity of hosts (groups and species). (Dilution effect vs pests) (e.g. Natura 2000 network)	3	3	1	7
High host density and/or intensive game management, inc. artificial feeding and game release (incl. Anseriformes)	2	3	2	7
Overwintering bird colonies and landfills leading to intermingling of different species, and high aggregation and densities	3	2	2	7
INTERFACE Direct contact with hunters and consumers of meat, regions where meat is commercialized	3	3	0	6
INTERFACE Direct/Indirect contact with pets, humans: outdoor recreational activities, farms and peri-urban areas & Parks	2	1	2	5
Where? At the borders of EU: eastern Europe, the Balkans, southern mediterranean region. Pathogen specific	3	1	1	5
Mammal sentinels: abundant and widelly distributed species (e.g. red fox, rats) often subject to control	0	3	1	4
Areas susceptible to rapid environmental change nearest to human setlements or domestic animals	1	1	2	4
Species capable of performing large migrations that could result in a cross-border spread	3	0	1	4
Vectors for target pathogens (vector borne) are presen (macroscale and microhabitat, e.g. *Ixodes*)	0	0	3	3

Geographical risks	
Interfaces/host diversity as a risk	
Epidemiological risks	

Score for risks	
0	No relevant at all or N.A.
1	Optional/not too relevant
2	Recommended
3	Highly recommended

Table 31. Ranking for risk factors for early detection associated to **non waterbirds** specific pathogens. The roles of non waterbirds as primary maintenance reservoirs (dark green), secondary reservoirs (light green) or not susceptible to infection (white) is indicated, as well as the potential role as sentinel species (dotted cells).

Risk factors of relevance for surveillance design and targeting	HPAI*	Swine Influenza	West Nile Disease	Lyme Borreliosis	Score
Pathogens, morbidity and/or mortality has been reported in livestock and/or wildlife, and humans (unusual episodes)	3	3	3	3	12
INTERFACE Farming Direct/indirect contact with livestock/poultry (inc. gamebird) (outdoor or extensive more relevant)	3	3	3	1	10
High diversity of hosts (groups and species) (dilution effect vs pests) (e.g. Natura 2000 network)	3	3	1	2	9
Wetlands, breeding ground, EU borders, migration paths overlaps, recent outbreaks in neighbour countries	3	3	3	0	9
INTERFACE Direct/Indirect contact with pets, humans: outdoor recreational activities, farms and peri-urban areas & Parks	2	1	2	3	8
High host density and/or intensive game management, inc. artificial feeding and game release (incl. Galliiformes)	2	3	2	1	8
Highy diverse waterbird community. Natura 2000, Ramsar sites	3	3	2	0	8
Pest & exotic birds in URBAN/PERI-URBAN Parks, such as pigeons (often subject to control)	3	3	2	0	8
Bird sentinels: can be set in natural/artificial water bodies, or song birds and/or zoo birds (e.g., at feeders)	3	2	3	0	8
Adapt to changes in birds migration risk maps (Euro Bird Portal EBP)	3	3	2	0	8
Mammal sentinels: abundant and widely distributed species (e.g. red fox, rats) often subject to control	0	3	1	3	7
Overwintering bird colonies and landfills leading to intermingling of different species, and high aggregation and densities	3	2	2	0	7
Where? At the borders of EU: eastern Europe, the Balkans, southern mediterranean region. Pathogen specific	3	1	1	1	6
Areas susceptible to rapid environmental change nearest to human setlements or domestic animals	1	1	2	2	6
INTERFACE Direct contact with hunters and consumers of meat, regions where meat is commercialized	3	3	0	0	6
Vectors for target pathogens (vector borne) are presen (macroscale and microhabitat)	0	0	3	2	5
Species capable of performing large migrations that could result in a cross-border spread	3	0	1	0	4

Geographical risks
Interfaces/host diversity as a risk
Epidemiological risks

Score for risks	
0	No relevant at all or N.A.
1	Optional/not too relevant
2	Recommended
3	Highly recommended

3.5. General recommendations for the first steps of sustainable wildlife zoonotic disease surveillance in the UE

Section 2.2 provided recommendations for integrated wildlife population monitoring and disease surveillance. The objectives of integrated wildlife monitoring will determine which population and diseases related variables to measure, such as, the pathogens to detect and the range of hosts (incl. the environment), and how to do it. It is best if different sectors coordinate their contribution over the different phases of monitoring to reduce overlap and effort as a function of their capacities and means, from planning (e.g., access to samples from wildlife), passing by execution of surveillance (e.g., wildlife syndromic surveillance and passive surveillance by wildlife departments, hunters) to join analysis of data and communication/reporting.

In this section we elaborate general recommendations for the first steps of sustainable wildlife zoonotic disease surveillance in the EU. A sound zoonotic disease surveillance system in wildlife, within the framework of integrated wildlife monitoring should detect all epidemics, pathogen incursions and important increases in risk of human infection, and they should be detected as early as possible.

The general recommendations to organize the first steps of sustainable wildlife disease surveillance under the OH approach for the list of cross-border pathogens that threaten the EU are:

- First, zoonotic disease surveillance under the OH approach requires interdisciplinary collaboration across stakeholders in human, animal (including wildlife) and environmental health representatives at all stages of surveillance efforts (i.e., design, implementation, management, and evaluation), if not, the system will be ineffective, less sustainable, and short-lived. There is a need to conduct analysis and needs assessment of stakeholders involved in wildlife surveillance systems, at regional and national level.
- Future OH wildlife surveillance programmes in Europe should employ a combination of general (passive) and targeted (active) wildlife disease surveillance because it is cost-effective to address the surveillance of several pathogens concurrently rather than individual or separate targeted surveillance programs specific to each pathogen. Passive and active surveillance components would ideally take place simultaneously, but the final choice depends on the evaluation of their cost-effectiveness for specific hosts, pathogens, geographical and epidemiological contexts:
    - If passive surveillance is prioritized, emerging diseases will be detected, but monitoring and assessment of interventions will be limited. It requires a multi-actor passive surveillance network using available infrastructure and data sources (e.g., public participation through citizen science tools (Lawson et al., 2015) or information derived from road kills (Schwartz et al. 2020, Fernandez-López et al. 2022), and covering a broad geographical range, to ensure early detection of disease emergence.
    - When only active surveillance is prioritized, the early detection of emerging diseases may be compromised. An active sampling scheme targeting selected (prioritized) hosts and diseases must be flexible enough to enable an adaptive approach, continuously improving surveillance strategies for target populations and diseases by incorporating new information on host demography and disease prevalence (Belsare et al. 2020).
    - There is an important role of diagnostic pathology in passive surveillance for the identification of new or unexpected pathogens and diseases, which also requires choosing what additional diagnostic tests need to be carried out (such as bacterial culture, PCR for certain pathogens. Thus, it is particularly important that the countries

to have, or if necessary, develop, adequate expertise and capacity in veterinary diagnostic pathology applied to general wildlife disease surveillance programmes and involve the contribution of relevant stakeholders, such as rescue centres.

- As for active surveillance, *antemortem* diagnostic tests are of limited value, depending on the pathogen, but especially for the host, since for many wildlife species test sensitivity and specificity have not been evaluated. Environmental detection of microbiological hazards is becoming a sensitive and cost-effective approach, but still needs to be developed for different pathogens and sampled matrices in order to become a reference technique for routine surveillance. This is relevant also to wildlife trade.

- There are multiple surveillance components (i.e., a single surveillance activity, defined by the source of data and the methods used for its collection, used to investigate the occurrence of one or more hazards in a specified population), the higher the number of them included, the higher the surveillance system sensitivity. Their selection of specific components in disease surveillance programs can be recommended for specific pathogen, hosts, epidemiological context and aims of surveillance in terms of cost-effectiveness. Under the OH approach, not only human and domestic animal, but wildlife and the environment component may need to be included in OH surveillance systems because they can serve as reservoirs of infection or infestation and/or as indicators of risk to humans and domestic animals. Therefore, an important and still needed discussion among the different compartments of OH is about the identification of criteria to guide the selection of zoonotic disease surveillance components (see section below).

- The nature, availability and sources of surveillance data may respond to different strategies, which are complementary. Under the OH approach, determining disease emergence, maintenance, and risk of transmission in multi-host communities is recommended, for which we need to focus surveillance on a diverse array of pathogens at once in a number of host community assemblages. This is the so-called "observatory approach" (see the European Observatory of Wildlife, https://wildlifeobservatory.org/). This network, ideally, should be designed under the premises of risk-based surveillance (see below) and incorporate a wide range of scenarios, and the more appropriate surveillance components to each case. Such a network addressing complex multi-host multi-pathogen systems offers the possibility to evaluate disease emergence not only when pathogens are found in new areas, but also to detect between-species jumps, and the emergence of new variants almost "in real time" (for which molecular tools are key), to report early and raise awareness about potential threats. The observatory approach therefore complements classical approaches which normally are fragmented in terms of target population (rarely entire communities of hosts) and pathogens are addressed, and opportunist spatio-temporal sources of samples/data.

- Risk-based surveillance approaches should be used and continuously informed by surveillance data as a cost-effective strategy addressing different components. This may be an especially important priority for initiating wildlife disease surveillance in settings where resources are limited. Namely, the main risk factors relate to

    - Ecological and anthropological factors: Epidemiological context (e.g., pathogen already present or not, just few incursions known, vectors present but not pathogen), risk period, variable multi-host communities, environmental and interfaces gradients, from natural areas, passing though farmland to urban and peri-urban scenarios. The relative importance of such environmental scenarios for surveillance of zoonotic pathogens is variable and context dependent. For a general surveillance strategy, covering all of them is an interesting initial option, which will be later improved through an adaptive process. The rapid intensification of agriculture, socioeconomic change, and ecological fragmentation have profound impacts on the epidemiology of zoonotic infectious diseases and the diverse wildlife-livestock-human interfaces must be included is surveillance strategies. These interfaces represent critical points for cross-species transmission and emergence of pathogens into new host populations.

  - Populations at risk, such as at the borders or wildlife migratory routes, in specific ecosystems/habitats (e.g., wetlands, bushlands, pasturelands), in specific interfaces (urban/peri-urban, farmland, nature/protected areas), and their combinations (stratified risk-based sampling). Traded wildlife (some are for hunting purposes) and exotic species must be considered as risky populations by definition.
  - Risk area, determined by previous risks, but also by purely biogeographic conditions, such as being in the border of EU at risk for a certain pathogen and/or vector. We recommend to map risk areas, for which is it needed to invest in the development of predictive species and pathogen modelling based on ecosystem data (e.g., mammal species richness, domestic livestock, their interfaces, and abundance, landscape changes) to map risk areas where to geographically target detection efforts. All this will allow to identify risk maps, which need to be continuously updated, for instance, about risk pathways and potential hot spots for zoonotic emerging wildlife diseases at the regional and national level. As a first approach for large scale design of wildlife surveillance, co-ordinately among countries, is considering the European bioregions (defined by official delineations used in the Habitats Directive 92/43/EEC and the EMERALD Network set up under the Convention on the Conservation of European Wildlife and Natural Habitats, Bern Convention).

More detailed recommendations on specific risks useful to design surveillance are presented in the section below.

- Where possible, initially select surveillance of wildlife at sites where:
  - Human or domestic animal surveillance is also occurring, which may help provide information on cross-species disease transmission risks.
  - Wildlife population and ecosystem monitoring are taking place, either highly available low precise data (such as hunting, which is available over large regions of Europe) or high precise density estimation data (observatory approach), ideally, a combination of both.
  - For that purpose and under the OH approach, applying the observatory approach to areas where the human and livestock interfaces are present is recommended.
- Sampling surveillance efforts for early detection, such as frequency of repeated sampling and its duration, can be adapted to specific pathogen risk and potential rate of introduction and spread of diseases within each MS.
  - However, available resources should guarantee minimum numbers and common sampling criteria for comparison purposes among study areas, health compartments, pathogens, and specific hosts.
  - Recommendations on specific the sampling effort required (this aspect is beyond the purposes of this report, for instance, the WOAH code for terrestrial animals provides design prevalence and detailed guidance for surveillance specific to several of the listed diseases, https://www.woah.org/en/what-we-do/standards/codes-and-manuals/terrestrial-code-online-access/) often must balance the need to collect sufficient data to make valid statistical inferences with the need to minimize cost and time expenditures. A representative sample of the population may be critical to detect changes in disease prevalence but for early detection of a disease, it may be more useful to have a sample of high-risk units (e.g., wild populations in proximity to risky areas for pathogen introduction). The actual number of animals, points, sites etc.

that should be sampled and the number of times each should be revisited during a particular field season will vary depending on the prevalence/incidence of the pathogen, difficulty to sample wild hosts species, variability of risk factors considered, surveillance design, the objectives of the monitoring program.

- The efficiency of a sampling design greatly depends on the characteristics of the target population, often distributed over large regions of Europe (e.g., wild boar, rodents), even variably according to the period and year (e.g., migratory birds):
    - If the target population can be divided into different spatial units that are relatively homogenous in nature, then stratification of sampling by type would result in a more efficient sampling design and more precise prevalence/incidence/detection estimates by type. In such cases adequate sample sizes need to be maintained for each stratum (rather than the population as a whole), and for low density populations or rare species, this is frequently not a feasible option. This can be a wise initial design, which can be adaptively modified and continuously adjusted to become more sensitive and cost-efficient as result of surveillance are obtained, analysed, and interpreted.
    - As for rare species occurring at low densities often relevant as sentinels (e.g., wolves), one can maximize the number of observations by standardizing timing of surveys (time and season), when individuals are more visible increasing detection probability. Again, an adaptive sampling intensity of sampling is dependent on initial sampling results.
- Diagnostic tests should be selected on a host species-pathogen specific basis, and it must be guaranteed the sufficient capabilities of laboratories to conduct the testing of recommended sample type and methods (https://rr-europe.woah.org/en/the-oie-national-focal-points/). The regional diagnostic capacity for wildlife diseases must be developed, standardized, and harmonized. Multi-host serological and pathogen detection tests are already used in wildlife literature, but their reliability often needs to be tested, as well as the sensitivity/specificity of PCR *vs* other tests (immunopathology, pathogen isolation). Environmental sampling through molecular techniques is already in use and its development is promising, but still, in most cases, its reliability and practical use need to be evaluated. This is essential to elaborate standardized protocols and guidance to be used in the necessary range of epidemiological, environmental contexts and pathogens.
- Wildlife zoonotic disease surveillance sensitivity for early warning of zoonotic pathogens and cost-benefit of adopted strategies need to be continuously evaluated to be optimized:
    - Representativeness of sampling, such as best frequency for repeated samples.
    - What and how many different surveillance components to consider, whose relevance may vary or need to be evaluated on a continuous basis.
    - The surveillance approach: risk-based sampling *vs* random/stratified, passive *vs* active, general *vs* targeted. Risk-based surveillance approaches should be used and continuously informed by surveillance data as a cost-effective strategy addressing different components.
    - The application of more appropriate diagnostic tests also needs continuous evaluation in wild animal species since, as above-mentioned, there is still lack of validation of some which may present challenges in selecting tests for specific pathogens and hosts.
- The continuous evaluation, including the monitoring of the implementation of agreed standards, will allow MS to take decisions based on cost-benefit as there are too many

different scenarios and considerations at local level to be done. This information, for harmonizing purposes, should be shared and discussed. There is need to establish interdisciplinary teams responsible for common data management, evaluation of surveillance, and risk-based epidemiological analysis. Presence of ecologists and epidemiologists on these teams for quantitative evaluation of surveillance data and risk-based analysis is also crucial. One relevant aspect is the assessments of economic aspects, measuring the costs and benefits of extending surveillance to wildlife for early detection. International groups (e.g., *ENETWILD*, Vectornet) should commit long-term support for continuous evaluation of zoonotic disease surveillance in wildlife at European level.

3.6. Specific recommendations of surveillance aimed at risk based early detection of pathogens in the main wild species groups

In this section we elaborate specific recommendations for sustainable wildlife zoonotic disease surveillance in the EU considering the listed pathogens and the main group oh hosts. These recommendations are to implement key aspects of an effective OH frontline surveillance system for a number of selected wildlife pathogens:

- To early detect them and raise awareness about potential threats;
- With a midterm perspective (once data is collected, analysed, and interpreted):
    - To inform on epidemiological risk specific to wildlife host and pathogens and improve the surveillance system;
    - To monitor the impact of prompt interventions.

Since there are many species of wildlife (here classified in a number of functional hosts groups), there are also varied risks of disease transmission pathways between and within species (also at the interfaces with domestics and humans), which may vary across in different regions or areas and host assemblages. Therefore, the value of the specific recommendations for surveillance aimed at early detection of pathogens in the main wild species groups should be interpreted under the light of national/local contexts. Individual experts may suggest different inputs and criteria to consider the relative importance of risks in a surveillance system, so we believe that there is a need to apply methods to combine knowledge, priorities, and preferences from a group of experts.

The specific recommendations of surveillance aimed at risk based early detection of pathogens in the main wild species groups are:

- Farmlands (particularly outdoor) should be priority areas to be incorporated to sampling strategies for wildlife in relation to most pathogens of the list.

  Hosts and pathogens: all hosts and pathogens. The presence of wild ungulates, carnivores, and lagomorphs at the interface with farms, and subsequent contacts with livestock was particularly relevant as a risk and accounted for most pathogens included in the list. This risk also ranked high for other hosts, such as micromammals and birds. Wild ruminants were relevant to the highest number of pathogens (at least 6: TBE, *E. granulosus*, CCHF, HEV, Q-fever and RVF).

- It is essential to develop best possible initial mapping of pathogen (or threat) presence and distribution, at least for those already present in the EU and nearby countries, for further development of risks-based surveillance (planning and sampling). Most pathogens here listed are communicable, however the disaggregation of data sources, lack of harmonization and interrupted data flow for these data, and neglecting published research, makes difficult to generate "live" maps on the distribution of pathogens over Europe.

<u>Hosts and pathogens</u>: all hosts and pathogens, considering that most pathogens already are variably present in the EU: as endemic (widely or in certain areas), few incursions only detected, or not present (RVF) but vector present.

- Currently, we are not ready to produce a complete range of good resolution maps of the spatial distribution of the wildlife/livestock interfaces in Europe, but only in some countries and for some species. If not at European level, at least countries should develop maps of the wildlife-livestock interfaces as a basis for designing future surveillance of zoonotic pathogens at such interfaces, paying special attention to outdoor livestock production (there is need to standardize the nomenclature of different types of production systems over Europe).

  Hosts and pathogens: all wildlife hosts (including domestic animals).

- The interface where direct and indirect contacts of wildlife with pets and humans occurs (outdoor recreational activities, farms, peri-urban areas, and parks) is a priority target for disease surveillance in most wildlife groups and should be considering during surveillance planning phase. The different stakeholders involved in surveillance in this interface (local veterinary services, rescue centres) and citizens should play a coordinated role.

  <u>Hosts and pathogens</u>: all hosts, pathogens, and vectors. Vectors that are maintained by wild hosts (ticks and mosquitoes), direct or indirect transmission, such as birds and HPAI, the presence of parasitic stages in the environment (e.g., *E. multilocularis* associated to peri-urban rodents and fox). Vector borne pathogens in the case of rodents, or for indirectly transmitted pathogens such as Q-fever in both rabbits and rodents. Micromammals may play a relevant role as sentinels at this interface.

- Regarding the risk posed by vectors where infected wildlife is present, efforts are needed to map at the finest possible resolution and at large biogeographical scales where hosts and vectors distribution overlaps, and to determine at local level, the habitat, land uses and features where both vectors and host sampling is recommended. It is also needed to elucidate where and when host and/or vector sampling is recommended for determining the presence of vectors borne pathogens to become more sensitive/practical/cost-efficient for early detection of specific pathogens, and in which conditions and for which pathogens and vectors. This information will provide a solid background for sustainable vector borne zoonotic disease surveillance in the future.

  <u>Hosts and pathogens</u>: all hosts and those pathogens which are vector borne (and vectors).

- The extent to which host species diversity and specific host assemblages influences transmission risk of infectious diseases in Europe (a highly impacted continent by human activities) is unknown for most pathogens and host communities. Therefore, a gradient of wild host biodiversity situations should be considered in surveillance planning. An adaptive approach is recommended, learning to improve early detection: from highly biodiverse to simplified host communities where one or a few species predominates, often as pest species (e.g., rodents in farmlands, or peri-urban). The EOW (https://wildlifeobservatory.org/), involving a wide range of scenarios (potentially including or coordinated with the Natura 200 network of protected areas) offers a possibility to work on this matter with a European perspective, since diverse well distributed host communities over Europe are included. Since the outcome of host richness changes for infectious disease risk depends on a community´s ability to support infection (community competence), the future European zoonotic surveillance should establish (as a complementary approach) a network of study areas with diverse host community assemblages (both wildlife and domestic) and environmental conditions where to develop jointly the surveillance of all priority pathogens and vectors.

  <u>Hosts and pathogens</u>: all hosts and pathogens (and vectors), of special relevance multi-host pathogens.

- Europe presents frequent and diverse situations where wildlife is abundant (referred to as overabundant) for numerous reasons, often associated to intensive management practices such as artificial feeding and game release, leading to simplified ecosystems where some pathogens become widespread and the risk for transmission to humans high. Therefore, a necessary first step for design disease surveillance strategies is mapping both **abundance** and **management schemes** of wild species over Europe, using standards for data collection to incorporate wildlife abundance to disease surveillance planning (i.e., as *ENETWILD* initiative does).

  Hosts and pathogens: all hosts and pathogens.

- Wildlife zoonotic disease surveillance should target where direct contact of wildlife with hunters, and consumers of meat are present. The possible role of wildlife as a source of zoonotic cases, such as HEV, should be listed in European reports on zoonosis. This can be the basis for prioritizing surveillance strategies in wildlife.

  Hosts and pathogens: all hosts and pathogens. Relevant are wild host species which are consumed and prone to carry zoonoses, such as wild boar.

- More evidence is needed on the potential role and practical use of wild species as potential sentinels for early detection of zoonosis, however their inclusion in wildlife zoonotic disease surveillance is recommended.

  Hosts and pathogens: all hosts and pathogens. Relevant are species carnivores which are on top of food chain.

- Wetlands and breeding grounds habitats/areas, and at larger scale, EU borders and where bird migration paths overlaps, especially where recent outbreaks occurred in neighbour countries are essential risk to be considered in surveillance sampling design.

  Hosts and pathogens: migratory birds and avian pathogens (HPAI). In these areas, non-migratory birds, and other species (e.g., predators) may play role as bridge hosts.

3.7. A final reflexion: integrating animal disease surveillance components (wildlife, domestic, environment) for early detection under OH approach

We all would agree that there is a need to concede more relevance to wildlife disease surveillance to assist in the detection of emerging infectious diseases, and that the integration of wildlife health into OH policy will be critical in better preparing the EU to prevent and manage the adverse impacts of zoonotic diseases on human health. However, there are a number of constraints that currently limit progress in developing risk-based disease surveillance in the wildlife, and this is even more notable for international surveillance programs. Probably, the most considerable constraint is the scarcity of published data to assist in the design of risk-based disease surveillance in wildlife, including the selection of cost/effective surveillance components. This is even more complicated in the OH context since surveillance components from different health compartments should meet and complementary be applied in an efficient way. This applies to data on the relative risk of wildlife populations in becoming infected due to the presence, absence or intensity of a given risk factor; the sensitivity/specificity of diagnostic tests; and the inexistence of data on previous infection for wildlife in a given locality or region. Studies into risk factors for pathogen introduction into wildlife population farms are very complex, since wildlife are more exposed to a larger variety of factor than, for instance, livestock.

In general, surveillance is aimed at demonstrating the absence of infection, determining the presence, distribution or introduction of infection, or detecting exotic diseases or emerging diseases as early as possible before they spread, cost human lives, economic, social, environmental damage and become difficult to control. An effective surveillance system may include one or more component activities that generate information on the health or disease, zoonosis in this case. Under the OH context, the early detection of zoonotic pathogens requires continuous robust and diverse components for early warning and response. Therefore, initially there is a need to select the components that are more effective to achieve the objectives and to prioritize data sources, considering the limitations of resources. Not only human and domestic animal, but also wildlife and the environment need to be included in OH surveillance systems because they can serve as reservoirs of infection or infestation and/or as indicators of risk to humans and domestic animals, and they can serve to detect pathogens earlier. This should also investigate the politics of National Reference Laboratories being the only way to (officially) report notifiable diseases, as this may limit international surveillance. Equally unvalidated and non-WOAH approved tests may be used, producing uncertain results. One difficult area may be eDNA sampling for pathogens which may produces positive un-validated results and not occur on a potential infected premises. These aspects may not be official positive cases but would benefit from being captured in some way.

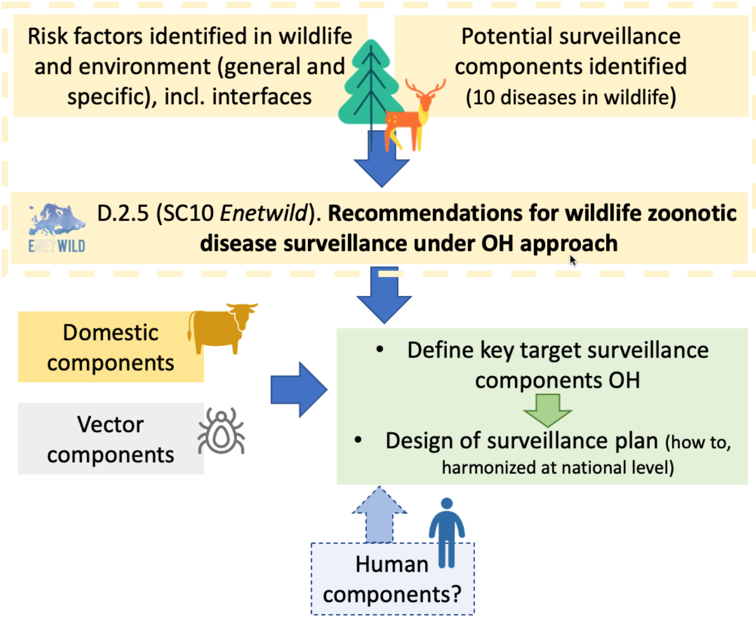

Figure 8. Scheme to identify criteria for further guiding the selection of the most cost/effective zoonotic disease surveillance components adapted to objectives.

Following, **some criteria to guide the selection of zoonotic disease surveillance components adapted to objectives and prioritizing cost/efficiency are identified** (Figure 8), for which we propose the following scheme:

III. Define **main targets** for the respective pathogens, which is our case is <u>early detection of</u>:
- Change in the geographic distribution/spread to new areas
- The introduction of the pathogen
- An increase in incidence, i.e., early epidemic detection
- The onset and duration of the period of increased risk

IV. To initiate the evaluation and optimization of a surveillance system, **all surveillance system components need to be identified and their utility to the aims described**. This step is essential and must be addressed/discussed jointly by the different health compartments for all priority pathogens.
- Sensitivity:
  - In which component of OH systems can we achieve the earliest detection?
    - Human (active/passively?)
    - Livestock (active/passively?)
    - Pets (active/passively?)
    - Wildlife (active/passive?)
    - Environment (active/passively?)
  - The ability to detect at least one positive unit given that the population is truly infected (considering that the sensitivity of a surveillance components depends on the level of disease in the population).
- Costs (comparing different options): economic, technical, and logistic aspects. Cost/effectiveness can be evaluated based on previous parameters.

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