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Monitoring of chronic wasting disease (CWD) (IV)

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Abstract

The European Commission requested an analysis of the Chronic Wasting Disease (CWD) monitoring programme in Norway, Sweden, Finland, Iceland, Estonia, Latvia, Lithuania and Poland (9 January 2017–28 February 2022). Thirteen cases were detected in reindeer, 15 in moose and 3 in red deer. They showed two phenotypes, distinguished by the presence or absence of detectable disease-associated normal cellular prion protein (PrP) in lymphoreticular tissues. CWD was detected for the first time in Finland, Sweden and in other areas of Norway. In countries where the disease was not detected, the evidence was insufficient to rule out its presence altogether. Where cases were detected, the prevalence was below 1%. The data also suggest that the high-risk target groups for surveillance should be revised, and 'road kill' removed. Data show that, in addition to differences in age and sex, there are differences in the prion protein gene (*PRNP*) genotypes between positive and negative wild reindeer. A stepwise framework has been proposed with expanded minimum background surveillance to be implemented in European countries with relevant cervid species. Additional surveillance may include ad hoc surveys for four different objectives, specific to countries with/without cases, focusing on parallel testing of obex and lymph nodes from adult cervids in high-risk target groups, sustained over time, using sampling units and a data-driven design prevalence. Criteria for assessing the probability of CWD presence have been outlined, based on the definition of the geographical area, an annual assessment of risk of introduction, sustained minimum background surveillance, training and engagement of stakeholders and a surveillance programme based on data-driven parameters. All positive cases should be genotyped. Sample sizes for negative samples have been proposed to detect and estimate the frequency of *PRNP* polymorphisms. Double-strand sequencing of the entire *PRNP* open reading frame should be undertaken for all selected samples, with data collated in a centralised collection system at EU level.

Keywords: chronic, wasting, CWD, surveillance, Europe, genotype

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Summary

Following the 3-year monitoring programme laid down by Regulation (EU) 2017/1972 that ended on 31 December 2020, and the extension of the intensified monitoring in Sweden until February 2022, the European Food Safety Authority (EFSA) was asked by the European Commission to deliver its scientific opinion by 31 March 2023 on the following terms of reference (ToRs): (1) To analyse the results of the monitoring programme carried out in Norway, Sweden, Finland, Iceland, Estonia, Latvia, Lithuania and Poland between 1 September 2017 and 28 February 2022, and in particular, to assess if the two objectives as set in the 2017 EFSA opinion on CWD in cervids have been met. (2) To describe any new knowledge on the epidemiology of CWD in EU/EEA countries. (3) To recommend, if considered appropriate, future CWD monitoring activities for the EU based on an assessment of the epidemiological situation. (4) Based on what is known about the epidemiology of CWD in EU/EEA countries, to describe the criteria relevant for considering an area not to be infected with CWD. (5) To provide the design of a genotyping protocol for positive samples, and for the negative samples of the 3-year monitoring programme stored as per point 3.3, section III.A of Annex III of Regulation (EC) No 999/2001, specifying which negative samples should be genotyped, the codons of the *PRNP* gene to be genotyped and recommending genotyping assay/s for the implementation of the requirement by the NRLs.

The data used in this assessment comprises the surveillance data submitted to EFSA and validated and published every year in the EU summary reports for TSE, and the additional data and information provided by Finland, Norway and Sweden. Following the description of the surveillance data, the intensified surveillance conducted in countries with cases and the caseload with the estimation of observed prevalence, the minimum detectable prevalence at country, species and at primary sample unit (PSU) level was calculated. Scenario-tree modelling was applied to combine samples from low- and high-risk target groups to estimate the surveillance system sensitivity (SSe) for detecting CWD in a cervid species for a two-stage sampling system in a country (PSU and within PSU). New knowledge on the epidemiology of CWD since the last published EFSA opinion on CWD was in part collated from relevant scientific literature and in part based on the outcome of the surveillance data analysed within this mandate. To inform a stepwise approach to the design of future monitoring activities, a SWOT analysis of the past mandatory surveillance (2018–2020) was conducted. Since developing criteria for considering an area not to be infected were judged not possible, criteria for the estimation of the probability of occurrence in a determined geographical area were developed. Finally, country- and species-specific samples size for genotyping was calculated for detecting new polymorphisms or estimating the frequency of known polymorphisms in the *PRNP*, and to test their association with susceptibility/resistance to CWD.

The eight countries tested a total of 155,660 cervids older than 12 months of age and of unknown age during the mandate period (1 September 2017–28 February 2022), distributed as follows: Estonia (2,153), Finland (3,457), Iceland (304), Latvia (3,087), Lithuania (3,784), Norway (130,836), Poland (3,505) and Sweden (8,534). The hunted and slaughtered fit for human consumption (HSHC) group was the most frequently tested overall in terms of number of animals (83.8%) in six of the eight countries, with road kills (RK) being the most tested high-risk target group in Finland and Poland. Roe deer was the most tested species in three MS (Estonia, Latvia and Poland). Through this statutory surveillance, prion diseases in cervids were detected for the first time in two countries (Finland and Sweden). During the mandate period, a total of 31 cases were confirmed in three countries (Finland, Norway and Sweden): 13 reindeer, 15 moose and 3 red deer. Sweden and Finland intensified the surveillance in and around areas where CWD cases were found ('affected areas'), testing both high- and low-risk target groups to increase overall probability of disease detection. The observed prevalence among HSHC in affected areas was 0.05% (95% CI 0.006–0.17%) in moose, 0.16% (0.04–0.57%) in red deer and 0.23% (95% CI: 0.12–0.4%) in reindeer. The observed prevalence was more than 10 times higher among moose and red deer submitted from high-risk target groups, emphasising the value of high-risk target groups to detect new CWD cases in these species. The results of the analyses revealed that RK should not be considered a high-risk target group.

The two objectives of CWD surveillance (detect disease and estimate prevalence) have partially been met, given the high variability in the implementation of surveillance at country, species, management systems and PSU level. As a result, for countries that conducted surveillance during the mandate period without finding CWD, the evidence is not sufficient to rule out the possibility of CWD being present. For those countries with cases detected, the observed prevalence is associated with uncertainty due to the sample-based monitoring.

Calculations of the minimum detectable prevalence at country level showed that it was possible to detect a prevalence in the general population (all species for the entire mandate period) close to the 0.1% referred to in the 2017 EFSA opinion. There is high variability between countries and between species within countries. The attained minimum detectable prevalence among high-risk animals was 10% or lower in only 15.3% of the monitored PSU. The result of the scenario tree modelling showed that the overall estimated sensitivity for all cervids except wild reindeer was 95% or greater to detect CWD in Norway, Sweden and Poland considering a scenario of a minimum 5% prevalence and a relative risk (RR) of 5 for high-risk animals compared to HSHC. For a scenario of a minimum 1% prevalence, only Poland (96%) and Norway (94%) reached ~ 95% sensitivity with their applied monitoring programme. In the analysis by species for RR of 5, only the following scenarios reached 95% sensitivity or higher to detect CWD: for semi-domesticated reindeer, Norway and Finland for 5% or higher prevalence and Norway for 1% or higher prevalence; for moose, Norway for 5% or higher prevalence; for roe deer, Norway and Poland for 5% or higher prevalence and Poland for 1% or higher prevalence. The rest of the assessed combinations (country/species) did not reach 95% sensitivity.

CWD has been detected in new areas within Norway but also in Sweden and Finland, and in a new species (red deer). The data showed two main disease phenotypes, distinguished by the presence (Ly+) or absence (Ly-) of detectable disease-associated PrP in the lymphoreticular tissues, in addition to that seen in the central nervous system. So far, the Ly+ phenotype has been observed only in wild reindeer and the Ly- only in moose and red deer. It was considered that these differences in tissue distribution could affect the transmissibility of the disease under field conditions and they were therefore considered as separate entities for the purpose of this epidemiological analysis.

In general, the geographic distribution of CWD Ly- in affected countries is patchy, while the CWD Ly+ was clustered in distribution. In areas where CWD has been found, the observed prevalence was low (< 1%), although the prevalence in adult males above 2 years old was 1.5% in the first area where CWD Ly+ was detected in wild reindeer in Norway. The prevalence of CWD Ly+ increases with age and males were more likely to have the disease than females, while cases of CWD Ly- were mainly in old (> 10 years) females (only a single male). Initial published data revealed genetic variations in Norwegian reindeer with some *PRNP*-alleles more frequently present in CWD Ly+ cases.

In the future, it is proposed that a minimum sustained background surveillance effort with a dedicated infrastructure and a good system for obtaining samples and testing should be available in every country. This effort should be focused on the testing of samples from relevant cervid species in high-risk target groups (clinical/sick(showing abnormal behaviour, locomotor disturbances or otherwise poor health) (SUS), fallen/culled (individuals found dead or killed for health/age reasons) (FC), hunted/slaughtered but declared unfit for human consumption (HSNHC)), systematically or opportunistically acquired. Beyond the minimum surveillance described above, specific surveillance activities can be implemented depending on the objectives set by risk managers. Common features of the proposed surveillance activities for four possible identified objectives are specific surveillance design for countries with/without previously detected cases of CWD, collection and testing of both retropharyngeal lymph node and brainstem samples; testing animals over 2 years of age if possible; maximising the sensitivity by prioritising the sampling of cervids from 'high-risk' target groups within each selected area and management system; consider a sustained rolling time frame for accumulating surveillance data; divide the area/region/country into sampling units based on the epidemiological and management knowledge of cervid populations present; and, in areas where disease is still undetected, set design prevalence based on the findings of this report or on new epidemiological data when available.

The criteria proposed for assessing the probability of CWD presence rather than 'for considering an area non-infected with CWD' include the definition of the geographical area by setting spatial boundaries; the annual assessment of the risk of introduction of CWD into the area to inform the surveillance design; a minimum sustained background surveillance with a dedicated infrastructure and a good system for obtaining samples and testing as described in ToR3; training and engagement of stakeholders, and an 'output based' surveillance programme based on data-driven input parameters.

All positive cases should be genotyped. Sample sizes for negative samples, ranging between 76 and 145 at country and species level, have been proposed to detect and estimate the frequency of *PRNP* polymorphisms and to ascertain susceptibility association. Double strand sequencing the entire *PRNP* open reading frame should be undertaken for all selected samples. A centralised data collection system at EU level is required, allowing the collation and extraction of data for analysis, containing the complete coding sequence of the animal *PRNP* in standard format, and the metadata associated with each sampled animal.

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1. Introduction

1.1. Background and terms of reference as provided by the requestor

After the notification by Norway in April and May 2016 of the first cases of CWD detected in Europe, EFSA delivered on 2 December 2016, a scientific opinion on CWD in cervids in the EU and EEA.

The EFSA opinion provided recommendations for the implementation of a three-year surveillance programme for CWD in cervids in Estonia, Finland, Iceland, Latvia, Lithuania, Norway, Poland and Sweden.

The EFSA opinion highlighted that the objectives, as stated in the ToR of the mandate, were to: 'detect CWD and/or estimating the prevalence of CWD in Norway, Sweden, Finland, Iceland, Estonia, Latvia and Poland, which are the EU and EEA countries with reindeer and/or moose populations, depending on the level of prevalence which is wished to be detected'.

Based on this opinion, the Commission adopted Regulation (EU) 2017/1972[1] of 30 October 2017 amending Regulation (EC) No 999/2001[2] as regards a three-year (2018–2020) monitoring programme for CWD in cervids in Estonia, Finland, Latvia, Lithuania, Poland and Sweden.

Chapter A. Section III of Annex III to Regulation (EC) No 999/2001 now lays down the basic sampling design, the sampling method and the laboratory testing to be applied by concerned Member States. Further, Section III point A(2.5) provides that, in case of a positive finding of TSE in a cervid, the number of samples from cervids collected in the zone where the positive TSE case was found must be increased, based on an assessment carried out by the Member State concerned.

Chapter B. Section I point A(9) establishes the reporting requirements for the concerned Member States. The annual report for the years 2018, 2019 and 2020 of these Member States must include:

The number of cervid samples submitted for testing, by target group according to the following criteria: — primary sampling unit (PSU) identifier, — species, — management system: farmed, captive, wild or semi-domesticated, — target group, — sex,

The results of the rapid and confirmatory tests (number of positives and negatives) and, where applicable, of further isolate characterisation investigations, the tissue sampled and the rapid test and confirmatory technique used.

The geographical location, including the country of origin if not the same as the reporting Member State, of positive cases of TSE.

The genotype and species of each cervid found positive for TSE.

Where tested, the genotype of cervids tested and found negative for TSE.

The three-year monitoring programme laid down by Regulation (EU) 2017/1972 ended on 31 December 2020, but Sweden extended its intensified monitoring until February 2022.

During the three-year (2018–2020) monitoring programme for CWD in cervids applied in the EU, Iceland and Norway have also implemented a monitoring programme for CWD in their own cervids population.

Data collected by the concerned Member States, by Norway, and by Iceland, have been submitted to the EFSA database following the above reporting requirements. Additional data for tested animals and cases, or information of the implementation of the monitoring programme, may be held at country level.

The disease is unlikely to disappear from Europe and it is important to draw from the data generated by the monitoring programme, a clearer understanding of the situation and epidemiology of the disease and learn from that experience to advise on the possible pursuit of a monitoring programme for CWD.

In addition, Point 3.3 of Section III.A of Chapter A of Annex III of Regulation (EC) No 999/2001 provides the following:

'The prion protein genotype shall be determined for each positive finding of TSE in cervids. In addition, for each cervid tested and found negative for TSE, either:

[1] Commission Regulation (EU) 2017/1972 of 30 October 2017 amending Annexes I and III to Regulation (EC) No 999/2001 of the European Parliament and of the Council as regards a surveillance programme for chronic wasting disease in cervids in Estonia, Finland, Latvia, Lithuania, Poland and Sweden and repealing Commission Decision 2007/182/EC.

[2] Regulation (EC) No 999/2001 of the European Parliament and of the Council of 22 May 2001 laying down rules for the prevention, control and eradication of certain transmissible spongiform encephalopathies.

the prion protein genotype of the animal tested and found negative for TSE is determined, or

a sample of a tissue, which may be the obex, shall be kept frozen until at least 31 December 2021, to allow for genotyping if so decided.'

On 15 December 2021, the Commission asked the concerned countries to not destroy the negative samples, which are kept frozen and have not been genotyped, as from 1 January 2022, but to keep them until a genotyping protocol for these samples has been defined.

It is important to rapidly provide, in consultation with the EU Reference Laboratory (EURL) for TSEs, the Member States with an appropriate genotyping protocol for positive and negative samples, in order to decide on the fate of these samples.

1.1.1. Terms of reference

EFSA is requested to provide a scientific opinion on the monitoring of CWD, based on the results of the above-mentioned monitoring programme including the statutory data available in the EFSA database, and any other monitoring data collected with the same epidemiological objective and having become available since the publication of previous EFSA opinions on CWD.

More specifically, EFSA is asked:

1) To analyse the results of the monitoring programme carried out in Norway, Sweden, Finland, Iceland, Estonia, Latvia, Lithuania and Poland between 1 September 2017 and 28 February 2022, and in particular, to assess if the two objectives[3] as set in the 2016 EFSA opinion on CWD in cervids have been met.
2) To describe any new knowledge on the epidemiology of CWD in EU/EEA countries.
3) To recommend, if considered appropriate, future CWD monitoring activities for the EU based on an assessment of the epidemiological situation.
4) Based on what is known about the epidemiology of CWD in EU/EEA countries, to describe the criteria relevant for considering an area not to be infected with CWD.
5) To provide the design of a genotyping protocol for positive samples, and for the negative samples of the 3-year monitoring programme stored as per point 3.3, section III.A of Annex III of Regulation (EC) No 999/2001, specifying which negative samples should be genotyped, the codons of the PRNP gene to be genotyped and recommending genotyping assay/s for the implementation of the requirement by the NRLs.

1.2. Interpretation of the terms of reference

1.2.1. Recent evidence that CWD in Europe is heterogeneous

When the 2017 opinion (EFSA BIOHAZ Panel, 2017) was published and Commission Regulation (EU) 2017/1972 was enforced, different phenotypes of transmissible spongiform encephalopathy (TSE) had not been identified in European cervids yet. The majority of cases were described in Norwegian reindeer and had disease-associated prion protein (PrP^{Sc}) deposits in lymphoid tissues and sometimes brainstem, resembling the patterns described in North American cervid species with CWD (for review see EFSA BIOHAZ Panel, 2018, 2019). Preliminary observations also suggested epidemiological features – e.g. multiple cases within affected population, infection prevalence higher in males and lower in calves and yearlings – resembling those reported from North American outbreaks (EFSA BIOHAZ Panel, 2018, 2019; Mysterud et al., 2019a). Two unrelated cases in moose had also been identified, with lesions restricted to central nervous system (CNS) and no detectable involvement of the lymphoreticular tissues.

Based on assumed similarities between the majority of cases and North American CWD cases, the 2017 opinion recommendations and subsequent regulations were developed by extrapolation from the considerable prior data and experience in North America, e.g. in the minimum detectable prevalence estimates used to determine target sample sizes for detection within a sampling unit (EFSA BIOHAZ Panel, 2017, Section 3.1 and Table 11).

However, the data continuing to emerge from *in vitro* and *in vivo* laboratory characterisations of the first detected cases in reindeer, and of the subsequently identified cases in European moose and red deer, indicate that at least five variations of cervid TSE are present in Europe, and that none of them

[3] 'to confirm or exclude the presence of CWD in countries where the disease has never been detected and (2) to estimate the prevalence and geographical spread of CWD in countries where CWD has been detected'.

appears to be a direct extension of North American CWD (reviewed by Tranulis et al., 2021). Specific isolate classification is outside the scope of this opinion, but it is clear that two main pathological phenotypes of disease can be distinguished in European cervids based on the presence or absence of detectable PrP^{Sc} in the lymphoreticular system.

It has been well established in other animal TSE (including CWD in North American deer) that abundant lymphoreticular involvement is associated with a greater likelihood of natural transmission under field conditions (EFSA BIOHAZ Panel, 2019), and it is therefore important to acknowledge this distinction when discussing disease epidemiology. For the purpose of this opinion, analysis will be subdivided, based on these two described phenotypes of CWD:

- phenotype 'Ly+' denotes case presentations with detectable PrP^{Sc} accumulations in lymphoid tissues (with or without deposits in brain tissue), and
- phenotype 'Ly-' denotes cases with detectable PrP^{Sc} accumulation in brain tissues, but not in lymphoid tissues.

Across CWD cases in multiple cervid species in North America and Asia, only the Ly+ phenotype has been recognised thus far. At present, CWD phenotype Ly+ has only been detected in reindeer in Europe. All CWD cases in European moose and red deer have, so far, been phenotype Ly-. Implications of these differences will be highlighted throughout this assessment.

1.2.2. Interpretation of 'geographical spread' in the context of ToR 1

ToR1 requests an assessment of whether the objective of estimating the 'geographical spread' of CWD in affected countries has been met by the monitoring programme. For the purposes of the current opinion, we interpret 'spread' as equating to 'geographical distribution' because the timeframe for sampling was too short to estimate *changes* in distribution (also considered as 'spread'). The expansion of the observed geographical distribution of CWD in Europe during the reference period is only a product of the increased surveillance efforts detecting new cases and disease foci in participating countries.

1.2.3. Definition of non-infected area

Some characteristics of animal prion diseases – particularly their low and slowly increasing prevalence and incidence, long pre-clinical incubation periods and long disease course, lack of practical ante-mortem screening tests, and multiple phenotypes and strains – make it difficult if not impossible to ascertain or declare areas as non-infected. Therefore, the criteria listed in Section 3.4 are considered relevant to ToR4, but not sufficient to rule out the presence of CWD. They could be useful 'in assessing the probability of CWD presence' rather than 'for considering an area non-infected with CWD.' As recommended in Section 3.4, these assessments must be supported by continuous collection and analysis of surveillance data, and the annual assessment of the risk of CWD introduction into the area of interest.

1.2.4. Surveillance vs. monitoring

Several alternative definitions have been proposed in the field of animal health to distinguish between the terms 'surveillance' and 'monitoring' (Doherr and Audigé, 2001; Salman, 2008). In the final report of a workshop of the International Conference on Animal Health Surveillance (ICAHS) in 2011 (Hoinville et al., 2013), a panel of experts agreed on the following definitions for surveillance: 'the systematic, continuous or repeated, measurement, collection, collation, analysis, interpretation and timely dissemination of animal health and welfare related data from defined populations. These data are then used to describe health hazard occurrence and to contribute to the planning, implementation, and evaluation of risk mitigation actions'. Monitoring was defined as 'the systematic, continuous or repeated, measurement, collection, collation, analysis and interpretation of animal health and welfare related data in defined populations when these activities are not associated with a pre-defined risk mitigation plan although extreme changes are likely to lead to action'. Based on its more general and operative definition, the term surveillance (instead of monitoring) has been used throughout this Opinion preferred and used instead of the term monitoring.

1.2.5. Timeline

The analyses reported in this opinion refer to two time periods with different starting and ending dates. The following terms distinguishing between these two periods will be used throughout the text:

Mandate period: 1 September 2017–28 February 2022. This is the entire period to be considered in responses to the terms of reference and includes voluntary surveillance activities undertaken before and after the regulation period (defined immediately below).

Regulation period: 1 January 2018–31 December 2020. This is the period when surveillance requirements were enforced in selected countries.

Time periods also are considered and referenced as appropriate. The overall timeline of surveillance activities undertaken by the eight countries included in the mandate is shown in Figure 1.

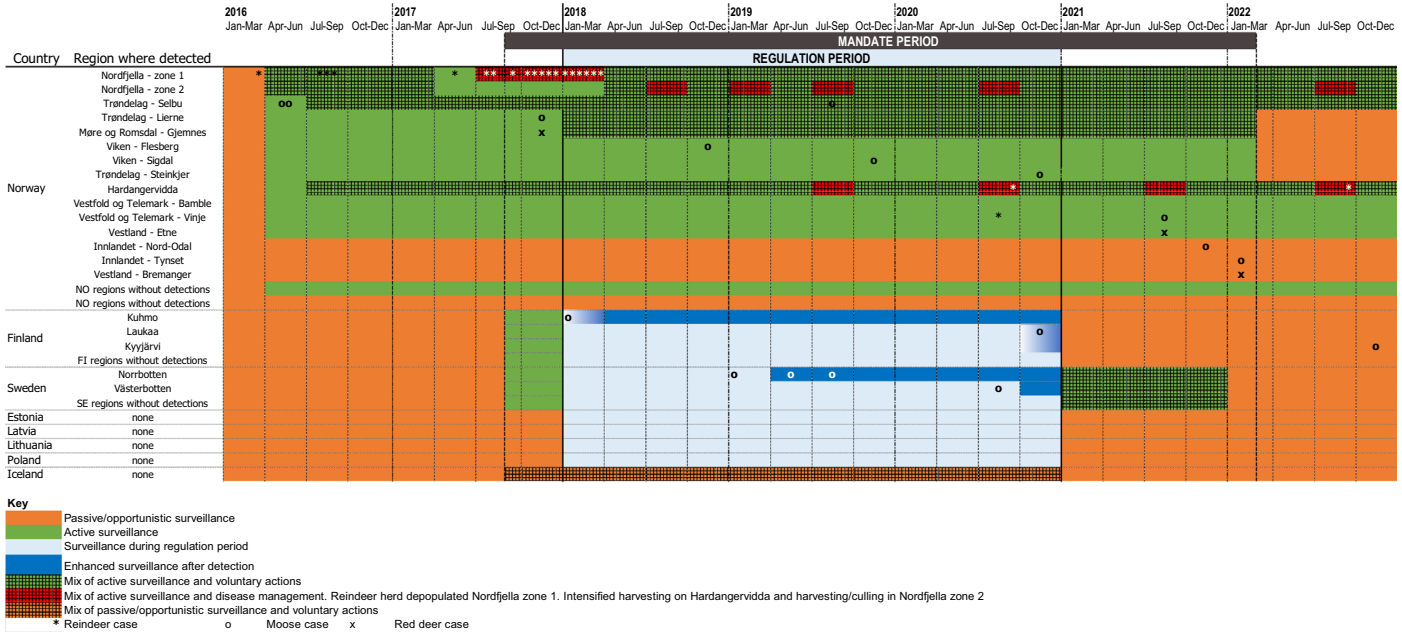

Figure 1: Overall timeline of surveillance activities undertaken by the eight countries included in the mandate. For the three countries that have detected chronic wasting disease cases, the timing of surveillance and related activities before and after case detection(s) within each affected region also is included to highlight similarities and differences. Sections 3.1.2 and 3.1.3 provide more comprehensive descriptions of surveillance activities undertaken by each participating country.

2. Data and methodologies

2.1. Data

2.1.1. EFSA data

According to point 9 Section 1.A, chapter B Annex III of Regulation (EU) 2011/999, for Member States covered by the 3-year CWD monitoring programme referred to in Part III.A of Chapter A of this Annex, the annual report for the years 2018, 2019 and 2020 shall include: (a) The number of cervid samples submitted for testing, by target group according to the following criteria: — primary Sampling Unit (PSU) identifier; species; management system: farmed, captive, wild or semi-domesticated; target group; sex. (b) The results of the rapid and confirmatory tests (number of positives and negatives) and, where applicable, of further isolate characterisation investigations, the tissue sampled and the rapid test and confirmatory technique used. (c) The geographical location, including the country of origin if not the same as the reporting Member State, of positive cases of TSE. (d) The genotype and species of each cervid found positive for TSE. (e) Where tested, the genotype of cervids tested and found negative for TSE.

According to the Commission Regulation (EU) 2017/1972 – amending Annexes I and III of the TSE regulation – MS which have a wild and/or farmed and/or semi-domesticated population of moose and/or reindeer (Estonia, Finland, Latvia, Lithuania, Poland and Sweden) shall carry out a 3-year monitoring programme for chronic wasting disease (CWD) in cervids, from 1 January 2018 to 31 December 2020. 'The collection of samples for the monitoring programme may, however, start in 2017'. Surveillance conducted in cervids as collected by EFSA and published in the TSE EU summary report since the confirmation of the first case of CWD in Norway, i.e. in the 2107 report (EFSA, 2018).

Data were extracted from the EFSA data warehouse and compiled in two data sets for analysis: aggregated data of cervids tested, and individual data for each CWD case. The surveillance data set of tested cervids contained the following fields: country, MS/non-MS, year, species, country PSU, management system, target group, age, gender, total number of animals tested. The data set of individual cases included the following fields: national case number, country, year, month, species, target group, country PSU, region, sex, age group, sample type, test type, analytical method and result.

2.1.2. Finland additional data

The Finnish Food Authority (Kuukka-Anttila, 2022) submitted additional information to EFSA, specifically explaining the design of the general and intensified surveillance in areas where positive cases were detected, thus enabling interpretation of the data already submitted to EFSA and the allocation of samples to either general or intensified surveillance. Maps were also provided showing how the intensified surveillance was geographically targeted.

In 2018, 100 PSU were selected according to the EU Regulation and included all the 54 reindeer herding associations, 45 game management associations and Åland Islands that is an autonomous part of Finland and has its own game management system. However, since there were not many samples received in 2018, all the 282 game management associations were included in the surveillance for the period 2019–2021.

2.1.3. Sweden additional data

The Swedish Board of Agriculture provided three documents to EFSA via the European Commission explaining the design of the intensified surveillance in areas where positive cases were detected and thus enabling interpretation of the data already submitted to EFSA and the allocation of samples to either general or intensified surveillance:

- Surveillance of chronic wasting disease in Sweden. Summary report explaining sampling strategies and intensified surveillance. Dated: 8 February 2022.
- Final information concerning CWD surveillance in Sweden dates on 31 March 2022.
- Appendix 1: The maps of wild cervid PSU, Sami village PSU and farmed red deer PSU. It also contained the list of Sami village PSU, including those in the intensified surveillance in Norrbotten. Dated: 31 March 2022.

2.1.4. Norway data

A continuously updated summary table of the surveillance results for CWD, established by the Norwegian Veterinary Institute, allows the public to obtain daily updated statistics on the number of tested animals depending on the year, cervid species, management system (farmed or wild), target groups (hunting or other) and geographic area.[4] The data are based on number of samples received and tested at the Norwegian Veterinary Institute (the only lab testing samples for CWD in Norway). The system is based on hunters or other persons submitting samples along with harvest code/or other ID number for culled animals and all additional information about the animal and the geographical management area, directly in the relevant web register ('hjorteviltregisteret', 'fallviltregister') or on the label following the sample. Missing information will be imputed and corrected from other target groups later, if available. Sometimes, parts of the data are cleaned and corrected after the data submission to EFSA. As a consequence, data submitted to EFSA and other data sets, extracted at later time points, do not match exactly the numbers for the same time period.

Information on the target group was missing for a high proportion of tested cervids from Norway. For example, looking at the samples of wild cervids for the mandate period, information on the target group was missing for 27% of the data (about 17% overall, if including semi-domesticated reindeer and farmed cervids). However, when data were submitted to EFSA, there was no possibility to use 'unknown' for target group. At that point, missing data were imputed with the most likely target group (missing information imputed in approximately the same proportions as the data with known target group) (Heier, 2023).

The most accurate information on target group for the positive CWD cases from Norway is found in the data available at the Norwegian Veterinary Institute website[5] (in Norwegian only). When retrospectively comparing the data in the EFSA database with the more certain data source for the positive CWD cases detected in Norway, we had to correct the target group for five (two reindeer and three moose) of the positive cases submitted to EFSA. Similar correction could not be done for negative samples.

The data set submitted from Norway to EFSA was pooled, and not specified at PSU level. Spatial information is based on two sources in this report: (1) the dynamic summary table (mentioned above) and (2) an additional data set for the mandate period (not including the test result) and extracted from the NVI database on 1.5.2022. This data set was used for the analyses of 'Estimation of the probability of detection' (Section 3.1.7). Overall, the number of tested cervids is similar to that reported in the data submitted to EFSA, but with minor differences.

2.2. Methodologies

2.2.1. Protocol

The protocol describing the methodologies applied, the approaches and evidence needs is available in Annex A. The Terms of Reference have been translated into a set of assessment questions (AQ) and subassessment questions (SAQ), which are addressed in the subsections of Section 3.

AQ 1.1 How was the surveillance conducted by the eight countries during the mandate period?

> SAQ 1.1.1 What surveillance data were reported to EFSA by each of the eight countries during the mandate period?
> SAQ 1.1.2 What approach(es) did each country apply to surveillance during the mandate period? What, if any, were the differences in monitoring activities between the countries?
> SAQ 1.1.3 To what extent has each of the six member states achieved the targets proposed in the 2017 EFSA opinion and in the subsequent requirements of (Reg. (EC). 2001/999? Did the other two non-MS achieve similar targets?

AQ 1.2 What are the outcomes of the surveillance conducted by the eight countries?

AQ 1.3 What do the surveillance data tell us about the achieved ability to detect or to estimate prevalence of CWD in the eight countries?

> SAQ 1.3.1 What is the minimum detectable prevalence at country/PSU/species levels using the surveillance data collected by the eight countries?

[4] https://apps.vetinst.no/skrantesykestatistikk/NO/#omrade
[5] https://apps.vetinst.no/skrantesykestatistikk/NO/#kasus

SAQ 1.3.2 What is the probability of detecting CWD (sensitivity of the surveillance system) at the design prevalence set out in the 2017 EFSA opinion using the surveillance data reported by the eight countries?

AQ 2.1 Since the last EFSA opinion (2019), what is the new evidence available about CWD in Europe?

AQ 3.1 What practical difficulties were encountered (e.g. logistics, training) in the delivery of the current surveillance programmes, and what have been the successful practices, that would influence the design of future surveillance activities?

AQ 3.2 What are the different potential objectives of future surveillance (e.g. cervid health and welfare, zoonotic potential, spill-over)?

AQ 3.3 Based on the results of AQ3.2, how would different surveillance objectives drive different monitoring strategies, and what would be the appropriate monitoring activities for each potential objective?

AQ 4.1 What are the criteria that would be needed to define an area as not infected with CWD?

AQ 4.2 What are the criteria that would contribute to the discrimination between an infected/non-infected area considering disease phenotypes and confirmation of cases?

AQ 5.1 What are the different objectives that should be pursued by the genotyping?

SAQ 5.1.1 For each objective, which is the appropriate subsampling strategy (target and sample size)?

SAQ 5.1.2 In the frame of CWD, what protocols/strategies for genotyping are currently available?

SAQ 5.1.3 How should the genotype data be collected in a harmonised centralised data system?

2.2.2. Descriptive analysis

Descriptive analysis of the surveillance data sets was conducted by reviewing and presenting in tabular and narrative format: the total number of animals tested by country, year or species with or without restricting to age-class and target group; the total number of cases by country, year, species and target group. Prevalence rates of CWD Ly+ and Ly-, defined as the number of cases per number of tested animals, were estimated with 95% confidence intervals (95% CI) using the exact binomial method. Crude prevalence rates and target group-specific rates were calculated both at species and sub-country level, country level or combining all the available national data.

2.2.3. Target groups reflecting different disease probability ('risk')

Samples collected in the course of CWD surveillance came from a variety of target groups ranging from apparently healthy animals to those showing signs consistent with end-stage clinical disease. Within infected cervid herds, individuals from different risk groups (e.g. 'healthy' vs 'sick') are known to have different inherent likelihood of harbouring detectable prion infection (Miller et al., 2000; Walsh and Miller, 2010; Walsh, 2012; EFSA BIOHAZ Panel, 2017; Jennelle et al., 2018).

According to the EU TSE Regulation, cervids ≥ 12 months of age must be selected from the following target groups:

- fallen/culled (individuals found dead or killed for health/age reasons) (FC),
- road- or predator-injured or killed (hit by road vehicles, by trains or attacked by predators) (RK),
- clinical/sick (observed as showing abnormal behaviour, locomotor disturbances or otherwise poor health) (SUS),
- hunted/slaughtered but declared unfit for human consumption (HSNHC) or
- hunted/slaughtered and considered fit for human consumption (i.e. apparently healthy) (HSHC).

An 'unknown' target group was not allowed in the EFSA data collection system.

For the purposes of some analyses supporting this opinion, FC, RK, SUS and HSNHC have been grouped together as 'high risk': this is to recognise that the probability of having the disease if CWD is present in the sampled population is higher in these groups than in HSHC (they were collectively termed as 'target groups' in the 2017 opinion [EFSA BIOHAZ Panel, 2017]). HSHC were analysed separately as 'low risk'.

2.2.4. Estimating the minimum detectable prevalence

The minimum detectable prevalence is a hypothetical prevalence of disease specified at the herd (herd design prevalence) or at the animal population level against which to measure surveillance sensitivity and interpret negative findings. The definition is based on the concept that if a particular pathogen is present, it is present in more than a specified proportion of the population (design prevalence) at a given level of statistical confidence (surveillance sensitivity) (FAO, 2014). So, if the minimum detectable prevalence is, for example, 10%, it means that by testing an appropriate number of animals there is a 95% probability (which corresponds to the surveillance sensitivity) of finding at least one case if 10% or more of the population is infected. If no cases are detected, then there is a 95% probability that the disease in the population, if present, is below a certain prevalence (here 10%).

Data have been used to calculate the minimum detectable prevalence at country/species/PSU levels (assuming a target 95% surveillance sensitivity), using the number of animals older than 12 months of age tested during the mandate period, the total for the calculation at country and species level, and those tested in the risk groups at PSU level. The analysis was performed using the package RSurveillance[6] in R.[7] The minimum detectable prevalence values obtained by these calculations contributed to the overall interpretation of the individual national surveillance approaches and their ability to detect the disease.

2.2.5. Estimating surveillance system sensitivity

To facilitate the reading of this Opinion, the scenario tree model used to estimate the surveillance system sensitivity is described together with its outputs in Section 3.1.7.1.

3. Assessment/results

The EFSA opinion on chronic wasting disease in cervids (EFSA BIOHAZ panel, 2017) proposed a surveillance system for CWD, following the mandate of the European Commission of May 2016 in which EFSA was requested *'to provide recommendations on surveillance of the cervid populations at the country level aimed at detecting CWD and/or estimating the prevalence of CWD in Norway, Sweden, Finland, Iceland, Estonia, Latvia and Poland, which are the EU and EEA countries with reindeer and/or moose populations, depending on the level of prevalence which is wished to be detected'* (Terms of Reference 1). The proposal consisted of a 3-year surveillance programme based on a two-stage sampling.

These proposals were incorporated into Commission Regulation (EU) 2017/1972, amending Annexes I and III of the TSE Regulation, requiring that *'MS which have a wild and/or farmed and/or semi-domesticated population of moose and/or reindeer (Estonia, Finland, Latvia, Lithuania, Poland and Sweden) shall carry out a 3-year monitoring programme for CWD in cervids, from 1 January 2018 to 31 December 2020, although 'the collection of samples for the monitoring programme may, however, start in 2017' (point 1,1)'*. The other MS may carry out monitoring for CWD in cervids on a voluntary basis. The 3-year monitoring programme for CWD in cervids is described in detail in Annex III, chapter A, Part III of the TSE Regulation.

As the EFSA proposal and the Regulation provisions differed in some regards, Table 6 is used to provide a summary and comparison of the CWD surveillance recommendations, requirements and overall outcomes reported by the relevant countries in Europe during the mandate period. Full transcripts of the surveillance proposal as in the EFSA opinion (EFSA BIOHAZ Panel, 2017) and of the surveillance requirements as in Annex III of Regulation (EC) No 999/2001 are displayed in Appendix A.

3.1. Analysis of surveillance data (ToR1)

3.1.1. Approaches applied by the countries during the mandate period (SAQ1.1.2)

The surveillance data reported to EFSA during the mandate period reveals very distinctive patterns in terms of time periods and country (see Figure 1). Before the statutory surveillance was enforced in

[6] https://www.fp7-risksur.eu/sites/default/files/documents/Deliverables/RISKSUR_%28310806%29_D6.24.pdf
[7] R Core Team, 2022. R: A language and environment for statistical ## computing. R Foundation for Statistical Computing, Vienna, Austria. Available online: https://www.R-project.org/

2018, only Norway was implementing a robust monitoring programme of testing multiple species, following the confirmation of cases in three species during 2016–2017, with the major emphasis on the testing of the wild reindeer population in the Nordfjella area. The rest of the mandate countries did not have systematic/specific surveillance programmes for TSE in cervids or had specific but limited programmes (like the testing of white-tailed deer in Finland), resulting in a very limited number of cervids tested per year and per country before 2018. In 2015, Finland tested 14 cervids. In 2016, Finland tested 56 cervids, Estonia 1 and Sweden 70. In 2017, Finland tested 114 cervids, Latvia 2 and Sweden 176 cervids (EFSA, 2016, 2017, 2018).

With the implementation of Regulation (EU) 2017/1972 and the start of the 3-year (2018–2020) CWD monitoring programme, the Member States concerned (Estonia, Finland, Latvia, Lithuania, Poland and Sweden) set up national programmes. As required by the Regulation, each MS preliminarily identified the PSU within which the CWD monitoring programme would take place. The criteria used by each MS to identify their PSU were different, and were described in the 2018 EU summary report on TSE (EFSA, 2019) as follows:

- Estonia: 15 PSU for both wild/semi-domesticated and captive/farmed cervids, corresponding to each of the Estonian counties.
- Finland: 54 PSU for semi-domesticated reindeer, based on administrative units of the reindeer herding cooperative (RHC); 295 PSU for wild cervids based on the local game administration units according to the game management association (GMA).
- Lithuania: each farm and each facility in which cervids are kept in an enclosed territory were considered as a PSU, approximately 655, based on permissions issued by the Ministry of the Environment (list not available); 51 PSU for wild cervids based on State Food and Veterinary Service (SFVS) territorial units.
- Latvia: 100 PSU based on territorial units for the determination of the number of animals continuous areas with defined natural boundaries, which included one or more hunting districts as undivided as possible, and which, according to the total hunting area, were not less than 5,000 ha for moose and red deer and 500 ha for roe deer.
- Poland: 16 PSU for both wild/semi-domesticated and captive/farmed cervids based on voivodeships (administrative units).
- Sweden: 109 PSU for farmed cervids (one for each farm), 51 PSU for semi-domesticated reindeer corresponding to the 51 Sameby (administrative units for reindeer herding) and 50 PSU for wild cervids, covering the whole country. The PSU of different management systems geographically overlap for reindeer.

Information on the PSU designation approach in Norway (see above Section 2.1.4) and Iceland was not submitted to the EFSA database because they are not MS. Details for the surveillance approach used by Iceland are not available.

3.1.2. Description of the surveillance data reported to EFSA (SAQ 1.1.1)

The reporting system was not set up to enable the separation of data from 'general' surveillance and 'intensified surveillance'. However, if the intensified surveillance (See Sections 3.1.3) was conducted using PSU as reported to EFSA, the data can be analysed independently for these two activities, before and after the confirmation of a case.

The eight countries tested a total of 155,660 cervids older than 12 months of age and of unknown age during the mandate period September 2017–February 2023, distributed as follows: Estonia (2,153), Finland (3,457), Iceland (304), Latvia (3,087), Lithuania (3,784), Norway (130,836), Poland (3,505) and Sweden (8,534). The hunted and slaughtered fit for human consumption (HSHC) group was the most frequently tested overall in terms of number of animals (83.8%) in six of the eight countries, with road kills (RK) being the most tested high-risk target group in Finland and Poland. Roe deer was the most tested species in four MS (Estonia, Latvia, Lithuania and Poland).

The number of cervids tested older than 12 months of age (or of unknown age) is presented by country and year as total tested (Table 1) or in the high-risk target groups (Table 2) or by country and species as total tested (Table 3) or in the high-risk target groups (Table 4). The species distribution of cervids > 12 months of age and of unknown age, tested during the mandate period is also displayed in Figure 2. The numbers of cervids > 12 months of age and of unknown age tested during the mandate period by target groups (HSHC and high-risk groups -FC, HSNHC, RK and SUS) are displayed Table 4.

Table 1: Number of cervids older than 12 months of age and of unknown age tested by country and year for the period September 2017 to February 2022 with positive cases in brackets

Country	2017	2018	2019	2020	2021	2022	Grand total
Estonia	0	204	505	1,436	7	1	2,153
Finland	66	657 (1)	1,115	1,211 (1)	398	10	3,457 (2)
Iceland	54	100	114	33	3	0	304
Latvia	1	1,042	1,075	964	5	0	3,087
Lithuania	0	1,835	1,105	844	0	0	3,784
Norway	21,508 (8)	32,570 (7)	30,061 (2)	22,416 (2)	21,551 (3)	2,730 (3)	130,836(25)
Poland	0	1,141	1,246	1,118	0	0	3,505
Sweden	107	200	2,928 (3)	1,398 (1)	3,316	585	8,534 (4)
Grand Total	21,736 (8)	37,749 (8)	38,149 (5)	29,420 (4)	25,280 (3)	3,326 (3)	155,660 (31)

Table 2: Number of high-risk (all except HSHC) cervids older than 12 months of age and of unknown age tested by country and year for the period September 2017 to February 2022

Country	2017	2018	2019	2020	2021	2022	Grand total
Estonia	0	118	192	308	6	0	624
Finland	60	560	1,081	1,086	373	10	3,170
Iceland	1	5	0	1	3	0	10
Latvia	1	59	75	77	5	0	217
Lithuania	0	278	313	202	0	0	793
Norway	1,251	4,325	3,119	2,820	3,458	491	15,464
Poland	0	862	1,073	1,018	0	0	2,953
Sweden	107	190	412	323	765	85	1,882
Grand total	1,420	6,397	6,265	5,835	4,610	586	25,113

Table 3: Number of cervids older than 12 months of age and of unknown age tested by country and species for the mandate period (September 2017 to February 2022) with positive cases (integers) in brackets. The proportions (%) in brackets represent the percentage of the total number of cervids tested that were 12 months of age or older

Country	Deer	Moose	Fallow deer	Red deer	Reindeer	Roe deer	Sika deer	White-tailed deer	Grand total
Estonia	5	525	0	271	0	1,352	0	0	2,153 (99.2%)
Finland	1	728 (2)	2	1	1,722	631	0	372	3,457 (99.8%) (2)
Iceland	3	0	0	0	301	0	0	0	304 (100%)
Latvia	0	654	1	923	0	1,509	0	0	3,087 (99.5%)
Lithuania	0	236	3	1,016	0	2,526	2	1	3,784 (100%)
Norway	1,949	28,233 (9)	216	27,129 (3)	64,836(13)	8,473	0	0	130,836 (99.3%) (25)
Poland	0	192	0	651	0	2,662	0	0	3,505 (100%)
Sweden	0	1,780 (4)	16	493	6,018	227	0	0	8,534 (100%) (4)
Grand total	1,958	32,348 (15)	238	30,484 (3)	72,877 (13)	17,380	2	373	155,660 (99.4%) (31)

Table 4: Number of high-risk (all except HSHC) cervids older than 12 months of age and of unknown age tested by country and species for the mandate period (Sep 2017–Feb 2022)

Country	Deer	Moose	Fallow deer	Red deer	Reindeer	Roe deer	Sika deer	White-tailed deer	Grand total
Estonia	5	47	0	18	0	554	0	0	624
Finland	1	517	2	1	1688	601	0	360	3,170
Iceland	3	0	0	0	7	0	0	0	10
Latvia	0	30	1	48	0	138	0	0	217
Lithuania	0	47	0	145	0	598	2	1	793
Norway	499	3,611	3	2,566	1,572	7,213	0	0	15,464
Poland	0	191	0	311	0	2,451	0	0	2,953
Sweden	0	715	16	96	833	222	0	0	1,882
Grand total	508	5,158	22	3,185	4,100	11,777	2	361	25,113

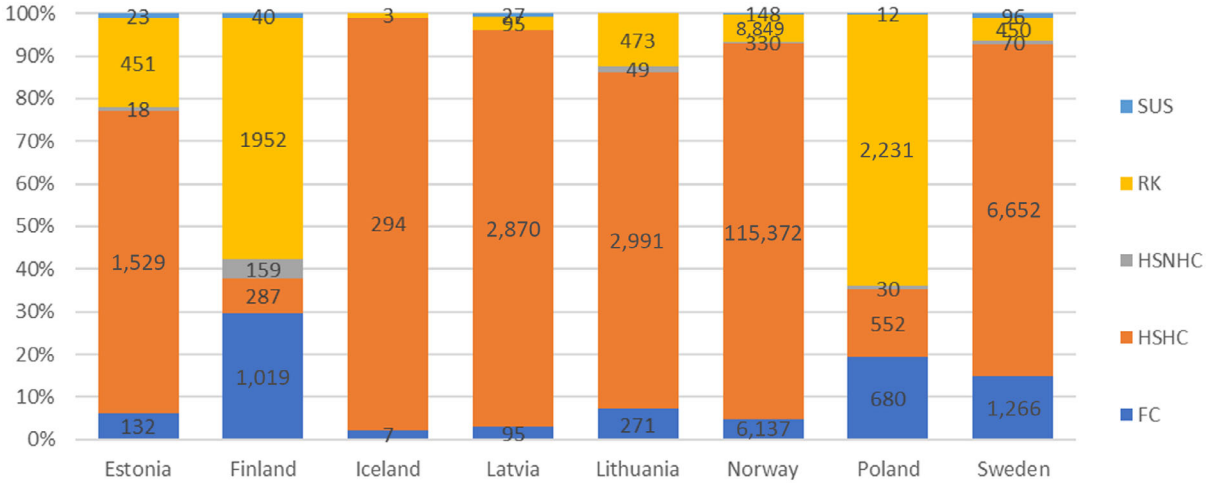

Figure 2: Numbers of cervids older than 12 months of age and of unknown age tested during the mandate period by target groups (HSHC and risk groups FC, HSNHC, RK and SUS). Norwegian data with unknown target group (ca. 17%) were distributed according to distribution in samples with known target group when submitted to EFSA

The following sections provide a description of the surveillance (by period) carried out by the six MS, before describing the surveillance carried out by Norway (Section 3.1.2.4) and Iceland (Section 3.1.2.5), respectively, during the overall mandate period.

3.1.2.1. Period 1 September 2017 to 31 December 2017, MS

In the last quarter of 2017, surveillance efforts were voluntarily intensified as part of the forthcoming regulation surveillance in 2018, with, Sweden testing 107 of the 176 cervids tested during the entire year, and Finland with 66 cervids tested out of 114.

3.1.2.2. Period 1 January 2018–31 December 2020 (regulation period)

The 3-year regulation period of surveillance brought the tally of tested animals from the six concerned member states to a total of 20,024 cervids older than 12 months of age (or of unknown age): 2,145 from Estonia, 2,983 from Finland, 3,081 from Latvia, 3,784 from Lithuania, 3,505 from Poland and 4,526 from Sweden. Of them, 14,921 (74.5%) were wild cervids, 4,480 (22.4%) were semi-domesticated and 501 (3.1%) were farmed cervids.

The most tested species was roe deer, with 8,730 (43.6%) samples, followed by reindeer with 4,524 (22.6%), moose with 3,461 (17.3%), red deer with 2,987 (14.9%), white-tailed deer with 306 (1.5%), fallow deer with 13 (0.06%), two sika deer and one deer for which the species was unidentified.

Estonia

Estonia tested 2,163 cervids during the regulation period, 2,145 of which were older than 12 months of age (or of unknown age) (99.2%). Of those adult cervids, 1,351 (63%) were roe deer, 522 were moose (24.3%) and 271 were red deer (12.6%). All except 2 were wild cervids. A total of 1,527 cervids older than 12 months of age (71.2%) were tested under the group Hunted/slaughtered fit for human consumption (HSHC): 52.2% roe deer, 31.2% moose and 16.6% red deer.

In terms of PSU, Estonia declared 15 PSU for both wild/semi-domesticated and captive/farmed cervids, corresponding to each of the Estonian counties. Cervids were tested in 13 of them (86.6%), with a median number of 89 cervids older than 12 months of age or of unknown age tested (range: 1–467) from each county.

Finland

Finland tested 2,990 cervids during the regulation period, 2,983 of them older than 12 months of age (or of unknown age). Of those adult cervids, 1,553 were reindeer (52.1%) of which 97.6% were semi-domesticated; 599 were moose (20.1%, all but five reported in the wild group), 525 were roe deer (17.6%, all but five reported in the wild group), 305 were white-tailed deer (10.2%, all but 10 reported in the wild group) and one was a wild red deer. Only 8.6% of all tested cervids were in the group HSHC, the greatest proportion of which were moose (78.1%), followed by reindeer (12.1%), roe deer (6.6%) and white-tailed deer (3.1%). The rest of the animals tested, 2,727 (91.4%) were from the risk target groups, with road kills being the largest contributor (63.8%).

Finland declared 54 PSU for semi-domesticated reindeer, based on administrative units of the reindeer herding cooperative (RHC), and 295 PSU for wild cervids based on the local game administration units according to the game management association (GMA). Although 54 PSU were initially declared, samples were reportedly collected from 59 PSU for semi-domesticated reindeer (109.2%), with a median number of 30 cervids tested (range: 1–58). For wild cervids, Finland tested animals in 193 of the 295 declared PSU (65.4%), with a median number of two cervids tested (range: 1–149). There are no farms producing meat from CWD susceptible cervid species in Finland, and just three zoos and very few game farms that keep susceptible cervid species to be released into the wild. As a result, Finland also reported tested animals in six PSU for farmed/captive cervids (median = 1, range: 1–13).

Latvia

Latvia tested 3,096 cervids during the regulation period, 3,081 (99.5%) of them older than 12 months of age or of unknown age. All but 146 of these cervids (145 red deer and one fallow deer) were wild. A total of 2,870 (93.1%) were cervids tested in the HSHC group, and 211 (6.9%) in the risk groups.

Based on territorial units for the determination of the number of animals, Latvia reported 100 PSU – continuous areas with defined natural boundaries – which include one or more hunting districts as undivided as possible, and which, according to the total hunting area, amount to 5,000 ha for moose and red deer and 500 ha for roe deer. Latvia reported tested farmed cervids in 12 PSU (median = 3, range: 1–14), and in 240 PSU wild cervids (median = 8; range: 1–43).

Lithuania

Lithuania tested 3,784 cervids during the regulation period, all of them older than 12 months of age (or of unknown age). Of these, 2,526 were roe deer (66.7%), 1,016 were red deer (26.8%), 236 were moose (6.2%) and three were fallow deer, two were sika deer and one was a white-tailed deer. Only 86 (2.2%) of the total throughput were farmed cervids, with the rest being wild cervids (3,698, 97.8%). The majority (2,991, 79%) of the cervids tested were reported in the HSHC target group, and only 793 (21%) were of the risk target groups: road kills (59.6% of all tested in the risk groups), fallen/culled (34.1% of all tested in the risk groups).

Lithuania reported that each farm and each facility in which cervids are kept in an enclosed territory would be considered as a PSU – ~655, based on permissions issued by the Ministry of the Environment (list not available); and 51 PSU for wild cervids based on State Food and Veterinary Service (SFVS) territorial units. Lithuania did not report PSU to the EFSA database when reporting test results.

Poland

Poland tested a total of 3,505 cervids during the regulation period, all of them older than 12 months of age (or of unknown age). Of these adult cervids: 2,662 were roe deer (75.9%), 651 were red deer (18.6%) and 192 were moose (5.5%). A total of 235 farmed/captive cervids were tested (6.7%), of which 233 were red deer and two were roe deer. The rest (3,270, 93.3%) were wild cervids. Cervids in the risk target groups amounted to 84.2% (2,953) of the total, with the road kills the most frequently tested group at 2,451 (83% of all tested cervids in risk groups). Only 552 cervids were reported in the HSHC group (15.7%).

Poland reported 16 PSU for both wild and captive/farmed cervids based on voivodeships (administrative units). The 16 PSU were sampled but captive cervids were tested in only five of them (median = 10, range: 5–113) whereas in all the 16 PSU wild cervids were tested (median = 149, range: 16–272). A total of 768 tested cervids were not assigned to any PSU.

Sweden

During the regulation period Sweden tested a total of 4,526 cervids, all of them older than 12 months of age: 2,971 were reindeer (65.6%), 2,964 of them semi-domesticated; 1,259 were moose (27.8%), 159 were roe deer (3.5%), 128 were red deer (2.8%) and 9 were fallow deer (0.2%). Sweden also tested 125 farmed cervids (2.7%), 116 of which were red deer, 7 were reindeer, one was a fallow deer and one was a moose. The rest, 1,437 (31.7%) animals, were wild cervids, of which 1,258 were moose (87.5%). In terms of target groups, 3,601 cervids were reported in the HSHC (79.6%), the majority of which were semi-domesticated reindeer. In the risk groups (925, 20.4%), the most frequently tested groups were fallen/culled, with 616 animals sampled (66.6% of all tested in the risk groups).

Sweden reported 109 PSU for farmed cervids (one for each farm), 51 PSU for semi-domesticated reindeer (corresponding to the 51 Sameby, the administrative units where reindeer are kept, and 50 PSU for wild cervids, covering the whole country). The PSU of different wild cervids and semi-domesticated reindeer geographically overlapped. A total of 125 animals (median = 3; range: 1–12) were tested from 33 PSU (30.3%) for farmed cervids; 2,964 cervids were tested (median = 20; range: 1–1,017) from 31 PSU (60.8%) for semi-domesticated reindeer. All the PSU for wild cervids were sampled (median = 11.5; range: 1–426).

3.1.2.3. First January 2021–28 February 2022, all countries

The patterns of surveillance after the end of the EC Regulation surveillance returned to pre-2018 levels for most of the countries, with the throughput being very low for the MS and high for Norway, despite the decrease to 21,551 cervids older than 12 months of age (and of unknown age) tested in 2021 in this country. The two exceptions are the MS (Finland and Sweden) in which cases of CWD had been confirmed during the 3-year period.

Compared to 2020, Finland reduced the sampling to 398 cervids in 2021, of which 152 (38.2%) were reindeer, mostly semi-domesticated, together with 100 roe deer (25.1%), 90 moose (22.6%), 55 white-tailed deer (13.8%) and one fallow deer.

The 3-year monitoring programme laid down by Regulation (EU) 2017/1972 ended on 31 December 2020, but Sweden extended the surveillance for CWD until the end of 2021. Sweden tested their largest number of cervids in a single year in 2021, (3,316), most of them semi-domesticated reindeer (2,526, 76.2%), followed by moose (433, 13%) red deer (290, 8.7%), roe deer (63, 1.9%) and fallow deer (3, 0.09%). The most frequent target group tested in Sweden in 2021 was HSHC (76.9%), followed by FC (14.3%), RK (7.6%), HSNHC (0.7%) and SUS (0.5%).

The first 2 months of 2022 showed a similar pattern, with 10 cervids older than 12 months of age tested by Finland, 585 by Sweden (84.5% semi-domesticated reindeer,) and 2,620 by Norway (81.4% semi-domesticated reindeer). In these latter two countries, the majority of the semi-domesticated reindeer tested were in the HSHC target group.

3.1.2.4. Norway

Norway started its surveillance programme in 2016, after the first case of an infected wild reindeer was detected and was run independently from the EU regulatory surveillance. The surveillance included samples from cervids being hunted, harvested at slaughterhouses and fallen stock.

PSU in Norway included 357 wild cervid municipalities, 24 wild reindeer management areas, 76 herding districts for semi-domesticated reindeer, all red deer farms (around 90) and zoos with wild

cervids (fewer than five). For the EU regulatory surveillance, Norway randomly selected 100 PSU, including 64 municipalities for wild cervids, 3 wild reindeer areas and 33 herding districts for semi-domesticated reindeer, and, in addition, included all (around 100) deer farms and zoos. For these selected PSU, the focus was to test high-risk animals (already part of the Norwegian surveillance programme). However, extra samples (from normal hunt or normal harvest) were tested to increase the chances of obtaining at least 30 samples from each of the selected PSU.

Cervids to be tested in the surveillance programme in Norway included:

1) All fallen stock (1 year and above) from wild and farmed cervids (from all municipalities, all reindeer herding districts, wild reindeer areas, from farmed populations and zoos).
2) All wild reindeer (1 year and above) hunted from all wild reindeer management areas.
3) All moose and red deer (2 years and above) harvested during hunting from regions/zones with cases of CWD 'Ly+' detected in reindeer (municipalities around Nordfjella and, from 2020, Hardangervidda), and initially also from areas with cases of 'Ly-'detected (see Section 3.1.3.1).
4) Cervids above 2 years old delivered to wild game processing facilities.
5) Samples from semi-domesticated reindeer from slaughterhouses in all reindeer herding districts. From reindeer districts in the northernmost counties (Nordland, Troms and Finnmark), testing was restricted to slaughtered animals older than 2 years, while from the southern counties, testing included all slaughtered animals above 1 year old.
6) As a part of the EU regulatory surveillance: moose and red deer at least 2 years old hunted (harvested) from randomly selected PSU. Ordinary harvest was included to increase the probability of testing a minimum number of 30 samples from each of the selected PSU.
7) Surveillance also included sampling of jaws (or a tooth) for age determination of reindeer in the Nordfjella region, and moose and red deer populations in selected municipalities.

The total number of cervids > 12 months of age (or of unknown age) tested during the mandate period by Norway was 130,836, of which 64,838 were reindeer (49.5%), 28,233 were moose (21.6%), and 27,129 were red deer (20.7%). A total of 78,112 (59.7%) were wild cervids, 49,987 were semi-domesticated (38.2%) while the rest were farmed cervids (2,737, 2.1%). The majority of the cervids tested were reported in the HSHC group: 115,372 (88.2%), and 15,464 (11.8%) were from the high-risk groups. Surveillance sampling from roe deer was more limited than for the other cervids (11% of the wild cervids tested) but was typically from fallen stock (mainly road kills, 63%). For the mandate period, the total number of samples from fallen stock of farmed red deer was 82 (3.5%).

3.1.2.5. Iceland

Iceland tested 54 wild reindeer in the last quarter of 2017, 247 wild reindeer during the regulation period (2018–2020) and three captive deer (unspecified species) in the post-regulation period (2021–February 2022), making a total of 304 cervids older than 12 months of age (or of unknown age) tested during the mandate period. The majority of the cervids were reported to be in the HSHC group (96.7%).

3.1.3. Description of the intensified surveillance undertaken by countries following case detection

According to the Regulation (EC) 999/2001, in additional to the implementation of the surveillance requirements (that can be referred to as 'general surveillance'), intensified surveillance was required following the detection of a positive case. '*2.5 In case of a positive finding of TSE in a cervid, the number of samples from cervids collected in the zone where the positive TSE case was found must be increased, based on an assessment carried out by the Member State concerned*.' This has been done by Norway (voluntarily), Sweden and Finland. After presenting the surveillance data as submitted to the EFSA database by all the countries included in the mandate, a brief description is given of the different approaches to intensified surveillance (i.e. additional sampling in areas where positive cases were found) applied by these three countries.

3.1.3.1. Intensified surveillance in Norway

After the first case of an infected wild reindeer being detected in 2016 (and in moose a few months later), Norway initiated and performed intensified testing for CWD, including sampling from cervids

being hunted during the hunting season, harvested at slaughterhouses and fallen stock. The total yearly number of samples tested for CWD increased from > 10,000 in 2016 to > 33,000 in 2018.

In Norway, wild reindeer management is aided by recreational hunting in 24 management areas. These areas provide the PSU for reindeer with some exceptions (Mysterud et al., 2023). The surveillance activities achieved a sample return rate of 61.5% out of a maximum of 22,123 reindeer aged 1 year or older, harvested for 2016–2021 (Mysterud et al., 2023). Samples from recreational hunting constituted 84% of the total sample size from wild reindeer. The final sample size including both relevant tissues (retropharyngeal lymph node (RLN) and brain) or in a few cases (1.3%), only RLN, was 9,412.

Surveillance upon CWD detection

In Norway, surveillance is intensified upon detection of CWD Ly+ cases. The same was initially the case upon detection of CWD Ly-, but as of 2020, this is no longer undertaken due to its sporadic appearance (Rolandsen, 2023a). Nordfjella Zone 1 was the area of first CWD Ly+ detection, through passive surveillance, in a reindeer in spring 2016. Regulation in the form of a *'CWD zone'* was put in place for the whole Nordfjella region (15 municipalities). Surveillance of the ordinary harvest was initiated from the hunting season in 2016 (see Section 3.1.2.4). There was some moderate expansion of harvest quotas for reindeer in 2016 prior to the full depopulation of reindeer from this area in 2017–2018 (Mysterud and Rolandsen, 2018; Mysterud et al., 2019b). There is still ongoing intensified surveillance in red deer and moose populations in the region. This involves increased harvest quotas and an extended hunting season to lower the population densities, and the risk of CWD spillover from reindeer, which also leads to larger sample sizes as more animals are hunted (Mysterud et al., 2020d). Governmental fees on harvest have been lifted, and aid is provided to lift carcasses out of remote areas. The CWD zone was extended with five municipalities of the Hardangervidda area from 18 September 2020. In addition, municipalities using Nordfjella for summer sheep grazing were included from 2019 to 2021. Similarly, due to detection of Ly+ on Hardangervidda, a zone was demarcated from 2021, and surveillance in 2021 also included areas using Hardangervidda for summer sheep grazing. From 2022, there is only intensified surveillance within the Nordfjella and Hardangervidda zones, and not in the areas using ranges for summer sheep grazing (Rolandsen, 2023b).

In 2016, Selbu municipality was the area where the first CWD Ly- cases were detected in two moose, through passive surveillance, following which a CWD zone was put in place (which included nine municipalities). Surveillance of the ordinary harvest was initiated in 2016 and is still ongoing. Lierne municipality was the area in which the third case of CWD Ly- was detected in moose in 2017. Similarly, surveillance of the ordinary harvest was initiated as of 2018 (including five municipalities) but ended in 2021. Gjemnes municipality was the area of first CWD Ly- detection in red deer in 2017; surveillance of the ordinary harvest was initiated as of 2018 and ended in 2021 (in a zone including five municipalities). The region of Flesberg/Sigdal (seven municipalities) was included from late fall 2018 (after CWD Ly- detection in moose) and ended in 2021. Steinkjer municipality was included from 2020, after CWD Ly- detection in moose), but this surveillance also ended in 2021. Following this, no further additional surveillance has been undertaken in areas (Vinje, Nord-Odal, Tynset) in which CWD Ly- has been detected (Rolandsen, 2023b). An exception was after detecting a Ly- case in red deer in Etne municipality in 2021. Upon detection, the neighbouring municipality Sauda was also included in the intensified surveillance in 2021. Etne municipality was already a part of the intensified surveillance as a municipality with sheep using Nordfjella and Hardangervidda, and all other municipalities neighbouring Etne were already part of this intensified surveillance being part of the Hardangervidda and Nordfjella zones. This intensified surveillance was therefore partly because of the proximity to Hardangervidda, with detection of Ly+ in reindeer (Rolandsen, 2023b).

Intensified surveillance to increase the probability of detecting CWD

The Norwegian Food Safety Authority and the Norwegian Environment Agency have implemented extra harvesting to increase sample size in areas Nordfjella Zone 1 and Nordfjella Zone 2, and on Hardangervidda. The intention was to establish a high confidence of freedom-from-CWD or to enable early detection. This involved larger but variable harvesting quotas, male-biased harvesting, extended hunting seasons and helicopters to aid hunters in retrieving carcasses from remote areas. A strongly male-biased harvesting has been at the core of these efforts. This was implemented due to an observed higher probability of CWD in adult males than females in Nordfjella, and the fact that the proportion of adult males does not affect population recruitment in this polygynous species (Mysterud et al., 2020c, 2021a). This has yielded greatly increased sample sizes. In 2019 alone, 47% of the adult

male population on Hardangervidda was removed, compared to an average harvest rate of 16% (Mysterud et al., 2021a). This has skewed the population sex ratio towards females and lowered the age of males in the population (Rolandsen et al., 2022).

The added harvest facilitated the detection of CWD, despite a very low disease prevalence, in Hardangervidda in 2020. Upon detection of disease, a CWD zone was put in place for the whole Hardangervidda region. The heavily male-biased harvesting in reindeer continued, and increased quotas of females were also set from 2021 (Rolandsen et al., 2022). The population size is therefore now lower and more female-biased than before initiation of CWD management. It is nevertheless unlikely that the population sizes would be sufficiently reduced to have a high likelihood of removing all CWD-infected individuals (Mysterud et al., 2021b).

Intensified surveillance in red deer and moose in the Hardangervidda region was also implemented from 2021 in a manner similar to that in the Nordfjella region (see above).

3.1.3.2. Intensified surveillance in Sweden

Sweden confirmed the first case of CWD in a moose in March 2019 in PSU 1045 (case #1), in Norrbotten. After its detection, an expert group was established with experts on cervid populations (wild and semi-domestic), hunting and disease surveillance as well as TSE diseases, to write a proposal for intensified surveillance. The competent authorities of Finland and Norway were also contacted to take into account the approaches taken in those countries.

Moose can migrate seasonally or be stationary and there are regional differences in migratory patterns (Van Moorter et al., 2021). The area where the first case was detected had been part of an extensive moose research project and data from that research project was used as a basis to determine the area where the positive case was likely to have spent its life, based on the knowledge of migration. Thus, the area was not limited to the PSU of detection. The first positive case had been GPS-tagged in the project and migratory patterns for the early part of the life was available (Figure 3). The area of surveillance was based on the moose migratory pattern data, both the specific moose but also the known east–west migratory pattern of moose in this area, but surveillance was not limited only to moose. The other predominant species in the region is semi-domesticated reindeer, and all Samebys (management areas for reindeer), which overlapped with the areas defined for moose, were included in the surveillance. Red deer and fallow deer are not present in the area, but a very limited number of roe deer are. Surveillance targets were set for the wild moose and for the semi-domesticated reindeer associated with the Samebys, which were treated as separate epidemiological units when setting surveillance targets. Sample size to detect CWD was calculated assuming a test sensitivity of 70% and the observed prevalence (0.7%) at that time in the Nordfjella wild reindeer population.

As it was not deemed possible to get sufficient animals from the high-risk target groups, it was decided to include samples from healthy hunted and healthy slaughtered animals. Although it has been shown that these target groups contribute less than high-risk animals, this was accounted for in the calculation of sample size. As there were already indications that the cases detected in moose seemed to be in relatively old animals, it was decided to collect part of the mandible to be able to determine the age of the surveyed animals. Hunters, wild game processing plants and slaughterhouses in the regions were involved in the surveillance, and targeted education and information sessions were held, together with the distribution of kits for sampling, and the creation of a dedicated transportation system of samples to the laboratory.

The first three cases were detected in the same area, which is roughly 200 km × 100 km. The fourth case was detected in Västerbotten. Once again, the expert group drafted a proposal for the intensified surveillance. As for the first case, the fourth case was detected in an area which had been involved in moose research. Based on the moose migratory data in the region (Singh et al., 2012), the area was determined based on the space-use patterns. This area was smaller as moose in the area are more stationary, compared to the area in Norrbotten. The design prevalence and diagnostic sensitivity used were as above. Some Samebys overlapping the new area were already included in the intensified surveillance for Norrbotten.

Since April 2019, when intensified surveillance was implemented, the number of cervids tested in the PSU where the cases were found increased and included healthy hunted moose and healthy slaughter reindeer. The PSU included in the intensified surveillance of Norrbotten were 1,043 and 1,045 for moose and 2014, 2028–2033 and 2037–2039 for semi-domesticated reindeer. The second case (case #2) was found in PSU 1,043 in May 2019. In September 2019, a second moose case was found in PSU 1045 (case #3). In the period March 2019–February 2022, a total of 4,571 semi-domesticated reindeer

(93.7% HSHC and 288 in the risk groups), 641 moose (97.8% HSHC and 11 in the risk groups) and one roe deer were tested in the intensified surveillance area of Norrbotten.

The fourth case was confirmed in a moose in PSU 1035 (case #4) in September 2020 in Västerbotten. The PSU included in the intensified surveillance of Västerbotten were 1,035 and 1,041. In the period September 2020–February 2022, 393 moose (95.9% HSHC and 16 in the risk groups) and one red deer were tested in the intensified area of Västerbotten.

A summary of the cervids tested in the two intensified surveillance zones in Sweden since the month of detection of the cases is shown in Table 5.

The map showing the location of the test cervids (including cases detected prior to intensified surveillance) can be seen at the web of the National Veterinary Institute (SVA) of Sweden at: (https://www.sva.se/amnesomraden/smittlage/overvakning-av-avmagringssjuka-cwd/karta-over-undersokta-viltlevande-hjortdjur/).

Table 5: Number of cervids older than 12 months of age including those of unknown age tested in the two areas of Sweden with intensified surveillance since the detection of the first cases in those areas. Numbers in brackets are counts of CWD-positive cases. The first four target groups listed (FC, HSNHC, RK, SUS) are considered high-risk target groups for analysis purposes

	Semi-domesticated reindeer	Moose	Roe deer	Red deer
Norbotten PSU identifiers	2014, 2028, 2029, 2030, 2031, 2032,2033,2037, 2038 and 2039	1043, 1045		
FC	133	10 (1)		
HSNHC	11	1		
RK	144	1		
SUS		1 (1)		
HSHC	4,283	627 (1)	1	
Total	**4,571***	**640***	**1**	
Västerbotten PSU identifiers		1041, 1035		
FC		11		1
HSNHC		1 (1)		
RK		1		
SUS		3		
HSHC		377		
Total		**393****		**1**

*: Tested during the period March 2019–February 2022.
**: Tested during the period September 2020–February 2022.

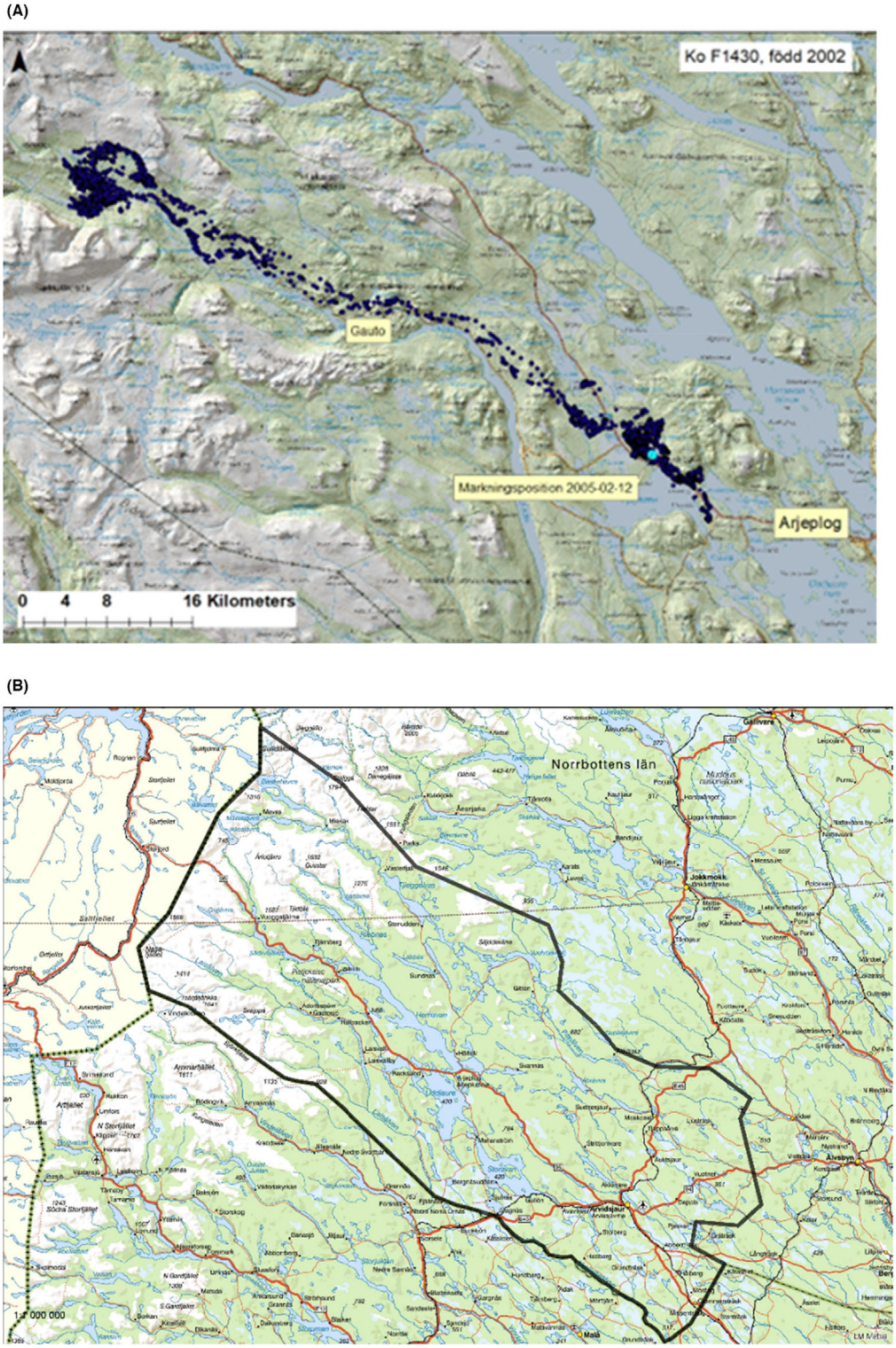

Figure 3: (a) GPS data from the first positive moose case. SGU Elevation model © SGU, GSD Road Map © Lantmäteriet, Moose positions © SLU Source: SLU. (b) Area of intensified surveillance in Norrbotten, corresponding to Älgförvaltningsområde 3, Source: County Board, Norrbotten. https://ext-geoportal.lansstyrelsen.se/standard/?appid=fc467ac65f7b4ddbad435187e17aa33f

Disclaimer: The designations employed and the presentation of material on this map do not imply the expression of any opinion whatsoever on the part of the European Food Safety Authority concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries).

3.1.3.3. Intensified surveillance in Finland

Between 2018 and 2020, there were two positive cases for CWD reported in Finland. An ad hoc CWD expert group was formed by the Finnish Food Authority to implement the surveillance program. The group was expanded to include experts/officials from Regional State Administrative Agency and Ministry of Agriculture and Forestry in Finland after the first positive case. Intensified surveillance in both cases was planned and implemented together with game management authorities.

Case #1 2018 (Kuhmo): The surveillance program in 2018 was targeted to 100 PSU. When case #1 was confirmed, surveillance was intensified by including all of the surrounding 11 game management associations in the surveillance (Figure 4). Hunters were asked to send samples that fulfilled the same criteria as in the surveillance program. In the autumn of 2018, during the moose hunting season, hunters in Kuhmo and surrounding areas were also asked to send samples from healthy, hunted moose, over 1 year old. The aim was to get as many samples as possible. Between 1.1.2018 and 31.12.2020, altogether a total of 107 moose samples were sampled in the area, all negative. The samples of healthy, hunted moose from this area are also included in the statistics of the surveillance program even if they did not fulfil the program's criteria (Kuukka-Anttila, 2022).

As mentioned above, Finland reported the first case of CWD in a moose in January 2018, in PSU P-0066679 (case #1). Looking at the number of cervids tested in the 11 (PSU) game management units (Figure 4) (i.e. Hyrynsalmen-Ristijärven riistanhoitoyhdistys P-0068087, Kajaanin riistanhoitoyhdistys P-0068315, Lieksan riistanhoitoyhdistys P-0066681, Nurmeksen riistanhoitoyhdistys (no samples), Paltamon riistanhoitoyhdistys P-0066420, Puolangan riistanhoitoyhdistys P-0069998, Sotkamon riistanhoitoyhdistys P-0066852, Suomussalmen riistanhoitoyhdistys P-0068015, Vaalan riistanhoitoyhdistys (no samples), Valtimon riistanhoitoyhdistys P-0069490, Vuolijoen riistanhoitoyhdistys P-0069480, included in the intensified surveillance area plus Kuhmon riistanhoitoyhdistys (P-0066679)), since October 2018 until February 2022, a total of 155 cervids were tested, 114 of them moose, and only 34 (21.9% of all tested moose) in the high-risk target groups.

Case #2 (2020, Laukaa): in 2020, a surveillance program was already in place in all the game management associations. When case #2 was diagnosed, the moose hunting season was ongoing, and surveillance was intensified by including in the monitoring activity also healthy hunted moose, over 1 year old in the surrounding seven game management associations (Figure 4). The target was to get as many moose samples from this area as possible and altogether 95 samples from moose were analysed and found negative in 2020. Samples from healthy, hunted moose from this area are included in the statistics of the surveillance program even if they did not fulfil the program's criteria.

Case #2 was reported in October 2020 in a moose in PSU P-0089018. Looking at the number of cervids tested in the seven (PSU) game management units (Figure 4) (i.e. Alakeiteleen riistanhoitoyhdistys P-0089120, Suolahden-Sumiaisten riistanhoitoyhdistys P-0089098, Konneveden riistanhoitoyhdistys P-0073964, Hankasalmen riistanhoitoyhdistysP-0089150,Toivakan riistanhoitoyhdistys P-0089091, Jyväskylän seudun riistanhoitoyhdistys P-0089095, Uuraisten riistanhoitoyhdistys P-0069495, plus Laukaan riistanhoitoyhdistys P-0089018, included in the intensified surveillance area) since October 2020 until February 2022, a total of 101 cervids were tested, 98 of them moose, but only 7 (6.9% of all tested) were from the high-risk target groups.

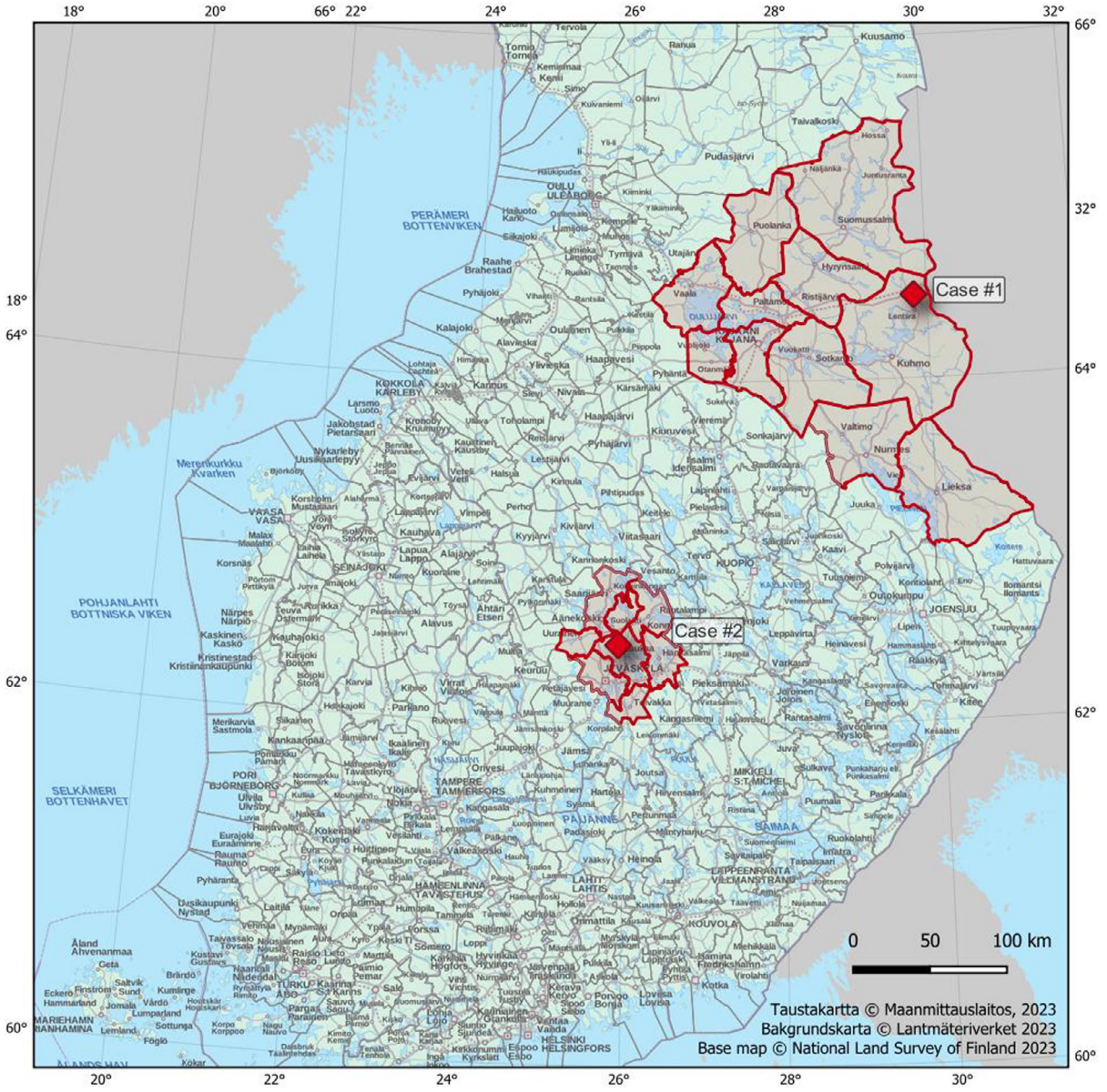

Figure 4: Game management associations included in the intensified surveillance around the location where case #1 and case #2 were found (Kuukka-Anttila, 2022)

3.1.4. Comparison between EFSA 2017 surveillance proposal, the EU requirements and approaches applied by the countries (SAQ 1.1.3)

The targets to be achieved by the countries listed in the Regulation were set with the aim of either identifying the disease (6 EU Member States where the existence of the disease was not yet known) or estimating prevalence in the cervid population where present (at that time only in Norway). The EFSA BIOHAZ Panel (2017) proposed a surveillance strategy that theoretically ensured representativeness and adequate sensitivity, and this was transposed in Commission Regulation (EU) 2017/1972) into a set of rules that countries would have to comply with. Firstly, it was requested that each country identified a set of primary sampling units (PSU) on a spatial basis. Lack of geographical representativeness was one of the main limitations of the previous CWD monitoring campaign (2008–2010). Secondly, the sample size was fixed for both the PSU and the number of animals to test. The EFSA indications were focussed on a risk-based strategy targeting adult animals belonging to target groups where the probability of disease is higher than in the healthy slaughtered animals (HSHC). Finally, compared with the EFSA indications, the EC regulations allowed HSHC to be added to achieve the same overall sample size (3,000 wild-semi-domesticated animals and 3,000 farmed animals, respectively) at the national level in each country. The main differences have been summarised in Table 6 below.

Focusing on wild populations, as shown in Section 3.2.1, over the 3-year period the overall target of 3,000 animals to be tested per country was largely achieved (range 2,047-4,401) in at least 5 out of 6 countries, but only if the low-risk target group (HSHC) is considered in the totals. Over the entire 2017–2022 period, only Finland achieved this by testing 3,130 risk animals; in the remaining 5 countries the figures ranged from 209 to 2,380. With regard to captive/farmed animals, in general each country tested only a small number (range 1–89). Moreover, in general, most countries (Table 1) were unable to prioritise monitoring testing of at-risk groups, and healthy animals contributed a large proportion of the overall sample. In summary, while the applied study designs allowed countries to approach the overall national sample size for wild/semi-domesticated animals, the proportion of testing in at-risk animals was small, which has compromised the effectiveness of the risk-based strategy and the ability to ensure the sensitivity of the surveillance.

In conclusion, some limitations emerged, and potential consequences can be deduced. First, where the identification of PSU was not followed by random or stratified selection, there could be a under or over-representativeness of PSU in the sample. Non-random selection of PSU for monitoring could have led to some areas being missed. It is also unclear whether a random sample extraction of 100 PSU was actually performed: From the data, it appears that in some cases surveillance involved most if not all of the PSU declared. Looking at the indications about sample size, there is variation in number of samples per PSU, and the data do not provide information about circumstances or efforts required to collect them. However, based on the available data, it seems countries may have prioritised reaching the national target (3,000 wild/semi-domesticated animals), thereby prompting some MS to test mainly healthy animals, a subgroup that is easier to sample. As discussed in the following sections, this approach may have compromised the benefits that would have accrued from a risk-based sampling strategy. On the other hand, the difficulties (see Section 3.3.1) in acquiring samples from risk groups should be acknowledged. A summary of the implementation of the mandatory surveillance against the legal requirements and the EFSA proposal in the six member states is displayed in Table 6.

Table 6: Summary of CWD surveillance recommendations, requirements and overall outcomes reported by participating country in Europe 2016–2022

Surveillance activities – proposed. Differences highlighted in red (for full text descriptions, see Appendix A)			**Surveillance activities (full details of all testing activity given in Section 3.1)**						
	EFSA opinion (2017)	**EU regulation**	**Estonia**	**Finland**	**Latvia**	**Lithuania**	**Poland**	**Sweden**	**Norway***
Countries	Estonia, Finland, Iceland, Latvia, Lithuania, Norway, Poland, Sweden	Estonia, Finland, Latvia, Lithuania, Poland, Sweden							
PSU definition	**Wild/ semi-domesticated:** Identify biologically defined population sampling units (PSU) within country. If > 100, select random subset of 100; if ≤ 100, use all.	**Wild/ semi-domesticated:** Select ≤ 100 PSU at random	15 declared; 13 sampled	54 semi-domesticated; 59 sampled 295 wild; 193 sampled	100 declared; 240 sampled	51 declared; sampling distribution not reported	16 declared; 16 sampled	51 semi-domesticated; 31 sampled 50 wild; 50 sampled	None declared 100 randomly selected wild PSU sampled (EU regularly surveillance) with maximum 30 samples. More PSU sampled in general (see Section 3.1.2.4) and intensified (see Section 3.1.3.1) surveillance
	Farmed/ captive: As above	**Farmed/ captive:** As above	15 declared; 0 sampled		12 sampled	665 declared; sampling distribution not reported	16 declared; 5 sampled	109 declared; 33 sampled	Around 100 in total; 81 sampled (2018–2020)
Target groups and numbers. All > 12 month	**Wild/semi-domesticated:** Within each PSU, examine a convenience	**Wild/semi-domesticated:** Within each PSU, sample all animals belonging to	2,141** (71.4% HSHC)	3,424 (8.3% HSHC)	2,941 (92.6% HSHC)	3,698 (79.2% HSHC)	3,270 (13.2% HSHC)	8,050 (77.7% HSHC)	From the EU regulatory surveillance 2924 (54.2% HSHC)

Surveillance activities – proposed. Differences highlighted in red (for full text descriptions, see Appendix A)			Surveillance activities (full details of all testing activity given in Section 3.1)						
	EFSA opinion (2017)	**EU regulation**	**Estonia**	**Finland**	**Latvia**	**Lithuania**	**Poland**	**Sweden**	**Norway***
	sample (cumulative, over 3 years) of 30 animals from target (=risk) groups: killed because sick or in poor body condition. hunted or slaughtered but considered not fit for human consumption. Road/predator kills or otherwise found dead	target groups over the 3-year period to total 30 animals per PSU Found dead or killed for health (clinical or sick)/ age reasons. Hunted or slaughtered but declared unfit for human consumption. Road- or predator-injured or killed Hunted wild game & slaughtered semi-domesticated cervids considered fit for human consumption (HSHC) if Member State has < 3,000 samples from other groups If unable to reach PSU target (30 tested over 3 – years), or if < 100 PSU identified,							All, including intensified sampling: 128,099 (88.0%) 128,099 (88% HSHC)

Surveillance activities – proposed. Differences highlighted in red (for full text descriptions, see Appendix A)			Surveillance activities (full details of all testing activity given in Section 3.1)						
	EFSA opinion (2017)	**EU regulation**	**Estonia**	**Finland**	**Latvia**	**Lithuania**	**Poland**	**Sweden**	**Norway***
		sampling may continue in PSUs having reached the 30 sample target, with objective of reaching a total of 3,000.							
	Farmed/ captive: As above	**Farmed/ captive:** As above	12 (0% HSHC)	33 (6% HSHC)	146 (99.3% HSHC)	86 (70.9% HSHC)	235 (51.5% HSHC)	484 (81.6% HSHC)	From EU regulatory surveillance: 1,049 (95% HSHC) All surveillance: 2,737 (96.2% HSHC)
Tissues to be collected	***All sampled animals:*** obex, retropharyngeal lymph nodes or tonsils or other head lymph nodes	***All sampled animals:*** brainstem *where feasible*, one of the following (listed in order of preference): retropharyngeal lymph nodes; tonsils; other head lymph nodes							Yes Yes (combined with obex for testing) After 2017, > 60% of yearly samples included lymph nodes
Intensified surveillance	Not addressed	*If cervid TSE case (s) found: then number of samples from zone yielding positive case(s) must be*	Not applicable	Yes	Not applicable	Not applicable	Not applicable	Yes	Yes

Surveillance activities – proposed. Differences highlighted in red (for full text descriptions, see Appendix A)			Surveillance activities (full details of all testing activity given in Section 3.1)						
	EFSA opinion (2017)	EU regulation	Estonia	Finland	Latvia	Lithuania	Poland	Sweden	Norway*
		increased, based on assessment by Member State concerned							

*: Norway did not submit to EFSA database the PSU in which cervids were tested but the surveillance was organised following the distribution of the country in PSU for wild cervids, wild reindeer management areas, herding districts for semi-domesticated reindeer, red deer farms (around 90) and zoos with wild cervids. Thus, Norway has been included in this table. Data available online: http://apps.vetinst.no/skrantesykestatistikk/NO/#psu Similar data were not available for Iceland.

**: Animals older than 12 months of age or of unknown age tested during the entire mandate period.

3.1.5. The outcome of surveillance. Cases detected (AQ 1.2)

Participating countries detected a total of 31 CWD cases during the mandate period (September 2017–February 2022). Seventeen of those cases were reported during the regulation period (Table 7). Three of the eight participating countries (including two of the six MS) reported cases from September 2017. Cases in Sweden (n = 4) and Finland (n = 2) were all moose of the Ly- phenotype. Norway first detected Ly+ phenotype cases in reindeer and Ly- cases in moose in 2016, but their additional cases from September 2017 did include areas where cases had not been detected previously and the first report of Ly- in red deer. Norway detected Ly- cases in moose (n = 5), as well as Ly+ cases in reindeer (n = 13) during September 2017–December 2020. The Norwegian Ly+ reindeer case in 2020 came from a neighbouring population (Hardangervidda) without prior detections.

Table 7: Caseload of chronic wasting disease (CWD) in Europe as of December 2022. In brackets, cases confirmed during the mandate period (September 2017–February 2022). Shaded rows indicate the 3 full years of the regulation surveillance period for member states

	Norway			Sweden	Finland
Country	**Reindeer**	**Moose**	**Red deer**	**Moose**	**Moose**
2016	4	2			
2017	9(6)	1(1)	1(1)		
2018	6(6)	1(1)			1(1)
2019		2(2)		3(3)	
2020	1(1)	1(1)		1(1)	1(1)
2021		2(2)	1(1)		
2022	1	2(2)	1(1)		1
Grand total	**21(13)**	**11(9)**	**3(3)**	**4(4)**	**3(2)**

The CWD cases detected during the regulation and mandate periods represent only a subset of the total detections since 2016. Prior to September 2017, Norwegian authorities had reported 7 Ly+ cases in reindeer (all from Nordfjella) and two Ly- cases in moose (Table 8). Since 2020, additional Ly- cases (four in moose, two in red deer) have been reported. A second Ly+ case has been detected in Hardangervidda since the end of the mandate period (in September 2022). As of December 2022, a total of 21 Ly+ and 21 Ly- CWD cases have been detected in European countries. Overall, 19 of Norway's 21 Ly+ reindeer cases have originated from the Nordfjella population where cases were first detected in 2016. By comparison, Ly- cases have been more geographically widespread.

Table 8: Species and target group of all 31 cases of CWD confirmed during the mandate period. The fifth target group – road-killed (RK) – has yielded no CWD cases in Europe thus far. Target group assignments for five cases from Norway were corrected as noted in Section 2.1.4

	Moose			Red deer			Reindeer	
	HSNHC	**FC**	**SUS**	**HSHC**	**FC**	**HSHC**	**HSNHC**	**HSHC**
FI		2						
NO	1	7		1	1	2	1	12
SE	1	1	1	1				
Total	**2**	**10**	**1**	**2**	**1**	**2**	**1**	**12**

3.1.5.1. An overview of all CWD cases in moose and red deer

Since it was first detected in 2016, and until December 2022, CWD has been detected in 18 moose, of which 11 were in Norway, 4 in Sweden and 3 in Finland, and also in 3 red deer in Norway (Table 7) (Mysterud et al., 2021c). All cases in moose and red deer were of the Ly- phenotype. Affected moose tend to be of an old age (mean age of 14.7 years) (Ågren et al., 2021; Tranulis et al., 2021), and thus far all but one of them have been females. The single case in a male moose was reported in a 13-year-old individual. There are few old males in the populations due to male-biased harvesting in Scandinavia, resulting in a much shorter life expectancy for males compared with females. In red deer,

only the first CWD case was examined to estimate its age (16 years). The heads of the other two red deer (2021, 2022) were discarded, so accurate ageing was not possible.

Most recently, in November 2022, Finland confirmed a third case of CWD in moose in Kyyjärvi, an area more than 100 km away from the previous cases. It was a 15-year-old female observed standing still with its head down, in poor body condition, and it did not run away from hunters or dogs. At the time of writing, the case has been confirmed positive in brainstem, and negative in the lymph node (i.e. Ly-).

3.1.5.2. Observed prevalence in affected areas

Observed or apparent prevalence (number positive/total sampled, expressed as a percentage) can serve as a metric for comparisons, e.g. of occurrence between disease phenotypes or target groups, or among affected PSUs. Apparent prevalence among harvested or otherwise randomly sampled animals is likely most representative of true prevalence and disease incidence within a CWD-affected cervid population (Miller et al., 2000; EFSA BIOHAZ Panel, 2017; Mysterud et al., 2019a; Miller and Wolfe, 2021). Apparent prevalence measured from pooled high-risk target groups tends to overestimate true prevalence but can be used to plan and evaluate surveillance data (EFSA BIOHAZ Panel, 2017).

Prevalence calculated from data pooled at the country level seems relatively uninformative because the denominator (total sampled) includes biases in sampling effort introduced by countries responding to the detection of cases before and during the mandate period. However, as reference, values for interpreting minimum detectable (or design) prevalence – the proportion of positive cases in the total samples tested from cervids older than 12 months (or of unknown age) during the mandate period – by the three countries that detected cases were: 0.058% (2/3,457) for Finland, 0.019% (25/130,836) for Norway and 0.047% (4/8,534) for Sweden. All three observed values were well below the assumed design prevalence of 0.1% at the country level (EFSA BIOHAZ Panel, 2017). This finding may be useful in planning future surveillance.

Similarly, the proportion of affected PSU detected within affected countries may be useful for future planning. From 2016, Norway detected Ly- cases in 12 (~ 3%) of 356 wild cervid PSUs (= municipalities), including nine with wild moose cases and third with wild red deer cases, as well as Ly+ CWD cases in 2 (of 23) defined wild reindeer population areas overlapping portions of 15 additional PSU. During the mandate period, Sweden and Finland also detected Ly- cases in 2 of 50 (4%) and 2 of 295 (~ 1%) of their respective wild moose PSUs. Overall, the observed proportion of affected PSUs was ~ 2.6% (18/701), which closely approximated the *a priori* assumption of 3% of PSUs affected for surveillance design stated in the 2017 opinion (EFSA BIOHAZ Panel, 2017).

Apparent prevalence estimated using only samples from known target groups varied somewhat across the local geographic areas that included affected PSU and, for Finland and Sweden, also PSU that neighboured the affected PSU. These are collectively termed 'sampled areas' for analysis purposes (Tables 9–11). The size (km^2) of these sampled areas varied by an order of ~ 25-fold or greater. Consequently, the estimates in Tables 9–11 illustrate the range of field observations during the mandate period but are not intended for direct area-to-area comparison or detailed analysis. Surveillance showed that the Ly- form was more widely distributed than the Ly+ form but was relatively rare in affected areas. Observed Ly- CWD prevalence ranged from < 0.1% to ~ 0.16% among low-risk target group (HSHC) moose in eight focal areas with ≥ 100 samples tested during the mandate period (Table 9). The range in prevalence among HSHC red deer from three focal areas was similar (< 0.1–~ 0.5%; Table 10). Overall, apparent prevalence calculated from data pooled across all affected 'sampled areas' was essentially the same (chi square 1.751, p = 0.186) among HSHC moose (~ 0.05%) and red deer (~ 0.16%), and substantially lower than the standard design prevalence of 1%. As predicted from experiences with North American CWD and other TSE surveillance (EFSA BIOHAZ Panel, 2018), the apparent prevalence of Ly- CWD in the affected areas was > 10-fold higher among moose and red deer sampled from high-risk target groups (Tables 9, 10; infection risk ratio vs. HSHC = 51.5; 95% CI 17.1–155.8 for the two species' data combined).

The Ly+ form has been detected in only two wild reindeer populations, with 19/21 cases coming from the Nordfjella population – specifically, Nordfjella management zone 1 – where CWD was first diagnosed in 2016. Apparent prevalence among HSHC sampled during the mandate period was ~ 0.68% at Nordfjella and 0.03% at Hardangervidda. Overall, high-risk target groups in the affected areas also yielded a > 10-fold higher proportion of Ly+ reindeer (Table 11), although with less certainty considering the wide confidence intervals (infection risk ratio vs. HSHC = 11.1; 95% CI 1.5–83.5).

The observed prevalence estimated using the combined data from all affected sampled areas and species listed in Tables 9–11 was 3.67% (95% CI 2.07–5.98%; 15/409) among samples from high-risk target groups and 0.15% (95% CI 0.09–0.24%; 16/10,653) among samples from the low-risk target group. The observed field values are considerably lower than the *a priori* design prevalence for affected PSU assumed in the 2017 opinion (10% among high-risk groups within a PSU [which equated to 1% among low-risk samples]; EFSA BIOHAZ Panel, 2017). It follows that these new data and lower values should be considered when making assumptions about the local (i.e. within sampling unit) design prevalence in future surveillance to detect new CWD Ly+ or CWD Ly- cases in Europe.

Table 9: Observed prevalence of CWD in moose in areas of NO, FI and SE where cases have been found by surveillance target groups (high-risk vs. low-risk). The variable size (km^2) of the sampled areas may have contributed to variation in observed prevalence. Data shown are from the mandate period and include only samples where the target group was known. See text for additional details of risk group definitions

Country	Sampled area	High-risk target groups (combined)				Low-risk target group			
		Positive	Total	Prevalence (%)	95% binomial confidence limits	Positive	Total	Prevalence (%)	95% binomial confidence limits
FI	Kuhmo	1	38	2.63%	(0.07–13.81%)	0	76	0.00%	(0–4.74%)
FI	Laukaa	1	7	14.29%	(0.36–57.87%)	0	96	0.00%	(0–3.77%)
NO	Viken – Flesberg	1	15	6.67%	(0.17–31.95%)	0	158	0.00%	(0–2.31%)
NO	Viken – Sigdal	1	10	10.00%	(0.25–44.5%)	0	238	0.00%	(0–1.54%)
NO	Innlandet – Nord-Odal	1	9	11.11%	(0.28–48.25%)	0	18	0.00%	(0–30.85%)
NO	Innlandet – Tynset	1	69	1.45%	(0.04–7.81%)	0	58	0.00%	(0–6.16%)
NO	Vestfold og Telemark – Bamble	1	13	7.69%	(0.2–36.03%)	0	17	0.00%	(0–19.51%)
NO	Vestfold og Telemark – Vinje	1	23	4.35%	(0.11–21.95%)	0	115	0.00%	(0–3.16%)
NO	Trøndelag – Lierne	1	11	9.09%	(0.23–41.28%)	0	579	0.00%	(0–0.64%)
NO	Trøndelag – Selbu	0	18	0.00%	(0–30.85%)	1	910	0.11%	(0.003–0.61%)
NO	Trøndelag – Steinkjer	1	53	1.89%	(0.05–10.07%)	0	952	0.00%	(0–0.39%)
SE	Norrbotten	2	14	14.29%	(1.78–42.81%)	1	627	0.16%	(0.004–0.89%)
SE	Västerbotten	1	25	4.00%	(0.1–20.35%)	0	382	0.00%	(0–0.96%)
Overall		13	305	4.26%	(2.29–7.18%)	2	4,226	0.05%	(0.006–0.17%)

Table 10: Observed prevalence of CWD Ly- in red deer in areas of NO where cases have been found by surveillance target groups (high risk vs. low risk). Data shown are from the mandate period and include only samples where the target group was known. See text for additional details of risk group definitions

Country	Sampled area	High-risk target groups (combined)				Low-risk target group			
		Positive	Total	Prevalence (%)	95% binomial confidence limits	Positive	Total	Prevalence (%)	95% binomial confidence limits
NO	Vestland – Etne	0	5	0%	(0–52.18%)	1	203	0.49%	(0.01–2.71%)
NO	Vestland – Bremanger	1	31	3.23%	(0.08–16.7%)	0	520	0%	(0–0.71%)
NO	Møre og Romsdal – Gjemnes	0	29	0%	(0–11.94%)	1	498	0.20%	(0.005–1.11%)
Overall		1	65	1.54%	(0.04–8.28%)	2	1,221	0.16%	(0.02–0.59%)

Table 11: Observed prevalence of CWD Ly + in reindeer in areas of NO where cases have been found, by surveillance target groups (high risk vs. low risk). Data shown are from the mandate period and include only samples where the target group was known

Country	Sampled area	High-risk target groups (combined)				Low-risk target group			
		Positive	Total(2)	Prevalence (%)	95% binomial confidence limits	Positive	Total(2)	Prevalence (%)	95% binomial confidence limits
NO	Nordfjella(1)	1	24	4.17%	(0.11–21.12)	11	1,613	0.68%	(0.34–1.18%)
NO	Hardangervidda	0	15	0%	(0–21.8%)	1	3,593	0.03%	(0.001–0.16%)
Overall		1	39	2.56%	(0.06–13.48%)	12	5,206	0.23%	(0.12–0.4%)

(1): The Nordfjella wild reindeer population inhabited two distinct management areas, denoted by Norwegian authorities as 'zone 1' (northern) and 'zone 2' (southern). All positive cases were from zone 1, and none of the 337 samples from zone 2 tested positive. Consequently, prevalence measured in zone 1 (high-risk target groups combined: 1/23 = 4.35%; low risk: 11/1,355 = 0.81%) are slightly higher than the estimates calculated from all Nordfjella samples of known risk target group shown in the table.

(2): Totals used for prevalence calculations do not include wild reindeer samples with an unknown risk target group (Nordfjella zone 1 = 36 samples, zone 2 = 78 samples; Hardangervidda = 1,103 samples). All of these samples were negative, and consequently, the estimates based on known low-risk samples are slightly different from (but have 95% confidence limits that include) the estimates based on all available samples (Nordfjella: 12/1,751 = 0.69%; Hardangervidda: 1/4,711 = 0.02%).

3.1.5.3. Sources of positive cases and implications for future surveillance

About half (15/31; 48.4%) of the CWD cases detected during the mandate period (Tables 9–11) were from target groups considered to be high risk (EFSA, 2017), even though samples from high-risk target groups comprised only 16.1% of the total samples screened from animals older than 12 months of age (or of unknown age). The majority (14/16) of positive samples from apparently healthy HSHC cervids came from areas where CWD was, or previously had been, detected and were collected in the course of either intensified surveillance following detection of an index case in the same or a nearby PSU (n = 3), or from disease management activities associated with depopulating the Nordfjella reindeer population (n = 11).

In 13 (81%) of the 16 areas where CWD was first disclosed during the mandate period, the first confirmed case came from an animal in one of the high-risk target groups. This includes both areas detected in Sweden, both areas detected in Finland and 9 of 12 new areas in Norway. The index cases in Norway reindeer and moose prior to the mandate period also came from high-risk target groups, leading to periods of intensified surveillance that greatly increased the number of HSHC submissions screened for CWD (Section 3.1.3). Notably, intensive screening of HSHC red deer in Norway contributed to the first detection in that species, and testing > 3,500 HSHC samples also led to the detection of CWD in the Hardangervidda reindeer population.

Samples from the four high-risk target groups identified in the 2017 opinion (Section 2.1.2; EFSA BIOHAZ Panel, 2017) combined had a ~ 5.6 (95% CL 2.8–11.3) infection risk ratio as compared to the low-risk HSHC submissions in areas where CWD was detected (Figure 7). The observed value was lower than the 10 times higher surveillance value assumed for high-risk submissions in the original 2017 opinion (EFSA BIOHAZ Panel, 2017). The simplest explanation for this discrepancy is that screening of road-killed (RK) cervids (n > 14,500 during the mandate period) has yielded no positive cases in Europe, even in the parts of NO, SE and FI where CWD has been detected by other means (Table 11). Considering surveillance data from the three known-affected countries, the proportion of positive cases detected within each risk target group – pooled across all cervid species sampled during the mandate period – was 0.35% (1/284) for SUS, 0.13% (11/8,424) for FC, 0.54% (3/558) for HSNHC, 0% (0/11,251) for RK, and 0.01% (16/122,310) for the lower risk HSHC, with the HSHC over-estimated because data from the Nordfjella depopulation are included in the totals. From these observations, RK submissions more closely resembled HSHC submissions (chi-square 1.472; p = 0.225) and differed markedly from the three other 'high-risk' target groups (chi-square values > 14; p < 0.00013; Figure 7).

Future surveys may benefit from either grouping RK submissions as low-risk or subdividing candidate road-killed submissions into high- and low-risk based on apparent body condition or other health problems (poor condition/health = high-risk; normal = low-risk). Comparing the affected countries' combined data from a redefined high-risk target group (SUS, FC, and HSNHC; 15/9,266) to the HSHC target group yielded a risk ratio of 12.4 (95% CL 6.1–25; Figure 5), which more closely approximates the 10 times higher surveillance value for high-risk submissions originally assumed (EFSA BIOHAZ Panel, 2017).

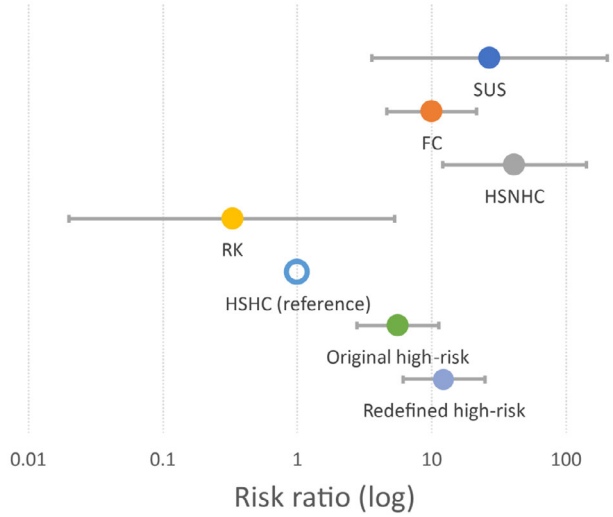

Figure 5: Risk ratios for the proportions of chronic wasting disease (CWD) positive cervids from the individual target groups originally designated as 'high-risk' (EFSA BIOHAZ Panel, 2017; solid circles) as compared to the proportion of positives among apparently healthy hunter/slaughtered fit for human consumption (HSHC; open circle) animals considered to be low risk. The pooled risk ratio for the target groups originally designated as high-risk (SUS, FC, HSNHC, and RK; 'original high-risk') is also shown. In the 'redefined high-risk' pooled risk ratio, RK has been excluded. Data are from cervids sampled in Finland, Norway and Sweden during the mandate period (see text for details). Horizontal bars represent 95% confidence intervals.

Of note, the proportion of cases among FC reindeer submissions would have been lower if multiple reindeer killed by lightning strikes or foot rot (digital necrobacillosis) had been counted in the FC target group. For example, in August 2016 at least 323 apparently healthy wild reindeer of the Hardangervidda population were found killed by lightning, among them 68 animals less than 1 year old. Although technically 'fallen', such animals were not considered high risk by Norway in the context of CWD surveillance. As a further refinement to CWD monitoring approaches, the surveillance sensitivity of the FC target group may be enhanced in future applications by following Norway's approach of focusing on individual 'found dead' cases and excluding 'fallen' cervids involved in mass casualty events (e.g. lightning strikes, avalanches, floods, other accidents or causes of death affecting multiple individuals at the same place and time) because the latter are more likely to involve otherwise healthy animals. Cervids from mass casualty events could still be screened for CWD but would be counted in the same low-risk risk bracket as apparently healthy road-killed and HSHC animals.

3.1.6. Estimation of the minimum detectable prevalence (SAQ 1.3.1)

Overall, and considering the total number of cervids tested older than 12 months of age and of unknown age (all target groups included) during the mandate period, the design (i.e. the minimum detectable) prevalence that the countries were able to detect at country level is, in general, quite low. Iceland had the highest: 0.98% (~ 1/100) and Norway the lowest with 0.0023% (2.3/100,000). In between are, Estonia (0.11%. 1.1/1,000), Finland (0.086%, 8.6/10,000), Latvia (0.09%, 9/10,000), Lithuania (0.08%, 8/10,000), Poland (0.085%, 8.5/10,000) and Sweden (0.035%, 3.5/10,000). Notably, the minimum detectable prevalence values for the five countries that did not detect CWD during the mandate period (0.08–0.98%) were somewhat above the highest observed value from affected countries (0.058%; Section 3.1.5.2).

The minimum detectable prevalence at species and country levels for the entire mandate period showed variability between countries. For moose, this ranged from the lowest in Norway (0.01%) to 1.5% in Poland. Similar differences appeared for red deer, with Norway the lowest (0.01%) and Estonia the highest with 1.1%. For reindeer the differences are greater, ranging from a very low minimum detectable prevalence for Norway (0.0046%) up to 1% in Iceland. Finally in roe deer, the differences between countries are smaller than a 10-fold magnitude, with Norway (0.04%) the country having the lowest minimum detectable prevalence and Sweden the highest (1.3%). For the other 'not specified' and exotic deer species, deer (not specified), sika deer, white-tailed deer and fallow deer,

either no, or very few, animals were tested (10 or fewer), making the calculation of the minimum detectable prevalence meaningless. Summaries of the minimum detectable prevalence achieved by country and species are shown in Appendix B.

When considering only the cervids older than 12 months of age (or of unknown age) and from surveillance groups other than HSHC (i.e. groups at higher risk) and during the entire mandate period, the minimum detectable prevalence is approximately 10-fold higher in all countries and species. In moose, for example, Norway has the lowest (0.08%) followed by Sweden, Finland and Poland, with 0.4%, 0.57% and 1.5%, respectively, and much higher minimum detectable prevalence are calculated for Estonia, Lithuania and Latvia. Similar differences appeared in the minimum detectable prevalence for red deer, with Norway the lowest (0.11%) and Poland (0.96%), at one extreme, and all other countries with a minimum detectable prevalence higher than 2%. For reindeer, the differences are smaller, ranging from a low minimum detectable prevalence in Finland and Norway (0.17% and 0.19%, respectively) to Sweden with 0.35%. Finally, for roe deer, the differences between countries are also large, Norway (0.04%) being the country with the lowest minimum detectable prevalence and Latvia with the highest (2.1%).

The calculations of the minimum detectable prevalence at the country level compared to the surveillance design as in the EFSA opinion (2017) revealed a detectable prevalence in the general population (all species for the entire mandate period), including all animals tested, close to the 0.1% estimated *a priori* but nonetheless higher than the overall observed prevalence in countries with cases. There is high variability between countries and between species within countries. By species, reindeer (only tested in four countries) is the one with the lowest overall minimum detectable prevalence (0.004%), followed by moose (0.009%), red deer (0.0098%), roe deer (0.01%), white-tailed deer (0.8%) and fallow deer (1.25%). When only high-risk animals are considered in the calculation, the estimated minimum detectable prevalence is approximately 10 times higher than the one when considering all animals tested.

When the analysis takes into account the geographical distribution of sampling at PSU level and the animals tested older than 12 months of age or of unknown age in the high-risk groups, there is a low proportion of PSU in which the minimum detectable prevalence attainable was 10% or lower: 40% of the declared PSU in Estonia, 4.5% of the declared wild PSU in Finland, 0% in Latvia, unknown in Lithuania, 23.5% of the declared semi-domesticated PSU in Sweden, 22% and of the declared wild PSU in Sweden. Only Poland (90% of the declared PSU) and Finland in semi-domesticated PSU (68.5%) had more than 50% of the PSU with a minimum detectable prevalence of 10% or lower. Table 12 shows the summary of the testing in PSU in the six member states.

The size and number of declared PSU substantially affect the interpretation of the calculation of the minimum detectable prevalence. Larger countries with lower numbers of PSU like Poland had better chances to achieve a lower detectable prevalence. However, representativeness of the sampling within large PSU cannot be assessed. Considering the surveillance findings to date, the relevance of using a country level, multispecies approach in calculating minimum detectable prevalence for future surveillance merits further discussion. For countries that conducted surveillance during the mandate period without finding CWD, the evidence is not sufficient to rule out the possibility of CWD being present. Details of the minimum detectable prevalence by country, species and PSU are included in Appendix B.

Table 12: Summary of the PSU tested in the six member states Animals older than 12 months of age or of unknown age

Country	Wild				Semi-domesticated				Farmed/captive				All			
	# PSU declared	# PSU tested	# PSU with more than 10 high-risk animals tested	# PSU with more than 10 high-risk animals tested and minimum detectable prevalence = < 10%	# PSU declared	# PSU tested	# PSU with more than 10 high-risk animals tested	# PSU with more than 10 high-risk animals tested and minimum detectable prevalence = < 10%	# PSU declared	# PSU tested	# PSU with more than 10 high-risk animals tested	# PSU with more than 10 high-risk animals tested minimum detectable prevalence = < 10%	# PSU declared	# PSU tested	# PSU with more than 10 high-risk animals tested	# PSU with more than 10 high-risk animals tested minimum detectable prevalence = < 10%
Estonia													15	13	12	6
Finland	295	221	213	31	54	62	48	37		9	1	0				
Latvia	100	249	0	0												
Lithuania																
Poland													16	16	16	15
Sweden	50	50	28	11	51	47	24	12	109	71	1	0				

3.1.7. Estimation of the probability of detection (sensitivity of the surveillance system) (SAQ 1.3.2)

3.1.7.1. Estimating surveillance system sensitivity

Surveillance system sensitivity (SSe) is the average probability that at least one infected individual will be detected by the surveillance, provided the disease is present in the population at a level equal to or greater than the specified minimum detectable prevalence. Scenario-tree modelling analysis is a tool to combine surveillance data of different target groups varying in risk of infection or probability of detection and can be used to evaluate probability of freedom from disease (Martin et al., 2007; Viljugrein et al., 2021). Simplified versions of the scenario-tree modelling tools for the analysis of complex surveillance systems to estimate SSe and to demonstrate freedom from disease are available at https://epitools.ausvet.com.au/. The analyses can also be run in R by utilising various R-packages with similar functions implemented. These R-functions may be utilised to also include uncertainty of the model parameters (parameters specified as stochastic distributions).

The function «rsu.sep.rb2st» from the R-package epiR (https://cran.r-project.org/web/packages/epiR/vignettes/epiR_surveillance.html) was used to estimate the surveillance system sensitivity (SSe) for detecting CWD Ly+ form in a cervid species for a two-stage sampling system in a country (PSU and individuals within PSU), including a single risk factor at animal level. With regard to the risk factor, tested animals were grouped according to whether they were fit for human consumption (HSHC) (low risk) versus unfit for human consumption (HSNHC) (risk animals). Animals from unknown target groups were included in the low-risk target group (fit for human consumption). All PSU were assumed to have the same risk for CWD.

The approach assumes all test results are negative. The focus is to estimate the probability of detecting at least one positive animal according to the specified design prevalence if the population (country) is infected. Here, the method was used for the CWD Ly+ form in a cervid species. The wild reindeer of Norway were not included in the analysis due to prior detection of CWD Ly+. The test results of wild reindeer of Norway were analysed and reported in Mysterud et al. (2023) (see Section 3.2.2.5). By assuming all tests were negative, it is also possible to compare the estimated SSe among PSUs also for the CWD Ly- form (with detected cases in moose and red deer). It is possible to compare the level of achieved SSe in a PSU with detected cases, relative to the level in PSU with no detected cases. However, it may be argued that a within-PSU design prevalence (average for all individuals in a PSU) of 1% is too high. For the Ly- form, also the sensitivity of the diagnostic test may need to be adjusted (or only include the brainstem samples).

The data submitted to EFSA do not include population data, so binomial approximations were used (assuming subpopulations to be relatively large compared to the number of samples). Hypergeometric assumption was used at the PSU-level if the total number of PSU were given. Samples tested from unknown sampling units were excluded from the analysis. Surveillance results were pooled together for the whole mandate period. Test results from animals registered as less than 12 months of age were excluded.

The analysis was run for all wild (and semi-domesticated) cervid species together, except the wild reindeer in Norway (due to prior detection of CWD Ly+). In addition, the analysis was repeated by single species and the relevant countries for semi-domestic reindeer, moose, red deer and roe deer, i.e. including countries reporting samples tested from more than one PSU and more than 100 samples in total from known PSU. By this criteria, Iceland and Lithuania were excluded from all the analyses. The approach was also applied to farmed red deer tested in Sweden and Poland (< 100 samples tested from Estonia and Latvia, and zero from Finland). The farm data from Norway were not included because it was not submitted to EFSA at PSU level.

For country-level prevalence (prevalence among PSU), 3% was used. However, if there were more than 100 PSU defined for a country, the prevalence was limited to three infected PSU out of total number of PSU. If a country had defined fewer than 33 PSU, the cluster-level prevalence was constrained to 1/total number of PSU. For all cervids together (except wild reindeer), the country-level design prevalence (minimum detectable prevalence) varied between 0.7% (Norway: 3 infected PSU out of 432, including 356 municipalities and 76 reindeer herding districts) and 6.7% (Estonia: 1 infected PSU out of 15). For Latvia, the country-level design prevalence was specified as 1.2% (3 infected PSU out of 240 PSU with test results reported). The country-level design prevalence (dependent on total number of PSUs) may also vary between species. For example, for Norway, the number of wildlife management units for cervids, except reindeer, corresponds to 356 Norwegian

municipalities. However, red deer, moose and roe deer have only partly overlapping spatial distributions in Norway (Mysterud et al., 2020b) and hunting data from Statistics Norway were used to define the approximate total number of PSU for moose (274), red deer (241) and roe deer (236), including only municipalities reporting at least one animal harvested for the last 3 years.

The analysis of the CWD surveillance data was run with two alternative levels of within-herd design prevalence (1% and 5%), and two alternative levels of relative risk of high-risk group animals compared to HSHC animals (2 and 5; see Table 18). The relative risk value of 5 approximated the overall relative risk of all high-risk submissions combined (~5.6) observed during the mandate period (Section 3.1.5.3). A higher relative risk of 10 or more was considered too high for the available sample sets because a relatively high proportion of risk animals were from the target group RK, and only a low proportion from SUS. The relative risk value of 2 approximated sampling scenarios where most of the animals tested were RK.

The within-PSU design prevalence (minimum detectable prevalence) corresponds to the average probability of a random selected individual (above 1 year old) in the PSU to be infected. Assuming a proportion of 5% high-risk animals in a PSU and a relative risk of 5, for a within-PSU design prevalence of 1%, the effective probability of infection (Martin et al., 2007) is 0.83% for the low-risk group and 4.2% (five times higher) for the high-risk group. For the same assumptions but increasing the within-PSU design prevalence to 5%, the effective probability of infection is 4.2% for the low-risk group and 20.8% for the high-risk group. Similarly, keeping the within-PSU design prevalence at 5%, but changing to a relative risk of 2, the effective probability of infection is 4.76% for the low-risk group and 9.5% for the high-risk group. Alternatively, it is possible to specify the within-PSU minimum detectable prevalence for the low-risk group (instead of a random selected individual in the PSU).

3.1.7.2. Diagnostic sensitivity

As an estimate for diagnostic sensitivity, we use a weighted average of the data from North American cervids summarised in the EFSA opinion II (EFSA BIOHAZ Panel, 2018) for the Bio-Rad TeSeE ELISA test, the weighted average being 92.6% and 83.9% for the diagnostic sensitivity of RLN and brainstem, respectively (Table 13). The data have some shortcomings as there is no information on whether the animals were clinical or preclinical, or if only one or both (RLN and brainstem (obex)) samples from an individual were positive.

The diagnostic sensitivity of CWD testing is known to be dependent on the individual infection stage of the disease. Therefore, another approach to obtain an estimate for the diagnostic sensitivity of testing is to use a simulation model to account for the earlier stages of infection when disease-associated accumulations of PrP are less widely distributed in the tissues tested (and possibly not yet detectable by the screening tests). With this approach, a disease detection model for how the likelihood of detecting CWD Ly+ infection develops during the course of infection and the type of sample tested may be utilised (Viljugrein et al., 2019). After the first months (stage 0), disease-associated PrP starts to become detectable in RLN (stage 1), while it takes a longer time before it starts to be detectable also in the brainstem (obex) (stage 2). In the last phase of the disease (stage 3), the detection probability is high, both in RLN and brainstem. In earlier stages of disease, it is more important to obtain a sample from the correct part of the brainstem (obex).

With this model, the diagnostic sensitivity is defined as a stochastic distribution to account for individual variation in disease progression and is dependent on tissue type and quality. A typical infection period of 2 (or 3) years from infection to death from disease is specified in the model. The maximum time after infection is restricted by the length of this infection period and age class; hence, mean diagnostic sensitivity is lower for yearlings than for adults (and low or negligible for calves). The diagnostic test sensitivity for yearlings (yearlings from wild cervids are likely to be ≤ 1.5 year old at harvest season, which may vary between species and countries) is low if only the brainstem (obex) sample is tested, and especially if the incubation period is longer than 2 years.

In the present analysis of the CWD surveillance data, we do not have the data to separate yearlings (1–2 years old) and adults (above 2 years old). Here, we apply a simpler approach, assuming a fixed diagnostic test sensitivity, dependent on the sampling scheme (type of tissue tested, pooled sample or tested in parallel) and ignoring the stages of disease that are not detectable. Re-running the analyses with mean test sensitivity (stochastic distribution) generated by simulations from the disease detection model gave only minor changes in the results (results not shown).

Test data from a PSU usually consist of different target groups and types of samples with different test sensitivity. The recommendation was to test the sample from RLN and brainstem, in parallel. Norway, due to the large number of tests to be run from the intensified surveillance, decided to

compromise, and a pooled sample of brainstem and RLN is tested in the screening. The test sensitivity of a pooled sample from the same animal is assumed to be lower than testing RLN alone (smaller sample volume from RLN) for detecting Ly+. The other countries were assumed to perform testing of a sample from RLN and brainstem in parallel. Because the results of the two tests are likely to be dependent on the infection stage of the disease, the test sensitivity of the two tests in parallel was assumed, as an approximation, to be an average between the test sensitivity assuming independence and the test sensitivity from testing only RLN.

We used weighted average (according to the proportions of the different sample categories: proportion tested with RLN versus only samples from brainstem) to specify the PSU-specific test sensitivity. If no data were given (for the countries other than Norway), 80% of the animals tested were assumed to have had samples from both brain and lymph nodes tested in parallel.

Table 13: Parameter values and assumptions used when estimating surveillance sensitivity for detection of CWD Ly + occurrence

Minimum detectable prevalence	**Value**	**Comment**
Design prevalence among PSU (cDP)	3 out of 100 (at least 1)	In cases with more than 100 PSU tested: 3/total PSU or 3/PSU tested
Design prevalence within infected PSU (uDP)	1% and 5%	EFSA working group decision
Risk factor		
Proportion of risk animals in adult population	5% (1%, 10%)	Stochastic, defined by expected, minimum and maximum value of a pert distribution (may vary by species, country/region)
Relative risk HSHC vs. high-risk target groups	1:2 and 1:5	EFSA working group decision. 1:5 used as baseline
Diagnostic sensitivity (Se) at individual animal level		
ELISA RLN test (Se1)	92.6%	Weighted average of data from 3 North American cervid species and two studies summarised (EFSA BIOHAZ Panel, 2018)
ELISA Obex test (Se2)	83.9%	Weighted average of data from 3 North American cervid species and two studies summarised (EFSA BIOHAZ Panel, 2018)
Brainstem and lymph node tested in parallel assuming independence (Se3)	(Se1 + Se2 – Se1 × Se2) = 98.7%	Assume independence of tests
Brainstem and lymph node tested in parallel (Se4)	(Se1 + Se3)/2 = 95.7%	Approximation to account for dependence in Se1 and Se2; assumption
Pooled sample lymph node and brainstem (Se5)	92%	Screening pooled material from RLN and obex, assuming slightly lower diagnostic sensitivity than for ELISA RLN (smaller volume tested); assumption
Diagnostic sensitivity at PSU level		
Proportion of animals tested with tissue samples from both brainstem and RLN, or only RLN (pRLN)	80% or data	Assumption (fixed) or species and PSU-specific data (Norway). The model is not very sensitive to this assumption.
Average test sensitivity at PSU	(1-pRLN) × Se2 + pRLN × Se4	For Norway: Se4 is changed with Se5 (testing pooled sample)

3.1.7.3. Results of the estimation of the surveillance sensitivity for different scenarios

Surveillance sensitivity (SSe) is estimated at country level (cSSe) and for each PSU with any sample tested (uSSe). At country level, cSSe is the average probability of testing at least one PSU positive if 3 out of 100 PSU are infected (cDP = 0.03; or if more than 100 PSU declared for a country, 3 out of total PSU). For each PSU with any sample tested, uSSe is the average probability of detecting at least one positive animal if the PSU is infected at the within-PSU design prevalence (uDP). SSe is calculated for different combinations of design prevalence (minimum detectable prevalence) within an infected PSU (1% and 5%) and for relative risk (RR) of high-risk target groups vs. HSHC (2 or 5) and is reported at

country level. Countries were ordered according to sample size (higher to lower). The surveillance sensitivity estimates for PSUs with any sample registered is summarised by the median value (median uSSe). In addition, Prob80 and Prob95 denote the number of sampling units reaching 80% or 95%-probability of detecting (i.e. surveillance sensitivity) at least one infected individual and can be compared with the total number of PSUs with any registered sample tested for CWD (PSU). The results of the model using RR 5 are displayed in Tables 14–19 whereas those using RR 2 are displayed in Tables C.1–C.6 of Appendix C. The number of samples from the tested animal species included in the analysis is detailed below.

The data sets and the code of the model to reproduce the results in the section below and in Appendix C can be accessed at: https://doi.org/10.5281/zenodo.7746016.

All cervids except Norwegian wild reindeer

The number of samples from all cervids except Norwegian wild reindeer included in the analyses ranged between 111,428 for Norway and 1,529 for Estonia. A high proportion (> 10%) of tested wild cervids were excluded due to unknown PSU for Latvia (11%, 329 animals) and Poland (23%, 768 animals). The number of PSUs with 0 or 1 sample registered were 39 (Norway), 5 (Sweden), 158 (Finland), 23 (Latvia), 0 (Poland) and 3 (Estonia), and these PSU were excluded from the analysis. The proportion of cervids reported in the high-risk group was 9.9% for Norway, 21.2% for Sweden, 91.7% for Finland, 4.1% for Latvia, 84.9% for Poland and 28.6% for Estonia.

The outputs from the analyses, combining the samples from the high-risk and the low-risk animals to estimate SSe, showed that the presence of PSU with CWD at a prevalence of 1–5% or higher is unlikely in Norway, Sweden and Poland. For those countries, the country-specific probabilities of detecting CWD in wild cervid animals (species combined excluding wild reindeer), and at a design prevalence of 5% within infected PSU, were higher than 95%. For Norway, and Poland also, the probability of detecting CWD at a design prevalence of 1% was higher than 90%, and in Sweden, there was a probability of 87%. Assuming the infection risk of the high-risk animals to be five times higher than for the low-risk animals, the probability of detecting CWD at 5% prevalence was 83% in Finland, 65% in Latvia and 74% in Estonia. At the PSU level, 69% (277 of 402 tested) PSU in Norway, 51% (50 of 98) in Sweden, 26% (73 of 279) in Finland, 0 in Latvia, 77% (10 of 13) in Estonia and 100% (16) of PSU in Poland reached a 95% probability of detecting CWD at the 5% minimum detectable prevalence (Table 14).

Table 14: Outputs of the disease detection model with estimates of the sensitivity of the surveillance by country for wild and semi-domesticated cervids

Country	cDP	uDP	RR	cSSe	Median uSSe	PSU	N PSU Prob 80	N PSU Prob 95
Norway	3/432	1	5	94.2	79.3	402	198	122
Norway	3/432	5	5	99.4	100	402	326	277
Sweden	3/101	1	5	87.2	45.0	98	20	9
Sweden	3/101	5	5	99.6	95.7	98	73	50
Finland	3/349	1	5	50.7	11.2	279	12	1
Finland	3/349	5	5	83.0	47.5	279	90	73
Latvia	3/240	1	5	23.9	6.9	240	0	0
Latvia	3/240	5	5	64.9	30.5	240	6	0
Poland	1/16	1	5	95.7	99.6	16	15	14
Poland	1/16	5	5	99.8	100	16	16	16
Estonia	1/15	1	5	58.8	82.5	13	7	4
Estonia	1/15	5	5	74.3	100	13	11	10

cDP: % Design prevalence among PSUs; 3 per 100 (or total) PSU. At least 1 per total PSU declared; uDP: % Design prevalence within infected PSU (detectable prevalence within PSU); RR: relative risk of high-risk target groups vs. HSHC; cSSe: % Country level SSe; uSSe: % SSe for PSUs, summarised by the median uSSe for PSUs with samples.; PSU: Number of PSU with samples registered; Prob80: Number of PSU with unit SSe reaching at least 80%; Prob95: Number of PSU with unit SSe reaching at least 95%.

Semi-domesticated reindeer

The number of samples from semi-domesticated reindeer included in the analyses ranged from 48,980 for Norway, 5,990 for Sweden and 1,677 for Finland. For Finland, although the tested reindeer were reported from 79 PSU, only 58 PSU with more than three animals tested were included in the analyses. The proportion of PSU with 0 or 1 sample registered was 3 of 76 for Norway and 5 of 51 for Sweden. The proportion of samples from the high-risk groups was 1.7% for Norway, 13.4% for Sweden and 98.2% for Finland.

Focusing on semi-domestic reindeer, the presence of PSU with CWD at a prevalence of 5% is unlikely in Norway, Sweden and Finland. The country-specific probabilities of detecting CWD at a design prevalence of 5% were higher than 95%. In Norway, the presence of PSU with CWD at a design prevalence of 1% was also unlikely. The country-specific probabilities of detecting CWD at a design prevalence of 1% were 95% (Norway), 66% (Sweden) and 82% (Finland), assuming the infection risk of the high-risk animals to be five times higher than for the low-risk animals. At the PSU-level, 83% (59 of 71) of tested PSU in Norway, 55% (28 of 51) in Sweden and 84% (49 of 58) in Finland reached 95% probability of detecting CWD at the 5% design prevalence. Similarly, while 5 (9%) PSU in Finland reached 80% probability, 36 (51%) in Norway and 8 (16%) in Sweden reached 95% probability of detecting CWD at the 1% design prevalence (Table 15).

Table 15: Outputs of the disease detection model with estimates of the sensitivity of the surveillance for semi-domesticated reindeer

Country	cDP	uDP	RR	cSSe	Median uSSe	PSU	N PSU Prob 80	N PSU Prob 95
Norway	3/100	1	5	95.3	95.2	71	47	36
Norway	3/100	5	5	99.0	100	71	65	59
Sweden	3/100	1	5	66.1	56.1	47	11	8
Sweden	3/100	5	5	91.9	98.6	47	35	28
Finland	3/100	1	5	82.1	71.6	58	5	0
Finland	3/100	5	5	99.0	99.9	58	51	49

cDP: % Design prevalence among PSUs; 3 per 100 (or total) PSU. At least 1 per total PSU declared; uDP: %Design prevalence within infected PSU (detectable prevalence within PSU); RR: relative risk of high-risk target groups vs. HSHC; cSSe: % Country level SSe; uSSe: % SSe for PSU, summarised by the median uSSe for PSU with samples; PSU: Number of PSU with samples registered for more than one (3 for Finland) animal; Prob80: Number of PSU with unit SSe reaching at least 80%; Prob95: Number of PSU with unit SSe reaching at least 95%.

Moose

The number of samples from moose included in the analyses ranged from 27,631 for Norway, 1,696 for Sweden, 687 for Finland, 654 for Latvia, 524 for Estonia and 137 for Poland. The number of PSU with 0 or 1 sample registered was 18 of 274 (Norway), 3 of 50 (Sweden), 189 of 295 (Finland), 36 (Latvia), 4 (Estonia) and 10 (Poland). The proportion of samples from the high-risk groups was 10% for Norway, 37% for Sweden, 70% for Finland, 5% for Latvia, 9% for Estonia and 100% for Poland.

The presence of PSU with CWD in wild moose at a prevalence of 5% is unlikely in Norway and Sweden. Assuming the relative infection risk of the high-risk animals to be 5, the probability of detecting CWD in wild moose for the design prevalence of 5% was 98% in Norway, 91% in Sweden and 55% in Finland. The probability of detecting CWD in wild moose, for the design prevalence of 1%, was 82% in Norway. At the PSU level, 49% of 256 tested PSU in Norway, 40% of 48 in Sweden, 2% of 182 tested PSU in Finland, 0.7% tested PSU in Latvia, 27% of 11 tested PSU in Estonia and 44% of 9 tested PSU in Poland reached 95% probability of detecting CWD at the 5% design prevalence (Table 16).

Due to the intensified surveillance in areas with positive cases, the uSSe for PSU with Ly- cases detected in moose were higher than the median for the respective country, except for one of the cases in 2022 in Norway (Nord-Odal). In Norway, the median uSSe for the PSU with Ly- cases detected was 91% (range: 39.7–99.9%) for the design prevalence of 1% (and RR for high-risk target groups vs HSHC = 5). Similarly (design prevalence = 1%, RR for high-risk target groups vs HSHC = 5), the uSSe for the two PSU with Ly- case detected in Finland were 25.4% (P-0066679) and 19.3% (P-0089018), and the uSSe for the three PSU with Ly- case detected in Sweden were 88% (PSU 1035), 83% (PSU 1043) and 97% (PSU 1045).

Table 16: Outputs of the disease detection model with estimates of the sensitivity of the surveillance for moose

Country	cDP	uDP	RR	cSSe	Median cSSe	PSU	N PSU Prob 80	N PSU Prob 95
Norway	3/274	1	5	81.9	41.6	256	62	21
Norway	3/274	5	5	97.9	94.4	256	164	125
Sweden	3/100	1	5	54.0	36.5	48	4	1
Sweden	3/100	5	5	91.4	91.5	48	32	19
Finland	3/295	1	5	17.6	7.6	182	0	0
Finland	3/295	5	5	55.1	34.8	182	11	4
Latvia	3/100	1	5	15.4	2.3	149	0	0
Latvia	3/100	5	5	50.4	11.1	149	1	1
Estonia	1/15	1	5	25.6	43.2	11	0	0
Estonia	1/15	5	5	54.7	94.4	11	6	3
Poland	1/16	1	5	21.1	27.0	9	0	0
Poland	1/16	5	5	40.9	82.1	9	5	4

cDP: % Design prevalence among PSUs; 3 per 100 (or total) PSU. At least 1 per total PSU declared; uDP: % Design prevalence within infected PSU (detectable prevalence within PSU); RR: relative risk of high-risk target groups vs. HSHC; cSSe % Country level SSe; uSSe % SSe for PSU, summarised by the median uSSe for PSU with samples; PSU: Number of PSU with samples registered; Prob80: Number of PSU with unit SSe reaching at least 80%; Prob95: Number of PSU with unit SSe reaching at least 95%.

Red deer

The number of samples from wild red deer included in the analyses ranged from 23,978 for Norway, 703 for Latvia, 362 for Poland and 253 for Estonia. The proportions of samples excluded due to missing information on PSU were 11% (Latvia) and 15% (Poland). The numbers of PSU with 0 or 1 sample registered were 24 (Norway), 31 (Latvia), 1 (Poland) and 11 (Estonia). The proportion of samples coming from the high-risk groups was 6.7% for Norway, 3.4% for Latvia, 45% for Poland and 5.2% for Estonia. Fewer than 30 wild red deer were tested from Sweden and Finland.

Assuming the infection risk for the high-risk animals to be five times higher than for the low-risk animals, the probability of detecting CWD in wild red deer in Norway was 70% and 94% for the design prevalence of 1% and 5%, respectively. Poland reached 84% probability of detecting CWD in wild red deer for the design prevalence of 5% in infected PSU. 35% of 217 tested PSUs for Norway, 0% PSUs for Latvia, 33% (4 of 12 tested) PSU for Poland and 1 out of 4 tested PSU for Estonia reached 95% probability to detect CWD at the 5% design prevalence (Table 17).

Due to the intensified surveillance in areas of positive cases, the uSSe for PSU with Ly- cases detected in red deer were high. The uSSe for the PSU with Ly- case detected in Norway were 91% (Etne municipality) and 99% (Bremanger and Gjemnes municipalities) for the design prevalence of 1% (and RR for high-risk target groups vs. HSHC = 5).

Table 17: Outputs of the disease detection model with estimates of the sensitivity of the surveillance for red deer

Country	cDP	uDP	RR	cSSe	Median uSSe	PSU	Prob 80	Prob 95
Norway	3/241	1	5	69.5	22.8	217	34	22
Norway	3/241	5	5	93.3	72.3	217	100	77
Latvia	3/141	1	5	12.0	3.1	141	0	0
Latvia	3/141	5	5	42.8	14.6	141	0	0
Poland	1/16	1	5	37.0	38.2	15	0	0
Poland	1/16	5	5	84.0	92.5	15	12	4
Estonia	1/15	1	5	7.7	12.8	4	1	0
Estonia	1/15	5	5	13.6	50.2	4	1	1

cDP: % Design prevalence among PSUs; 3 per 100 (or total) PSU. At least 1 per total PSU declared; uDP: % Design prevalence within infected PSU (detectable prevalence within PSU); RR: relative risk of high-risk target groups vs. HSHC; cSSe % Country level SSe; uSSe % SSe for PSU, summarised by the median uSSe for PSU with samples; PSU Number of PSU with samples registered; Prob80 Number of PSU with unit SSe reaching at least 80%; Prob95 Number of PSU with unit SSe reaching at least 95%.

Roe deer

The number of samples from roe deer included in the analyses ranged from 8,409 for Norway and 2,003 for Poland to the lowest numbers collected for Finland (619) and Sweden (225). The proportions of samples excluded due to missing information on PSU were 15% (Latvia) and 33% (Poland). The number of PSUs with 0 or 1 sample registered were 31 (Norway), 0 (Poland), 1 (Estonia), 29 (Latvia), 259 (Finland) and 27 (Sweden). The proportion of samples from the high-risk groups (mainly road kills) was 69% for Norway, 91% for Poland, 41% for Estonia, 5.2% for Latvia, 95% for Finland and 98% for Sweden.

The presence of PSU with CWD in roe deer at a prevalence of 5% is unlikely in Norway and Poland. For Poland, the presence of PSUs with CWD in roe deer is even unlikely at a prevalence of 1%. Assuming the infection risk in the high-risk animals to be five times higher than for the low-risk animals, the probability of detecting CWD in wild roe deer in Norway was 77% and 96% for the design prevalence of 1% and 5%, respectively. Poland reached 95% probability of detecting CWD in wild roe deer even for the design prevalence of 1%. 8% of 226 tested PSUs for Norway, 81% of 16 tested PSUs for Poland, 1.5% of 13 tested PSUs for Estonia, 0% for Latvia, 1.3% of 75 tested PSUs for Finland and 0% for Sweden reached 95% probability to detect CWD at the 1% design prevalence (Table 18).

Table 18: Outputs of the disease detection model with estimates of the sensitivity of the surveillance for roe deer

Country	cDP	uDP	RR	cSSe	Median uSSe	PSU	N PSU Prob 80	N PSU Prob 95
Norway	3/226	1	5	77.1	31.8	226	43	18
Norway	3/226	5	5	96.4	86.6	226	124	96
Poland	1/16	1	5	94.6	98.6	16	15	13
Poland	1/16	5	5	99.8	100	16	16	16
Estonia	1/15	1	5	51.1	74.3	13	3	2
Estonia	1/15	5	5	72.4	99.9	13	10	9
Latvia	3/198	1	5	16.4	4.6	198	0	0
Latvia	3/198	5	5	53.3	21.2	198	1	0
Finland	3/295	1	5	13.1	3.9	75	3	1
Finland	3/295	5	5	30.4	19.5	75	17	11
Sweden	3/100	1	5	18.6	11.2	32	1	0
Sweden	3/100	5	5	46.3	47.6	32	9	5

cDP: % Design prevalence among PSUs; 3 per 100 (or total) PSU. At least 1 per total PSU declared; uDP: % Design prevalence within infected PSU (detectable prevalence within PSU); RR: relative risk of high-risk target groups vs. HSHC; cSSe: % Country level SSe; uSSe: % SSe for PSU, summarised by the median uSSe for PS with samples; PSU: Number of PSU with samples registered; Prob80: Number of PSU with unit SSe reaching at least 80%; Prob95: Number of PSU with unit SSe reaching at least 95%.

Farmed red deer

The number of samples from farmed red deer included in the analyses was 466 for Sweden and 146 for Poland. The numbers of PSU with 0 or 1 sample registered were 56 (Sweden) and 12 (Poland). The proportion of samples from the high-risk groups was 16% for Sweden and 45% for Poland.

The surveillance resulted in a low probability of detecting the potential presence of CWD in farmed red deer. Assuming the infection risk in the high-risk animals to be five times higher than for the low-risk animals, the country-specific probabilities of detecting CWD at a design prevalence of 5% were 48% for Sweden and 14% for Poland. At the PSU level, 1 out of 70 farms tested in Sweden and 1 out of 4 tested PSU in Poland reached 95% probability of detecting CWD at the 5% design prevalence (Table 19).

Table 19: Outputs of the disease detection model with estimates of the sensitivity of the surveillance for farmed red deer

Country	cDP	uDP	RR	cSSe	uSSe	N total.u	units	N PSU Prob 80	N PSU Prob 95
Sweden	3/109	1	5	15.2	3.9	109	69	0	0
Sweden	3/109	5	5	48.3	19.4	109	69	4	1
Poland	1/16	1	5	7.8	13.4	16	4	1	0
Poland	1/16	5	5	13.8	51.2	16	4	1	1

cDP: % Design prevalence among PSUs; 3 per 100 (or total) PSU. At least 1 per total PSU declared; uDP: % Design prevalence within infected PSU (detectable prevalence within PSU); RR: relative risk of high-risk target groups vs. HSHC; cSSe: % Country level SSe; uSSe: % SSe for PSU, summarised by the median uSSe for PSU with samples; PSU: Number of PSU with samples registered. For farmed deer in Sweden, PSU refers to individual farms; Prob80: Number of PSU with unit SSe reaching at least 80%; Prob95: Number of PSU with unit SSe reaching at least 95%.

3.1.8. Concluding remarks on ToR1

- Heterogeneity in surveillance approaches: The implementation of surveillance for CWD in the eight countries (Estonia, Finland, Iceland, Latvia, Lithuania, Norway, Poland and Sweden) included in the mandate has shown great heterogeneity in terms of species, number of samples, surveillance groups, management systems and time. Surveillance to detect CWD ranged from the extensive (voluntary) efforts of Norway since the detection of first case of CWD in the country in March 2016, to Iceland with only a few hundred reindeer tested during the mandate period. The implementation of the European Commission statutory surveillance in the six MS (Finland, Estonia, Latvia, Lithuania, Poland and Sweden) also varied widely in terms of the design (number, size and characteristics of the declared PSU), the number of cervids tested in general and per PSU in particular and the distribution of testing by species and high-risk target groups. The hunted and slaughtered fit for human consumption (HSHC) group was the most frequently tested overall in terms of number of animals (83.8% of the total tested older than 12 months of age and of unknown age) in six of the eight countries, with road kills (RK) being the most tested high-risk target group in Finland and Poland. Roe deer was the most tested species in three MS (Estonia, Latvia and Poland). Setting aside the extensive sampling done by Norway, for the seven other countries the overall number of roe deer (8,417) and reindeer (8,028) tested were nearly equal, and about double the number of European moose (4,032) or red deer (3,172).
- Detection of CWD: The statutory surveillance detected prion diseases in cervids in two new countries (Finland and Sweden). During the mandate period, a total of 31 cases were confirmed: 13 reindeer, 15 moose and 3 red deer.
- Detection in new species: Red deer cases were detected for the first time on the European continent, in western Norway whereas no cases were detected among 17,380 roe deer ≥ 12 months of age and of unknown age tested, including 9,331 collected from the three countries that detected CWD in other species. Overall, expanded surveillance during 2017–2022 revealed that CWD was more widely geographically distributed than had been assumed in 2017 based on previous surveillance done in Europe.
- Application of intensified surveillance: Per statutory requirements, intensified surveillance in affected and surrounding PSU was implemented by Sweden and Finland in areas where cases of CWD had been found, expanding the testing to animals including HSHC in order to maximise sample size. Two additional cases (both Ly-) were detected in one of the four areas subjected to intensified surveillance. Prevalence in affected areas: apparent (observed) prevalence of CWD was low in locations where cases were detected. For the Ly- form, now detected in three countries, prevalence among HSHC moose sampled from affected areas was ~0.05% (95% CI: 0.006–0.17%), and was ~ 0.16% (95% CI: 0.04–0.57%) among HSHC red deer from affected areas in Norway. For the Ly+ form, thus far detected only in Norwegian reindeer, apparent prevalence also was $< 1\%$ (0.23%; 95% CI: 0.12–0.4%) among HSHC animals sampled from both affected populations, while it reached higher levels in adult males.
- Value in sampling high-risk animals: As expected, apparent prevalence was higher ($> 10\times$) among moose and red deer submitted from high-risk target groups, emphasising the value of high-risk target groups in surveillance to detect new CWD foci or cases of CWD Ly-. This relationship also held for the Ly+ phenotype among reindeer, although with less certainty considering the wide confidence intervals. Comparison among target groups revealed that RK

submissions yielded no cases and most closely approximated HSHC submissions in terms of infection probability, suggesting merit in excluding RK submissions from the high-risk target groups (SUS, FC and HSNHC) in future surveillance.

- Country-level minimum detectable prevalence: The calculations of the minimum detectable prevalence at the country level revealed a prevalence in the general population (all species for the entire mandate period), including all animals tested, close to the 0.1% estimated in the EFSA opinion (2017). There is high variability between countries and between species within countries. By species, reindeer (only tested in four countries) is the one with the lowest overall minimum detectable prevalence, followed by moose, red deer and roe deer.
- Variable spatial representation in sampling effort: When the analysis takes into account the geographical distribution of sampling at PSU level and the animals tested older than 12 months of age or of unknown age in the high-risk groups, there is a low proportion of PSU in which the minimum detectable prevalence attainable was 10% (EFSA BIOHAZ Panel, 2017) or lower: 40% of the declared PSU in Estonia, 4.5% of the declared wild PSU In Finland, 0% in Latvia, unknown in Lithuania, 23.5% of the declared semi-domesticated PSU in Sweden, 22% and of the declared wild PSU in Sweden. Only Poland (90% of the declared PSU) and Finland in semi-domesticated PSU (68.5%) had more than 50% of the PSU with a minimum detectable prevalence of 10% or lower. The size and number of declared PSU affect substantially the interpretation of the calculation of the minimum detectable prevalence. Larger countries with lower numbers of PSU like Poland had better chances to achieve a lower detectable prevalence. However, representativeness of the sampling within large PSU cannot be assessed. Considering the surveillance findings to date, the relevance of using a country-level, multispecies approach in calculating minimum detectable prevalence for future surveillance merits further discussion.
- The two objectives of CWD surveillance (detect disease and estimate prevalence) have partially been met, given the high variability in the implementation of surveillance at country, species and PSU levels. As a result, for countries that conducted surveillance during the mandate period without finding CWD, the evidence is not sufficient to rule out the possibility of CWD being present. For those countries with cases detected, the observed prevalence is associated with uncertainty due to the surveillance design affected by sampling errors.
- Scenario tree modelling has been used to estimate at national level the surveillance system sensitivity (SSe) and to evaluate the probability of species-specific presence of disease at a prevalence of 5% or 1% (within infected sampling units) and a relative risk (RR) high-risk vs. HSHC of 5 (baseline assumption) and 2. For each country, it was assumed that 3% of PSU were infected, or if more than 100 PSU were defined, three PSU were assumed infected.
- For all cervids (except Norwegian wild reindeer and farmed deer) combined, results show that the presence of PSU with CWD at a prevalence of 1–5% or higher is unlikely in Norway, Sweden and Poland (5% design prevalence: all cSSe > 95%, 1% design prevalence: cSSe = 94%, 96% and 86% for Norway, Poland and Sweden, respectively). For Finland, Latvia and Estonia, the country-level probability of detecting CWD at the 5% design prevalence (and RR of 5) was 83%, 65% and 74%. At the PSU-level, 277 (69%) PSU in Norway, 50 (51%) in Sweden, 73 (26%) in Finland, 0 in Latvia, 10 (77%) in Estonia and 16 (100%) in Poland reached 95% probability of detecting CWD at the 5% design prevalence.
- Presence of PSU with CWD for different species and probability of detection at PSU-level:
  - Semi-domestic reindeer: (a) presence of PSU with CWD at a prevalence of 5% is unlikely in Norway, Sweden and Finland (cSSe = 99%, 92% and 99%, respectively); (b) country-level probability of detecting CWD at a minimum detectable prevalence of 1% was high in Norway (cSSe = 95%; cSSe = 66% and 82% for Sweden and Finland, respectively); (c) at PSU level, 83% (59 of 71) of tested PSU in Norway, 55% (28 of 51) in Sweden and 84% (49 of 58) in Finland reached 95% probability to detect CWD at the 5% design prevalence.
  - Moose: (a) presence of PSU with CWD at a prevalence of 5% is unlikely in Norway and Sweden (cSSe = 98% and 91%, respectively); (b) For 1%, none of the six countries assessed had at least 95% sensitivity; (c) at PSU-level, 49% (125 of 256) of tested PSU in Norway, 40% (19 of 48) in Sweden and 2% (4 of 182) in Finland reached 95% probability of detecting CWD at the 5% design prevalence.
  - Red deer (in wild): (a) presence of PSU with CWD at a prevalence of 5% or higher is unlikely in Norway and, with relatively high confidence, also in Poland (cSSe = 93% and

84%, respectively); (b) None of the four countries assessed (Norway, Latvia, Poland and Estonia) achieved 95% sensitivity to detect CWD in red deer if present at a minimum of 1% or 5% prevalence; (c) at PSU level, 35% (77 of 217) of tested PSU in Norway, 27% (4 of 15) in Poland, 1 of 4 in Estonia and 0 for Latvia reached 95% probability of detecting CWD at the 5% design prevalence.

- Roe deer: (a) presence of PSU with CWD at a prevalence of 5% or higher is unlikely in Norway and Poland (cSSe = 96% and 99%, respectively); (b) For Poland, it was also unlikely at a prevalence of 1% or higher (cSSe = 95%); (c) at PSU level, 42% (96 of 226) of tested PSU in Norway, 16% (5 of 32) in Sweden, 15% (11 of 75) in Finland, 100% (16) in Poland, 69% (9 of 13) in Estonia and 0% for Latvia reached 95% probability of detecting CWD at the 5% design prevalence. 81% (13 of 16) of tested PSU in Poland reached 95% probability of detecting CWD at the 1% design prevalence. However, if assuming a relative risk ratio of 2 instead of 5 for the high-risk group (mainly road kills), only 1 PSU in Poland reached 95% probability of detecting CWD at the 1% design prevalence.
- Farmed red deer: (a) The presence of PSU with CWD at a prevalence of 5% or higher is uncertain in farmed red deer tested for Sweden and Poland (cSSe = 49% and 20%, respectively); (b) One of 70 farms tested in Sweden and 1 of 4 tested PSU in Poland reached 95% probability to detect CWD at the 5% design prevalence. Norway tested more farmed red deer (2,364, 3.5% in high-risk group) than the other countries together, but data were not submitted to EFSA at farm level.

3.2. New knowledge on the epidemiology of CWD (ToR2)

3.2.1. Description of the available epidemiological knowledge until the last EFSA CWD opinion

There is a substantial body of published work on the epidemiology of CWD in North America, where this disease has been recognised for more than 50 years. A comprehensive review of the relevant literature was undertaken for the previous CWD opinions (EFSA BIOHAZ Panel, 2017, 2018, 2019) and the key points from those opinions most relevant to the current opinion are summarised briefly here, for historical context and ease of reference. (For individual references please refer to the original EFSA opinions: EFSA BIOHAZ Panel, 2017, 2018, 2019).

3.2.1.1. The North American situation

CWD has been reported in a range of cervid species in North America, including small numbers of moose, captive red deer and a case in a captive reindeer. North American CWD cases detected thus far have been of the phenotype denoted in the current opinion as Ly+, although cases have been encountered with PrP^d detected only in brain tissue. Based on experimental oral infections, the estimated incubation period preceding onset of clinical signs is about 15 months in mule deer and white-tailed deer and between 12 and 34 months in wapiti, with longer incubations observed in some *PRNP* genotypes of each host species. Most clinical cases are observed in animals between 2 and 7 years of age. Affected animals shed prions naturally via multiple routes throughout most of the disease course, suggesting a role for excretions and secretions in contagious lateral transmission, with oral exposure appearing to be the main natural infection route, following interactions with an infectious host or prion-contaminated environmental sources (e.g. infectious carcass remains, food, water, soil). Prions have been shown to resist degradation and may persist for years in some environments. Indirect transmission greatly complicates CWD control strategies.

The occurrence of CWD in affected populations or species can vary. Consequently, caution is needed when considering field data. Prevalence may be low in captive herds where CWD introduction was thought to be recent, but over time has approached 100% in research facilities in which the disease is endemic. In affected free-ranging populations, reported prevalence has ranged between < 1% and 30% or more. North American deer (*Odocoileus* spp.) generally show higher prevalence than syntopic wapiti or moose, but high rates have been described in captive and free-ranging wapiti so patterns in the field may be more a function of social and foraging behaviour differences than differences in susceptibility.

Surveillance activities have been applied extensively to CWD in North America, but one of the most common flaws in CWD control efforts to date has been an initial underestimation of the affected area

(often based on inadequate surveillance and erroneous assumptions about how long disease has been present). Random sampling (e.g. from harvested animals) seems better suited for providing relatively unbiased prevalence or incidence estimates, while a risk-based strategy focusing on subpopulations at expected higher risk may be particularly efficient when detection is the primary aim. It is also necessary to apply an appropriate disease distribution model within the population. For example, a random disease distribution model is not considered realistic for CWD, while a more realistic disease distribution model is given by the clustering of diseased animals within the population, with only a few outlying cases.

Reviewing North American data underscored the heterogeneity of CWD distribution. The observed pattern of CWD distribution in the eastern US that apparently resulted from multiple foci emerging over the previous two decades or longer offered a conceptual model for considering how CWD might appear to 'emerge' in Europe given the similarities in size, complexity and flawed surveillance approaches shared by the geographically distinct areas where CWD has now been detected. Data from an uneven surveillance effort in the eastern US that was spatially incomplete and spatially biased could be misleading. For example, sampling for the first time in areas adjacent to recently detected foci can give the superficial appearance of spatial 'spread' if the lack of prior surveillance is not considered. Spatial heterogeneity is therefore an important challenge when designing and interpreting surveys for detecting CWD.

Assessing large-scale temporal trends in North America has been problematic. In general, until the mid-1990s only two US states (Colorado, Wyoming) and a zoo in Canada (Ontario) had reported cases; however, few other jurisdictions were looking for CWD. Over the subsequent years, a far wider distribution was observed. Although described by some as 'rapid spread', it has been argued that the pattern reflects ever widening efforts to detect disease and is not a 'real time' representation of geographic spread. It has been observed that – in the absence of control efforts – the prevalence of CWD at any particular point in time is correlated with temporal distance since introduction at that location, as a surrogate for the time required for disease spread or 'disease history'.

Field and modelling data from North America suggest that CWD epidemics develop relatively slowly as compared to other infectious diseases in wildlife. Prevalence likely remains low, and infections spatially localised, for a decade or more after the introduction of the disease into natural cervid populations. One likely outcome of focusing detection on standard thresholds (e.g. 1% design prevalence) in North America has been that CWD may have been present for 10–20 years before the first case was identified in a cervid population unit. An even longer period of time likely would to be needed for a CWD outbreak to expand across an entire political jurisdiction (e.g. a state, province or country). It is in part for this reason that updated recommendations on CWD surveillance in the EU included consideration of dividing cervid populations into multiple, biologically relevant spatial units within each MS in order to increase detection probabilities.

In general, males of North American deer species experience higher apparent risk than females (e.g. in a cohort of mule deer, prevalence among the sampled adult male deer was about twice the prevalence among adult females). This difference seems likely to be explained by different behaviour. For both sexes, the risk of infection appears to increase in early adulthood, resulting in relatively high prevalence in adult (> 2-year-old) mule deer as compared with juveniles and yearlings, and in a decline in older age classes.

Multiple risk factors have been suggested to facilitate the introduction and spread of CWD. The incursion of the disease into unaffected populations or areas may be due to the natural movements of cervids and/or the human-assisted translocation of infected animals or perhaps fomites.

Genetics must also be considered as a risk factor. Polymorphisms in the *PRNP* appear to influence susceptibility even though this remains less understood for CWD than the well-documented and strong genetic influence on TSE susceptibility of the *PRNP* in small ruminants, for example.

From North American observations, the natural host range of CWD is known to include white-tailed deer, mule deer/black-tailed deer, moose, wapiti, reindeer (captive) and red deer (captive). European red deer and muntjac deer have been shown susceptible to CWD following experimental oral challenge. Fallow deer, however, have proven to be relatively resistant.

Experience in Colorado, Wyoming and Wisconsin has shown that the probability of finding a CWD-positive animal may be greater among sick-looking animals than the general population. Animals involved in road accidents or killed by predators also may have a higher probability of infection than the general population. Although screening sick-looking animals may help increase detection probabilities, when sampling collection depends on the voluntary reporting of sick or dead wild animals it may be difficult to obtain a large number of samples for several reasons: (a) most animals with

clinical signs will not be observed/detected in the wild; (b) it may be difficult to gain access to the carcass (deep in the forest, in rivers); (c) the need of transport from remote areas; (d) low quality of the material (advanced autolysis so there is no brain left).

3.2.1.2. The European situation

During the period 2006–2010, a survey was carried out in the EU,[8] based on recommendations from Scientific Steering Committee (SSC) and EFSA opinions, with the aim of detecting the possible presence of CWD and other TSE in the EU farmed and wild cervid populations. No TSE-positive results were encountered. A subsequent EFSA opinion reviewed the results and concluded that there was not a cervid TSE epidemic in the EU but that, based on available data, the 'occurrence of cases of TSE, especially in remote and presently unsampled geographic areas, may not be excluded in cervids in the EU'. The Bayesian approach used to arrive at prevalence estimates was based on an aggregated sample, but the assumption that free-ranging or captive cervids in the EU represent a single homogeneously mixed population is not supported by biological or epidemiological data. The assumption of homogeneous disease distribution was also untenable. The actual implementation of surveillance in 2006–2010 was also deemed to have had logistical and practical limitations, and where additional data on geographical distribution of sampling were available it confirmed that there were discrepancies in its geographical representativeness.

The monitoring activities in cervids carried out throughout the EU since 2010 have been sporadic, not supported by any specific study design or targeting strategies (with the exception of one study in Germany) and examined relatively few animals. Between 2011 and 2014, the testing activity in the MS was minimal. In 2015, based on available official data from the TSE annual reports submitted by the MS to the European Commission, only Finland and Hungary and one non-MS (Norway) reported test results for TSE in cervids. None of the samples tested positive, but the number of tested animals over the 2011–2015 period was insufficient to draw any epidemiological conclusions.

The nearly simultaneous detections of CWD in reindeer and moose from separate geographic locations in Norway during 2016 led to a greatly expanded Norwegian cervid surveillance programme that generated substantial new data, but the temporal dynamics of CWD in European cervid species remain unclear given its recent detection and the aforementioned inadequacies of historical surveillance.

The pattern of PrP^{Sc} distribution observed in the earliest detected reindeer cases (n = 8) showed lymphoid tissue involvement in all infected individuals, with CNS involvement in five of these, which supported the initial assumption that European CWD presented with a phenotype (referred to in the current opinion as Ly+) that resembled CWD in North American deer and wapiti. It followed that the expected natural (e.g. horizontal) transmission and epidemic dynamics would be similar to that described for North American cervids.

Comparing the point estimate of CWD prevalence among 'adult' (>1 year old) wild reindeer harvested (including hunted and found dead/injured/diseased) in Nordfjella Zone 1 in 2016 (3/310: 0.97%; 95% C.I.: 0.2–2.8%) and during 2017 (up to 27 November 2017: 5/738: 0.68%; 95% C.I.: 0.22–1.6%) with the epidemic curve for mule deer, it was considered plausible that CWD had become established in Norway more than a decade earlier.

Retrospectively assessing surveillance data from Norway collected during the slaughter season for semi-domesticated reindeer (December 2003–February 2004) revealed that 792 animals were screened for TSE, with all found negative. A further 2,163 cervids were found negative between 2004 and 2015, leading to the conclusion that there was no CWD epidemic in Norwegian cervid populations. However, the sample size was very limited as only 10 wild reindeer and 130 moose were analysed, none of them originating from the areas in which CWD has since been identified. Consequently, low-level occurrence of CWD during this period could not be excluded.

The first case of CWD in Norway was the first case of naturally occurring CWD in reindeer worldwide, but it has been known that the species is susceptible to the disease since researchers had reported successful experimental oral transmission of North American CWD to reindeer. It is also notable that CWD cases in moose seem to be a rare occurrence in North America, whereas two of the first five CWD cases detected in Norway were in moose. This difference might be due to the fact that the Scandinavian moose population is much larger than that in the parts of North America where CWD

[8] 2007/182/EC: Commission Decision of 19 March 2007 on a survey for chronic wasting disease in cervids (notified under document number C(2007) 860) (Text with EEA relevance).

occurs, or due to differences in CWD strains or exposure probabilities in moose between the two continents.

Differences in phenotype are also reported: A natural case of CWD in a hunted moose in Colorado presented with PrP^{Sc} in both the brain and lymphoid tissue, whereas the Norwegian moose cases do not have any detectable involvement of the LRS. This absence of detectable PrP^{Sc} in non-neural tissues in the Norwegian moose cases has been hypothesised to be linked to a lower (or possibly absent) shedding of prions, which may impact on its transmissibility under field conditions. It may also affect detection, as surveillance in North America often targets lymphoid tissues only.

3.2.2. Description of the new epidemiological knowledge since the last EFSA CWD opinion (AQ 2.1)

3.2.2.1. Apparent novelty & heterogeneity of CWD in Europe

The first Norwegian moose cases described in 2016 showed patterns of immunolabelling in the brain and molecular profiles of PrP^{Sc} from Western blot that were different from patterns seen in North American CWD isolates (regardless of species) and also from the Norwegian reindeer cases (Pirisinu et al., 2018). At the time of the last EFSA opinion (EFSA BIOHAZ Panel, 2019), several bioassay studies were underway to explore these apparent differences. Those studies have now been completed, and their results have verified (1) that from a total of eight isolates (from three species) at least five biologically distinct types of cervid TSE (potentially representing different strains) are present in northern Europe, and (2) that none appears to be a direct extension of North American CWD (reviewed by Tranulis et al., 2021 and by Otero et al., 2022).

As reflected in data presented earlier in this opinion (Section 3.1.5), differing epidemiological patterns seem to be associated with the Ly+ and Ly- CWD cases encountered thus far (also reviewed by Tranulis et al., 2021). The Ly+ cases (to date, all in reindeer) have clustered in two neighbouring populations (Figure 6), with a relatively high apparent prevalence in one (Mysterud et al., 2019a). By comparison, the Ly- cases in moose and red deer have been rare but more geographically scattered, with most presenting as seemingly isolated cases in older aged animals (Tranulis et al., 2021). Considerable uncertainty remains regarding the origin and epidemiology of both CWD phenotypes infecting European host species. Given the differences in epidemiological patterns observed thus far and the evidence supporting the hypothesis that multiple different strains are involved in the CWD cases encountered in Europe, direct extrapolation from North American data and experiences to the European context has been undertaken with caution in recent years.

3.2.2.2. Demographic infection pattern of reindeer from Nordfjella

Analysis of data from the CWD outbreak in Nordfjella has now been published (Güere et al., 2019; Mysterud et al., 2019a). A total of 19 CWD cases in reindeer were confirmed in the population of Nordfjella, Norway, out of a total of 1,081 males and 1,278 females being tested from spring 2016 to spring 2018. Of these, CWD was detected in six females aged between 3 and 4 years of age and 13 males aged 1.5–8.5 years of age. Infection was not detected in calves, and only in one yearling (1.5 years old). There was a higher CWD prevalence in adult males compared to adult females (adults above 2 years old), with infection being 2.7 times (95% CI 1.0, 7.2) more likely in adult males after accounting for age. The apparent prevalence was 1.5% in adult males and 0.5% in adult females, while the true prevalence (adjusted for detection probability) was 1.8% in adult males and 0.6% in adult females (Mysterud et al., 2019a). Prevalence increased with age in males. Adult males $\geq$ 5 years had an estimated true prevalence of 3%. These estimates are associated with large uncertainty due to the low number of cases. All of the 19 reindeer were of the Ly+ phenotype, with brainstem samples from 10 of them (52.6%) also testing positive (Rolandsen et al., 2019).

3.2.2.3. *PRNP* variation in cervids in Europe

It is well established from epidemiological investigations and experimental inoculations that *PRNP* genetic variation can profoundly influence prion disease susceptibility and disease progression in humans and animals. Among sheep and goats, the effect of *PRNP* genetic variation on classical scrapie susceptibility is particularly well described and utilised in disease controlling breeding programs (EFSA, 2018, 2019, 2020, 2021).

PRNP variation can emerge in a population as random genetic mutation, but its fixation, with an increased frequency in the population, can be the result of different factors. It can be a casual effect such as genetic drift, with a resultant loss of the variant, mainly in populations of limited dimensions.

Another possibility is that increased frequency of a particular polymorphism is the result of natural selection. The variants can be under the effect of positive or balancing selection. In the first case, a variant is positively selected due to its advantageous effect on carriers, favouring their reproduction and leading to an increase in its frequency in the population. In balancing selection, the diversity at a locus is maintained in a population either by frequency-dependent selection or because it is the heterozygote, rather than homozygote, status that confers an advantage. In both cases, genetic variants already exist in the population. An increase in those traits only occurs when a change in selection pressure causes them to favour reproduction.

The major European cervids, namely, roe deer (*Carpreolus capreolus*), red deer (*Cervus elaphus*), reindeer (*Rangifer tarandus tarandus*) and European moose (*Alces alces*), all share a common variant of *PRNP* giving rise to a PrP molecule with identical amino acid composition. This variant of *PRNP* is in this opinion called the 'wild-type' (wt) allele. Mammals carry two alleles of any given gene. An animal with two wt *PRNP* alleles is homozygous for this allele and its *PRNP* genotype is wt/wt.

In roe deer, this is the only observed *PRNP* allele. Thus, they appear to be monomorphic for the wt-allele (Peletto et al., 2009; Wik et al., 2012; Robinson et al., 2019; Güere et al., 2022).

PRNP genetic variation is also very limited in European moose encoding only one *PRNP* allele in addition to the wt (Wik et al., 2012; Güere et al., 2022). The variant stems from a mutation at position (codon) 109, replacing lysin (K) with glutamine (Q). The variant allele is given as 109Q. Consequently, moose can have the three *PRNP* genotypes: wt/wt, wt/109Q, 109Q/109Q.

In red deer, variation has been recorded at codons 59, 98, 168, 226 and 247 (Peletto et al., 2009; Pitarch et al., 2018; Robinson et al., 2019), of which substituting glutamine (Q) with glutamate (E), at codon 226 is by far the most common. Variation at codons 59 and 168 appear to be very rare, while variation at codon 98 (98A), has been observed at close to 30% in some Scottish populations, while apparently absent in red deer in Southern England and Norway (Robinson et al., 2019).

In reindeer, the *PRNP* genetic variation is high and comparable to that in sheep. In addition to the wt-allele, six variant *PRNP* alleles, giving rise to different coding sequences have been reported (Güere et al., 2020, 2021). Four of which are single amino acid substitutions; 176D, 207 M, 211Q and 225Y. In addition, one allele involves three codons (2, 129 and 169): 2M129S169M. Finally, a *PRNP* allele where eight amino acids are deleted, a type of variation also seen in other mammals, has been also reported. The allele is called 'deletion'.

PRNP allele frequencies will normally vary considerably between subpopulations of the same species, reflecting the origin and history of separate populations. In Table 20, therefore, allele frequencies are given primarily to indicate which alleles that are most common in a species. Subsequent analysis of distinct subpopulations might reveal different allele proportions. For semi-domesticated reindeer, this is illustrated by allele frequency range when comparing five herds of reindeer.

Table 20: *PRNP* variant positions in cervid species

***PRNP* type**		**2**	**Octarepeats**	**20**	**59**	**95**	**96**	**98**	**100**	**109**	**116**	**129**	**132**	**138**	**168**	**169**	**176**	**207**	**209**	**211**	**225**	**226**	**Reference**
Consensus amino acid sequence	**Freq.**	V	5	D	G	Q	G	T	S	K	A	G	M	S	P	V	N	K	M	R	S	Q	
Subfamily Cervinae																							
Rocky Mountain wapiti																							O'Rourke et al. (1999)
																						E	White et al. (2010
132 L													L									E	Perucchini et al. (2008)
Red deer																							Peletto et al. (2009)
59S	0.2%				S																	E	Peletto et al. (2009)
98A	8%							A														E	Peletto et al. (2009), Robinson et al. (2012)
168S	0.2%														S							E	Peletto et al. (2009), Robinson et al. (2012), Pitarch et al. ((2018)
226Q	44%																						Peletto et al. (2009), Robinson et al. (2012), Pitarch et al. (2018)
Sika deer[(c)]																							Jeong et al. (2007)
100G	3%								G													E	
226Q	48%																						
Fallow deer																							Rhyan et al. (2011)
monomorphic	100%													N								E	
Subfamily Capreolinae																							
Reindeer (semi-domesticated)[(d)]																							Güere et al. (2021)
225Y	(31.7–70)%																					Y	
Deletion	(0–1.7)%		4																				
176D	(8.6–26.7)%																D						

PRNP type		2	Octarepeats	20	59	95	96	98	100	109	116	129	132	138	168	169	176	207	209	211	225	226	Reference
2M129S169M	(10–35)%	M										S				M							
207 M	0–1.2)%																	M					
211Q	(0–0.1)%																			Q			
White-tailed deer																							Johnson et al. (2003), Johnson et al. (2006)
95H	2%					H																	Kelly et al. (2008)
96S	26%						S																O'Rourke et al.(2004)
116G	13%										S												Wilson et al. (2009)
226 K	0.5%																					K	Heaton et al. (2003)
138 N pseudogene	15%													N									Brayton et al. (2004)
Mule deer																							Jewell et al. (2005)
20G	9%			G																			Wilson et al. (2009)
225F	5%																				F		Heaton et al. (2003)
138 N pseudo gene	~ 100%													N									
Moose																							
209I	45%																		I				Huson and Happ (2006)
109Q										Q													Güere et al. (2021), Wik et al. (2012)
Caribou																							Happ et al. (2007)
129S	2%											S											
138 N	30%													N									
2 M/129S/169 M	4%	M										S				M							
Roe deer																							Peletto et al. (2009)
monomorphic	100%																						
Chinese water deer																							Jeong et al. (2009)
100 N	na								N														

(a): Adapted from Robinson et al. (2012).
(b): When blank, data on other polymorphisms not reported by the publication.
(c): Sika deer (*Cervus nippon*) are now included in the list of species naturally susceptible to CWD (Sohn et al., 2020 and references therein). Amino acid codes: A, alanine; D, aspartic acid; E, glutamic acid; F, phenylalanine; G, glycine; H, histidine; I, isoleucine; K, lysine; L, leucine; M, methionine; N, asparagine; P, proline; Q, glutamine; R, arginine; S, serine; T, threonine; V, valine.
(d): Range of allele frequencies are based on analysis of five Norwegian herds of semi-domesticated reindeer.

3.2.2.4. *PRNP* alleles associated with susceptibility to CWD – observations from Norway

The distribution of *PRNP*-genotypes between CWD cases and controls differed in reindeer in Nordfjella (Güere et al., 2020), with a significantly increased CWD risk in reindeer carrying two copies of the wt allele coding for serine in position 225 (Ser225) or in those carrying this allele with the 24 bp deletion. 52.8% of CWD+ cases were wt/wt, while the proportion of wt/wt among controls was 14.9% in Nordfjella. CWD cases were also observed in genotypes wt/225Y and 225Y/deletion. The single male on Hardangervidda provided a new positive genotype (wt/176D) (Güere et al., 2022). For the first 20 CWD+ reindeer cases, 50% were wt/wt, 20% wt/225Y, 20% wt/deletion, 5% wt/176D and 5% 225Y/deletion (Güere et al., 2022).

A wider screening of *PRNP* variation in 365 reindeer, 105 red deer, 137 moose and 46 roe deer from Norway has been reported (Güere et al., 2022). The susceptible wt allele was more frequent in wild reindeer compared with semi-domesticated reindeer, in which alleles 225Y, 176D and 2M129S169M were present at higher frequencies. This suggests that many of Norway's wild reindeer populations are genetically more susceptible to CWD than most semi-domesticated herds, given the overall higher frequency of susceptible wild-type *PRNP* in wild reindeer (on average 55%) compared to 20% in semi-domesticated reindeer. Differences in *PRNP* composition between wild and semi-domestic reindeer have also been found in Russia (Kholodova et al., 2019, 2022).

The single CWD+ red deer sequenced was 226E/226E, which is common in Norwegian red deer. Among CWD+ moose, six had 109 K/109 K and two had 109Q/109Q. No moose cases have been confirmed in heterozygous 109 K/109Q.

Generally, data from North America and Norway associating *PRNP* alleles with CWD susceptibility suggest that the wt allele must be considered as a 'susceptibility allele', although the real-life susceptibility of any given species may be influenced by factors beyond its *PRNP* genetic makeup.

3.2.2.5. Status of geographic distribution of CWD among wild reindeer populations in Norway

A serious development of the CWD situation in Norway was the first detection of a CWD Ly+ reindeer in a new population: Hardangervidda (Figure 6). The detection was in a prime-aged male aged 8.5 years *(PRNP* genotype wt/176D) shot 3 September 2020 (Ytrehus et al., 2021). The male was lymph node positive only, suggesting an early stage of infection. Hence, the animal may have been infected after the elimination of the Nordfjella population (terminated 1 May 2018). An assessment of genetic relatedness suggested that this infected male originated from Hardangervidda and not Nordfjella, and hence, it is unlikely he had migrated from the Nordfjella mountain range. The Hardangervidda area harbours the largest population of reindeer in Europe, historically (1986–2021) ranging from 5,000 to 15,000 individuals, and most of the area has a status as a National Park (Mysterud et al., 2021a). There is currently no plan to depopulate, and this case therefore raises concern that CWD-Ly + will become endemic in Europe. The prevalence in the Hardangervidda population was estimated at ~ 0.1% before hunting in 2020. There was no further detection of cases in 2021 among 1437 tested individuals (Rolandsen et al., 2022), but a new CWD Ly+ female reindeer was shot on 27 September 2022 (aged 8.5 years with *PRNP* genotype wt/wt).

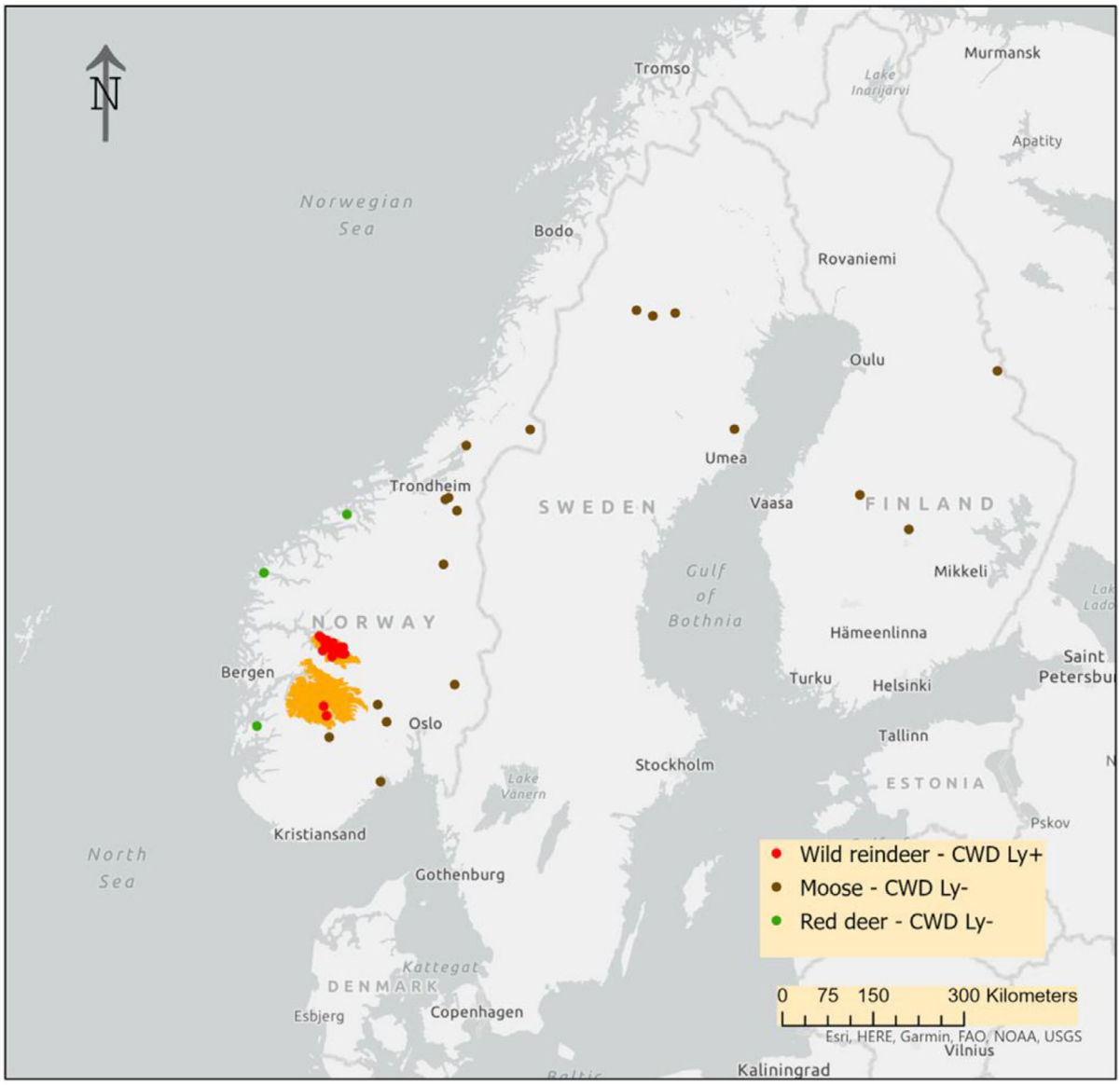

Figure 6: An overview of spatial locations of all detected CWD cases in Europe until November 2022. The GPS positions were provided by the national cervid register and the Norwegian Environment Agency for Norway, by the Swedish National Veterinary Institute for Sweden, and by the Finnish Food Authority for Finland
The map is curated by the Norwegian Institute for Nature Research (contact Dr. Christer M. Rolandsen) and updated on (https://www.hjortevilt.no/skrantesjuke-cwd/cwd-in-norway-english/) © Norwegian institute for nature research.
Disclaimer: The designations employed and the presentation of material on this map do not imply the expression of any opinion whatsoever on the part of the European Food Safety Authority concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries.

3.2.2.6. Status of geographic distribution of CWD among semi-domestic reindeer populations in Norway and Sweden

So far, no cases of CWD have been detected in semi-domestic reindeer that are raised in Norway and Sweden.

Nordfjella is bordered in the north-east by the semi-domestic reindeer population of Filefjell with about 3,000 individuals (Viljugrein et al., 2021). A (scenario tree) model for this specific management system was developed using three risk categories: sample target group, demographic group, and deviations in behaviour or physical appearance (Viljugrein et al., 2021). The model makes the

assumption that since herders stress the animals in penned areas during slaughtering in late fall, animals with deviant behaviour or physical appearance will be removed. Thus, it is likely that animals in the clinical stages of CWD will be included in the group of animals that is culled, if the population is infected. All clinical suspects, fallen stock and reindeer from the ordinary harvest tested negative. The likelihood of CWD absence is well above 99% even with the strict design prevalence of four individuals used by the Norwegian Food Safety Authority.

3.2.3. Concluding remarks on ToR2

- The geographic distribution in affected countries is patchy, and in areas where the disease has been found, the observed prevalence is low (< 1%).
- The geographic distribution, pattern of spatial clustering and age and sex distribution of infected individuals differed for cases of CWD phenotypes Ly- and Ly+. Analysis of a proportion of these cases (n = 8) indicates that they represent at least five distinct biological entities, and none of them aligns fully with CWD cases in North America.
- The Ly- cases have been reported in moose and red deer. Ly- cases in moose have been detected across large areas of Norway, Sweden and Finland and with limited geographic clustering. All cases have been reported in old individuals (> 10 years of age). So far all but one case has been detected in females.
- In Europe, the Ly+ phenotype of CWD has only been detected in reindeer in Norway. The 19 Ly+ cases (2016–2018) were clustered in the Nordfjella Zone 1 at higher prevalence than Ly- cases in moose and red deer. Ly+ was detected in six females aged between 3 and 4 years of age and 13 males aged 1.5–8.5 years of age. Infection was 2.7 times more likely in adult males compared to adult females. The apparent prevalence was 1.5% in adult males and 0.5% in adult females. Prevalence increased with age in males.
- The distribution of *PRNP*-genotypes between CWD+ cases and controls differed in reindeer in Nordfjella. In Nordfjella Zone 1, five *PRNP* alleles were observed. A higher susceptibility to Ly+ was linked to the presence of alleles wt and deletion, compared to 225Y, 176D and 2M129S169M.
- Two more cases of Ly+ wild reindeer were reported in the population of a new region, Hardangervidda: one adult male (8.5 years old) with wt/176D genotype in 2020 and one adult female (8.5 years old) with wt/wt genotype in 2022. The estimated prevalence is very low (<0.1%), but this may represent a new outbreak of the Ly+ phenotype.
- Despite surveillance efforts in areas bordering the main reindeer CWD outbreak, the disease has not been detected, to date, in semi-domestic reindeer.
- Roe deer are assumed to be susceptible to CWD based on genetic similarity to susceptible species (EFSA BIOHAZ Panel, 2017), and the data presented in this opinion do not rule out the possibility that roe deer are susceptible to CWD.

3.3. Recommendations for future monitoring activities for CWD (ToR3)

Further monitoring for CWD in Europe appears to be warranted given the findings to date. Future monitoring activities for CWD seem likely to be multifaceted and may be structured around individual or multiple surveillance aims, risk management or trading requirements. The scale and complexity of any future monitoring activity will also depend on how much information is already available in the context of the specific species/population/geographic area in question. Consequently, it is not possible, or appropriate, to propose any single approach for future monitoring at a European-wide level. However, findings summarised in this opinion can be used to inform the general approaches to passive or enhanced passive monitoring (i.e. the practice of opportunistic, 'suspect'-focused testing) and to conducting more formal surveys moving forward. Customised approaches can then be tailored to meet needs based on specific aims, context and deliverability. In the following section, the challenges and successes of the initial mandate surveillance activities are assessed and used to inform a stepwise approach to the design of potential future surveillance activity.

3.3.1. SWOT analysis (strengths, weaknesses, opportunities and threats) of surveillance systems

The previously implemented European surveillance efforts for CWD, as described in this opinion, have been assessed by means of a strengths, weaknesses, opportunities and threats (SWOT) analysis

with special focus on the mandate period. Weaknesses and threats are, respectively, the internal and external obstacles that must be addressed to improve a surveillance system; strengths and opportunities are elements that can be used to remove these obstacles. The purpose of the analysis was to define the development opportunities, which result from exploiting strengths and limiting weaknesses. It is useful to define which strengths to focus on or weaknesses to intervene on and which threats can be turned into opportunities.

Strengths

- Appropriate design of surveillance: The surveillance approach suggested by EFSA and reflected in Regulation (EU) 2017/1972 provided a more structured geographically based surveillance system compared to the previous surveillance with a clear focus on testing risk animals.
- Regulatory backing: e.g. Commission Decision 2007/182/EC, surveillance was made mandatory, hence a more standardised approach had to be implemented by the MS concerned. Moreover, in Regulation (EU) 2017/1972, there was a requirement to undertake intensified surveillance in areas where positive cases were detected, which has contributed to an increased understanding of the occurrence of the Ly+ and Ly- disease phenotypes, and their differing epidemiology.
- A harmonised data collection system at EFSA: It had already been established for TSE in a range of species and enabled joint analysis of surveillance data. Collecting data as required about species, sex and whenever feasible determining the age of positive cases, has contributed to an increased understanding of the Ly+ and Ly- case phenotypes.
- Diagnostic expertise: The continuous monitoring of TSE in other species (cattle and small ruminants) has led to the development of a network of laboratories both within the EURL-framework and OIE-reference laboratory framework with the required expertise to diagnose prion diseases.
- Target tissues: Analysing both brainstem and lymph nodes enabled both the detection of disease, and the differentiation of cases into Ly+ and Ly-, when implemented.
- Multi-aim sample collection: The samples collected can also be used to increase knowledge of genetic composition of European cervids, and to define the prion strain diversity found in European cervids at both a European and a global level.
- Collaboration between animal health and wildlife experts: incorporating such collaboration into the intensified surveillance investigations, in particular, improved the overall interpretation of findings, and positively influenced further surveillance.
- High stakeholder involvement was achieved through active work by involved authorities: This stimulated and facilitated the identification and sampling of appropriate animals/carcasses.

Weaknesses

- Despite the proposed intention for surveillance efforts to focus on risk target groups, the data and surveillance activity described in this opinion from the countries involved indicate that this has not always been achieved due to practical/logistical difficulties (e.g. detection and sampling in remote, vast forested areas with low human population density, difficult climatic conditions; sick or fallen animals can be taken away by predators and scavengers). As a result, the sampling targets in the Regulation have been met by the addition of sufficient numbers of healthy hunted and healthy slaughtered animals for wild and semi-domesticated populations, but at the expense of the sensitivity of the surveillance in some areas. Targets for farmed animals were not met.
- In the absence of evidence to the contrary, all species of deer, except fallow deer, were considered potentially susceptible to CWD and acceptable targets for surveillance. However, at the time of designing the surveillance programme, the disease had only been detected in reindeer and moose, and so only those countries with reindeer and moose populations were included in the mandatory surveillance. Subsequent identification of cases in red deer challenges this decision.
- National legislation and organisation/arrangement/distribution of responsibilities among authorities has hindered the collection of samples from risk animals in at least one MS, thus decreasing surveillance sensitivity.
- Relative species susceptibility to CWD infection was not known and may not be equal. Targets were not set by species. The composition of the throughput may have affected sensitivity.

- Surveillance in wildlife requires organisational efforts substantially greater than those applied to livestock where it is easier to account for animals on-farm, or at abattoirs with dedicated staff and facilities.
- Central funding from the EU only covered a proportion of the laboratory testing costs, leaving MS with varying additional costs, particularly related to locating animals and retrieving samples, which may have impacted on the ability to deliver comprehensive surveillance activities as envisaged.

Opportunities

- Continued European surveillance would further increase our understanding of the geographical extent, host range and phenotypic diversity of CWD in Europe as a basis for future management decisions with regard to both animal and public health.
- Using the data and experience accumulated during the mandate period to inform future CWD surveillance efforts would benefit sampling design as well as improve practicalities and stakeholder communication. The existing database could be used to continue to collect data, which would facilitate joint data analysis across Europe.
- Education and training as well as distribution of kits for sampling was shown to enhance the reporting and sampling from animals found dead, displaying symptoms, discarded after hunting (hunted but not used for consumption because of anything abnormal, or poor body condition) or discarded at slaughter inspection (game slaughtering, reindeer slaughtering) as well as hunted animals in areas of intensified surveillance. Ensuring this type of outreach communication, building greater awareness of CWD and its spread, is a cornerstone of future surveillance activities and would increase cooperation and engagement within the relevant stakeholder groups (e.g. reindeer owners, hunters and farmers, etc.). It would improve stakeholder-led disease detection and would also build networks through which sampling kits could be proactively distributed, improving the proportion of appropriate animals that can be sampled, and the timeliness of sample submissions.
- Experience from the countries involved in the surveillance emphasises the importance of the production/adoption in each MS of specific guidelines on harmonised surveillance activities for CWD, accommodating the practical difficulties encountered by the MS to improve the overall sensitivity of future surveillance.
- Future surveillance activities will benefit from current improvements in national TSE diagnosis and genotyping capabilities and dedicated efforts to gather and use cervid population data. It is clear from the surveillance already conducted that HNSHC allow easy sampling of fresh tissues, as does sampling of HSHC. Data from the initial surveillance could be used to build a weighted system for calculating the surveillance value of samples from different target groups to enable MS to use a wider range of target groups, if required, and adjusting numerical targets to prevent compromising the overall surveillance sensitivity.

Threats

- The nature of the disease (strain variation and phenotype) and especially the unknowns in relation to transmissibility and species susceptibility affect the objectives and design of surveillance. This in turn may affect prioritisation and funding at the EU-level and within countries. CWD is currently regulated by Regulation (EC) No 999/2001 which came into force to protect public health; the disease is not regulated in relation to wildlife health and conservation.
- If future surveillance is undertaken without effective communication on the aims and the importance of an output-based (aiming for 'conclusions can be drawn') rather than input-based (aiming only to 'reach a fixed number of samples') surveillance, the target groups maybe affected with a subsequent decrease in surveillance sensitivity.
- Sample collection from high-risk target groups may be time consuming and can require expensive logistic solutions like dedicated chains of transport for samples, and/or adequate storage capacity for carcasses.
- Lack of funding may also drive sampling towards samples which are easier to collect (healthy hunt, healthy slaughter), but less informative, thus decreasing surveillance sensitivity.
- Lack of necessary legislation, or conflicting legislation or areas of responsibility on national level may affect sample collection.

- Stakeholder involvement is necessary but conflicting objectives or interests, or a lack of trust in the authorities, may affect their engagement.
- Policy and risk management decision-making may be misled by surveillance and monitoring data that are incomplete or inconsistently gathered, and result in future surveillance efforts being misguided or insufficient.

3.3.2. General considerations for future surveillance

CWD cases can only be detected when sampling and testing are in place, so it is important to have at least a small background surveillance programme operating. In this way, each country will have the necessary infrastructure and a good routine for obtaining samples and testing each relevant cervid species for CWD.

Currently, according to Article 12 of the Regulation (EC) 2001/999, 'any animal suspected of being infected by a TSE shall be either placed under an official movement restriction until the results of a clinical and epidemiological examination carried out by the competent authority are known or killed for laboratory examination under official control'. Because there is limited probability that clinical CWD suspects in wildlife are easily detected or readily recognised (Miller and Wolfe, 2021), sampled and tested, it is therefore recommended that a background surveillance program for CWD should include more cervids than just those animals suspected of being infected (SUS). The recommendation is to expand background surveillance to include testing of cervids in any of the three high-risk target groups (SUS, FC, HSNHC) that are systematically or opportunistically acquired by all countries. For these purposes, RK should no longer be included as a high-risk target group based on the analysis of available data from affected European countries as described elsewhere in this Opinion (see Section 3.1.5.2; Tables 9–11). The same holds also for cervids fallen or found dead in mass-casualty events (e.g. lightning strike, avalanche, intoxication, etc.).

At least for the positive cases, determination of age is relevant to contribute to knowledge building. Similarly, testing of both lymphoid tissue and brainstem is important to increase the sensitivity of the surveillance and enable the classification of any case into specific CWD phenotypes.

It is hypothesised, based on naturally occurring TSE in other species, that cases presenting with a disease phenotype that includes widespread lymphoreticular involvement are more likely to transmit disease naturally under field conditions. It is likely that animals presenting with CWD Ly+ could result in other cervid species being exposed to them as sources of infection, creating the potential for cross-species transmission to susceptible animals. The geographical ranges of cervid species differ even if they overlap. The habitat preferred by each species varies, and direct contact is rare between species, so the patterns of exposure will be largely species-specific.

In Europe, CWD Ly+ has so far only been detected in wild reindeer in Norway. Semi-domestic reindeer are the same species as wild reindeer and therefore susceptible, although some semi-domestic reindeer populations have a higher proportion of apparently less susceptible *PRNP* genotypes (see Section 3.3.2).

TSE surveillance in both bovines and small ruminants has shown that the detection of so-called 'atypical' cases of BSE and scrapie, which share the apparently sporadic distribution pattern of Ly- cervids, has been strongly correlated with the implementation of extensive active surveillance in different countries. Only a limited number of cases have been detected due to passive surveillance (clinical suspects) or prior to the implementation of increased surveillance programmes. Since such cases are likely to stay undetected without active surveillance, it cannot be excluded that these cases have been present for a long time without detection or that they may be present in places where there is inadequate, or no, surveillance at all.

The majority of Ly- cases in moose and red deer were animals found dead, animals with abnormal behaviour or clinically sick animals that were euthanised. Also, the first detection of CWD Ly+ in Norway (similar to the detection of CWD in many locations in North America and Asia) was in a clinically sick animal. It follows that, as a minimum, future surveys for CWD in Europe should focus on the sampling of cervids from the redefined high-risk target groups (see Section 3.1.5.3).

Based on the knowledge accumulated so far and presented above, a number of recommendations and practical suggestions for future EU CWD surveillance have been compiled and summarised in Table 21. A target design prevalence is suggested based on the findings of the report. With sufficient justification, countries could choose another design prevalence, based on new epidemiological data when available.

The target sample size shown in Table 21 is based on a surveillance design prevalence (95% confidence) of ~3% among cervids from the high-risk target groups in CWD-affected areas – a value estimated based on pooled data from all affected areas observed during the mandate period – or 0.3% among low-risk samples based on an assumed ~10-fold difference in apparent prevalence between the high-risk target groups and the low-risk target groups (Section 3.1.5.2; Tables 9–11), resulting in the need to collect 100 samples from high-risk target groups or 1,000 samples from low-risk target groups per area and species of interest.

Given the complexity of the disease and the populations that may be affected, the formation of an expert advisory group to inform on CWD surveillance approaches is recommended. This expert advisory group should be consulted on the following topics: definition of area or population of interest, design of surveillance strategies and analysis and interpretation of the results. At a minimum, the expert group should include experts on cervid species and their population structure in the affected region, epidemiologists with knowledge of disease surveillance and CWD experts.

To allow for knowledge building and analysis both on national and European level, data collection and reporting on both national and European level is advisable. Data collection and reporting to the existing EFSA database would be advisable.

Table 21: Toolkit for future chronic wasting disease surveillance in European countries

Aspect of surveillance	Recommendation	Practical tips
Spatial representation	Divide country into primary sampling units (ideally 50–300 for most countries).	Subunits within a country can be defined using, e.g. epidemiological knowledge, population data, pre-existing administrative or management boundaries for convenience.
Species representation	Assess/analyse data for each cervid species separately.	If resources are limited, then the emphasis should be on sampling the known susceptible species within given primary sampling units as a first step.
Management system representation	Assess data for wild, semi-domestic and captive or farmed cervid populations separately.	Customise education & training and facilitate logistic solutions to encourage surveillance participation within each system.
Surveillance sensitivity	Maximise surveillance sensitivity by emphasising sampling of 'high-risk' cervids within each primary sampling unit and management system.	The most valuable animals to sample for detecting CWD are 2 years of age or older and from the SUS, FC and HSNHC target groups.
Design (minimum detectable) prevalence	Design surveys to detect the disease at or above data-supported values, accounting for expected patchy spatial distribution and low prevalence within affected areas. The following are examples based on the data compiled in this opinion: Proportion of sampling units with cases: About 3% of the primary sampling units in affected Scandinavian countries yielded cases. Prevalence within affected sampling units: Observed prevalence was ~3% among high-risk target groups (or ≤0.3% among 'low-risk' samples) within positive units in the countries that detected CWD.	Choosing a design prevalence *a priori* based on observed data will help to minimise the probability of a survey failing to detect CWD because sample sizes were too small. Country-level design prevalence – if used – should be the product of the assumed proportion of sampling units expected to be infected (e.g. ~3% observed; see Section 3.1.5.2) and the expected prevalence within affected sampling units (e.g. 0.3% of low-risk samples). From the data presented in this opinion, the country-level calculation would be: 3% × 0.3% ~ 0.01% of low-risk samples from the entire country.
Target sample sizes	Based on detected prevalence examples described above: 100 samples from 'high-risk' target groups *per sampling unit per species* (accumulated over time, as below) -or-	If it becomes necessary to combine data from high-risk and healthy target groups to achieve surveillance targets, the value of each test-negative healthy hunted/slaughtered or road-killed sample is 1/10th (0.1×) the value of a negative high-risk sample. Healthy hunted/slaughtered animals tested should be at least 2 years old.

Aspect of surveillance	Recommendation	Practical tips
	1,000 apparently healthy hunted/ slaughtered and road-killed samples *per sampling unit per species* (accumulated over time, as below) -or- Every fallen animal from small defined sampling units such as individual domestic herds (accumulated over time, as below)	
Tissues to collect & test	Both retropharyngeal lymph node and brainstem samples should be screened to maximise detection of CWD in European cervid species.	
Data collection and reporting	Data should be collected as available, inducing information about species, sex, target group, geographic location and age.	To enable knowledge building and analysis both on national and European level, continued reporting of detailed data to the existing EFSA database is advisable.
Timeline	Surveillance data should be accumulated over several years. Inferences on disease absence should be limited to the timeframe established. Risk of introduction during the timeframe should also be considered.	Consider establishing a rolling timeframe (e.g. 5–10 years?) for accumulating surveillance data, adding the newest year's data and dropping the oldest.
Interpretation of negative findings	Interpret negative findings in the context of the minimum detectable prevalence or the detection probability achieved based on the number of samples tested and their risk groups. Limit inferences to the spatial units represented in sampling. In the example, finding no cases among 100 high-risk samples (or among 1,000 samples from apparently healthy animals) would provide reasonable confidence (95%) that CWD was not present above the design prevalence within the specified sampling unit.	Tools are available to estimate the probability of CWD detection for sample totals above or below the target values presented as an example here. Disease status for a country as a whole could be represented, if required, as a composite of the data gathered for individual sampling units, species and management systems.

3.3.3. Objectives of, and recommendations for future surveillance (AQ 3.2) (AQ 3.3)

The outcomes of the recent mandatory surveillance activities described above suggest that there is a need for some level of further surveillance and monitoring for CWD in Europe. However, appropriate objectives and approaches for future surveillance and monitoring may be more numerous and varied given the complexities already revealed.

Possible objectives for future surveillance activities might include:

- **Generate epidemiological data and knowledge** on, e.g. frequency of detecting disease and how prevalence varies between potential risk groups (target group, sex and age category), changes in temporal and spatial distribution, host range and age structure, different variants, potential control measures and their effectiveness.
- **Provide support for statement of disease status:** collect data helpful in assessing the probability of CWD absence.
- **Screen for evidence of spillover:** i.e. monitor for evidence of spillover/cross-species infections between cervid species, between free-ranging and captive animals and/or between cervid and other food animal species.
- **Generate data and knowledge on population/case genetics:** i.e. increase knowledge of the genetic composition of species in the host range and its impact on disease susceptibility/ resistance, and of the potential for disease control intervention.

Surveillance recommendations supporting the specific listed objectives are displayed in Tables 22–25 and Figure 7.

3.3.3.1. Recommendations for 'generate epidemiological data and knowledge'

Table 22: Surveillance recommendations for 'generating epidemiological data and knowledge'

CWD already detected in the country/ area?	YES	NO
General objective	– Increase epidemiological knowledge (including risk factors) about the temporal and geographic distribution and host range of CWD within countries/ areas where cases were previously detected.	– Contribute to epidemiological knowledge about the presence of disease, geographic distribution and host range of CWD and changes over time in Europe.
Specific objectives	– Detect disease in new locations. – Detect disease in new susceptible species. – Estimate prevalence in species affected in an area (where disease has been detected). – Assess changes in temporal and spatial distribution of the disease in affected species.	– Detect disease – Detect disease in new susceptible species.
Target species	– All cervids species (to date known to be susceptible) combined and present in the area of concern (country/region).	
Animal categories	– Adult animals (> 2 years of age)	
	– Detection: High-risk target groups including SUS, FC, HSNHC – Prevalence estimation: adult animals from high-risk groups ± HSHC for detecting range expansion or from HSHC for estimating prevalence within affected areas. (Mixing high-risk and HSHC target groups for prevalence estimation not recommended without adjustments for bias).	– High-risk target groups including clinical suspects, FC, HSNHC.
Management system	– Data collection and analysis separately for wild, – semi-domesticated, captive cervids.	
Target sample size	– Intensified surveillance in and around affected areas; relevant to define area based on what is known about the species and the population. – Sample size to be calculated (1) based on scenarios of expected prevalence and error and (2) large enough to identify differences in prevalence and geographic distribution. – Sampling to detect CWD in other CMA or PSU: the expert group should set a relevant design prevalence. From the experience in the Nordic countries, prevalence in areas of intensified surveillance has been low. Within selected area: a design prevalence of 3% would be more appropriate than 10%, proposed by the EFSA's CWD I scientific opinion (EFSA BIOHAZ Panel, 2017), among high-risk animals.	– See the original scheme proposed by the EFSA's CWD I scientific opinion (EFSA, 2017). – However as suggested in Table 21, a design prevalence of 3% (rather than 10%) among high-risk animals could be more appropriate.

Furthering knowledge about the geographic distribution and host range of both CWD phenotypes would be logical baseline objectives moving forward. These objectives could apply to continued systematic efforts to detect cases in countries where CWD is not already known to occur, as well as to refining knowledge about geographic distribution in affected countries. Minimally, new mandates for surveillance would cover countries with relevant species, i.e. those known to be susceptible (moose, red deer and reindeer). Consideration should also be given to other potentially susceptible species, such as roe deer and white-tailed deer.

Assessing change in the distribution of CWD over time also seems a logical future objective. However, achieving this will require a more complete understanding of current or baseline distribution in Europe. In the absence of that understanding, 'new' detections may be misinterpreted as evidence of expansion when in fact they are simply endemic areas that had eluded prior detection. Problems with the misinterpretation of sparse surveillance data have plagued CWD surveillance efforts in North America (EFSA BIOHAZ Panel, 2018). Gathering additional data (especially those regarding the age of the animals) to better inform estimates of design prevalence for the CWD Ly- phenotype and better understand its epidemiology could be an additional objective. Such estimates would be especially useful for interpreting surveys wherein no cases had been detected.

3.3.3.2. Recommendations for 'provide support for statement of disease status'

Table 23: Surveillance recommendations for 'Providing support for statement of disease status'

CWD detected in the country?	YES	NO
General objective	– Assess the probability of CWD presence in an area (or specific subareas or individual farms) of concern	
Specific objectives	– Maintain status – Regain status after confirmation of cases in the area of concern	– Substantiate status
Target species	– All cervid species.	
Animal categories	– Adult animals (> 2 years of age) from high-risk target groups (see generate epidemiological data and knowledge above) – High-risk animals as above. – HSHC to increase body of evidence via analytical methods of an appropriate nature (see Section 3.4.2)	
Management	– Dedicated strategies by management system – (wild vs. semi-domesticated vs. captive)	
Target sample size	– Scheme as recommended for ToR4	

3.3.3.3. Recommendations for 'evidence of spillover'

Table 24: Surveillance recommendations for 'evidence of spillover'

CWD detected in the country/ area?	YES	NO
General objective	– Gather evidence of potential transmission of prion diseases between species	
Specific objectives	– Detect infection in new, or between, species	
Target species	– All cervids species (known to be susceptible or not) present in the area of concern (e.g. country/region) – All livestock susceptible ruminants present in the area of concern	– All cervid species (known to be susceptible or not) in areas where prion disease identified in other livestock susceptible ruminants

CWD detected in the country/ area?	YES	NO
Animal categories	– Adult cervids (> 2 years of age): HSHC and high-risk target groups (see Table 26)	– Adult cervids (> 2 years of age) in high-risk target groups
Management system	– Farmed, wild and semi-domesticated cervids – Livestock – susceptible ruminants	– Farmed, semi-domesticated, wild cervids
Target sample size	– No quota. All species of interest represented	– Opportunistic, no quotas; geographic reference to locations of cases in other species

3.3.3.4. Recommendations for 'generate data and knowledge on population/case genetics'

Table 25: Surveillance recommendations for 'generate data and knowledge on population/case genetics'. For countries with/without cases detected

General objective	**Generate knowledge on the impact of host genetics on disease**
Specific objectives	**Increase knowledge on *PRNP* variability in European cervid populations** **Generate data to assess susceptibility/resistance** **Assess feasibility of use of genetics for disease management**
Countries/areas	– With/without CWD
Target species	– All cervids species
Target groups	– All target groups proportionally represented
Management system	– All types
Target sample size	– Based on the specific aim: – (a) Animals of all ages; all species; healthy and high-risk animals – (b) As in (a) but considering also disease cases (all of them to be genotyped) – (see also scheme as recommended for ToR5 in Section 3.6)

3.3.3.5. Public health considerations

Given the uncertainty about the zoonotic potential of European CWD isolates, a country may choose to take measures to prevent/minimise human exposure via the food chain. Systematic testing cannot be considered a surveillance strategy for data collection, but it is a risk mitigation measure. The EFSA opinion on chronic wasting disease III (EFSA BIOHAZ Panel, 2019) recommended the measures listed below to reduce/eliminate human exposure to CWD:

- Systematic testing: only allowing human consumption of meat, meat products and offal sourced from animals that have been tested negative for CWD.
- Targeted measures: prohibition of harvesting/hunting susceptible species or the introduction of compulsory testing of animals before human consumption in/from declared infected premises/ areas (e.g. a farm, or a surveillance PSU (Section 3.6), region, country, etc.).
- Systematic removal of high-risk tissues from all cervids intended for human consumption with no requirement for testing.

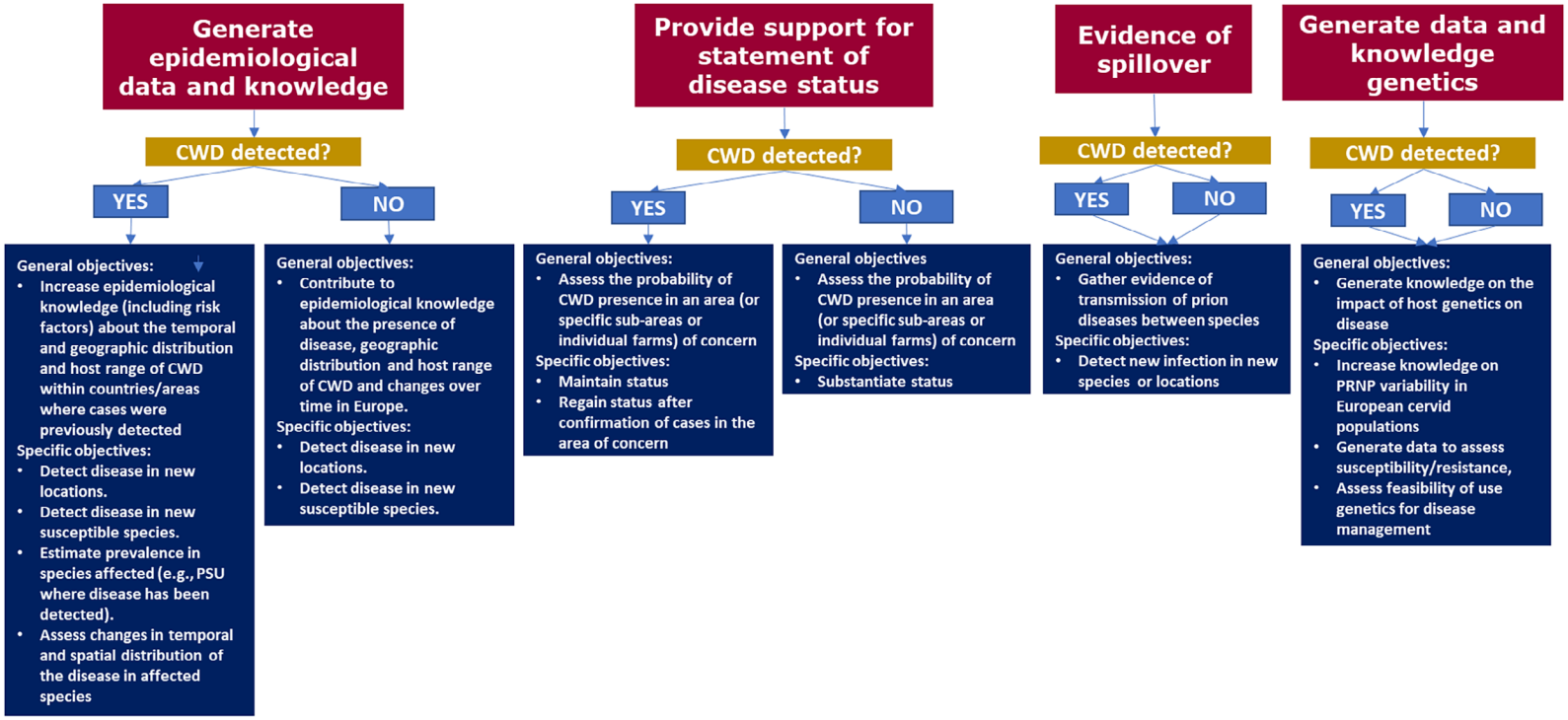

Figure 7: Flow charts describing the objectives of the four surveillance recommendations

3.4. Criteria for non-infected areas (ToR 4)

3.4.1. Feasibility for the definition of criteria for the establishment of non-infected area (AQ 4.1)

The requirement to define the host and geographic distribution of CWD or (better) to demonstrate its absence in areas of EU/EEA countries is understandable from a policy and risk management perspective. Unfortunately, certain characteristics of animal prion diseases – in particular, their low and slowly increasing prevalence and incidence, long preclinical incubation periods and long disease course, lack of practical antemortem screening tests, multiple forms and strains – hampers the application of the established pathways to demonstrating 'disease freedom' that are used for other animal diseases.

With CWD, these difficulties are compounded by its potential to occur in hosts from within farmed, semi-domestic and wildlife populations, with the latter presenting inherent spatial, sociobiological, temporal and practical obstacles to the application of traditional disease surveillance and monitoring tools (EFSA BIOHAZ Panel, 2017, 2018). The recognition that the definition of 'CWD' now comprises novel phenotypes and multiple prion strains (Pirisinu et al., 2018; Otero et al., 2022; Sun et al., 2023) and that there is uncertainty about the precise duration of, or circumstances giving rise to, CWD in either Europe or North America (EFSA BIOHAZ Panel, 2017, 2019) add further to uncertainty about assurances of sustained disease freedom anywhere where cervid hosts may reside.

The findings from the mandate period (summarised in earlier sections of this opinion) describe patterns in the occurrence and distribution of CWD that could extend to other parts of Europe that have not been investigated yet. The outcomes also illustrate the challenges of assembling rigorous surveillance data sufficient to detect CWD or estimate its prevalence. Experiences to date in Europe (this opinion) and in North America (EFSA BIOHAZ Panel, 2018) suggest that a failure to detect CWD within a jurisdiction does not ensure its absence. In concept, establishing relevant criteria for considering an area or an entire country not to be infected by CWD seems impractical and impossible. Instead, as noted in Section 1.2, it seems more appropriate to establish criteria useful in assessing the overall probability/likelihood of CWD being present. Details of such an approach are offered in the sections that follow.

3.4.2. Criteria for assessing the probability of CWD presence

As the previous section indicates, the complexity of CWD may require some modification of the standard approaches for the assessment of the probability of occurrence of CWD. In particular, it is likely that inferences will need to be limited to a specific area or a population, and perhaps to individual species within those areas.

The development of knowledge in relation to the epidemiology of the different phenotypes of CWD needs to be closely monitored and is of relevance for the approach to assess the risk of CWD in a region or a population. Available data suggest different epidemiological patterns of CWD can occur within the same country (Tranulis et al., 2021).

In order to provide the most comprehensive assessment of CWD occurrence, surveillance efforts should be designed to detect all phenotypes, with sufficient data to enable the phenotypes to be separated for the purposes of analysing and interpreting the results, if required, as illustrated in this opinion. For example, if independence between the two phenotypes is assumed, then surveillance results can still support the assumption that an area or a population could have low probability of presence of Ly+ CWD despite having Ly- cases detected. But such assumptions about independence would need to be supported by continuing analysis of the prion strains and improved epidemiological data (e.g. any changes in prevalence, or the age profile of cases). It also needs to be borne in mind that detailed laboratory investigations of the initial cases indicate that there is potential strain variability both between the two main cervid phenotypes, and within the Ly- phenotype.

Before the criteria below are addressed at country/area level, an expert advisory group should be established by the competent authorities interested in assessing the probability of occurrence of CWD and consulted for the (1) definition of area or population of interest, (2) design of surveillance strategies, (3) risk assessment and (4) analysis and interpretation of the results. The expert group should at least include (1) experts in the cervid species of concern as regards the population structures, specifically in the region concerned, (2) epidemiologists with knowledge in the field of disease surveillance (3) disease experts with knowledge of CWD.

The criteria useful in assessing the probability of CWD presence (Section 1.2.3) should include:

A) Defining the geographical area

The first step is to define the geographical area and set its spatial boundaries.

With domestic livestock or farmed deer, most contacts between herds are controlled and, in many cases, recorded. In contrast, most wild cervid populations cannot be clearly demarcated on the landscape. There is considerable variation in the spatial extent of movement depending on species, and within species depending on geographical area, and the age and sex of the individual animals. Administrative units often do not reflect the extent of cervid populations, which may even overlap national boundaries. For continuous populations, surveillance is very sensitive to the spatial extent of the surveillance area. Semi-domesticated cervids have owners and are herded, but the herds are not herded continuously. The animals walk freely over large areas part of the time, and varying extents of interaction with neighbouring flocks/herds may occur.

Based on the North American experience, it seems reasonable to assume that live animals infected with CWD may be contagious to other cervid species, creating the potential for cross-species infection in susceptible animals. Moreover, in general, 'on field' carcasses of animals affected by any form of CWD (either Ly+ or Ly-) could be a potential source of exposure and/or give rise to environmental contamination (EFSA BIOHAZ Panel, 2019). Therefore, all cervid species in an area need to be taken into account when defining populations or geographic areas of reference.

As seen in North America, as well as in Norwegian wild reindeer, cases of the Ly+ phenotype tend to be clustered in space and time. This needs to be considered when defining the area and population of interest, and the size of the area as well as population(s) will have implications for the sample size depending on the aims of any surveillance (see below).

When planning surveillance activities, it is important to include cervid specialists and stakeholders with knowledge of the different cervid species and their regional abundance, management practices and migratory patterns. The rationale for defining a region or population should be well explained.

The following criteria are relevant to consider:

Wild and semi-domestic cervids:

- Cervid species present in the area: extent of the population included in the defined area;
- Extent of the population, or existence of borders (natural or man-made);
- Migratory patterns, if species is migratory;
- Approximate population sizes (based on hunting bags or other available data);
- Interaction between species;
- Interaction between herds (semi-domesticated);
- Interaction with farmed cervids.

Farmed cervids:

- Interaction or not with wild cervid species (including fence-line contact);
- Documentation of movements and contact between farms.

B) Assessing the risk of introduction into an area

The risk of introduction of CWD to an area of concern needs to be assessed as a basis for the surveillance. The risk assessment would inform both the surveillance design and the interpretation of results. Factors to consider are e.g. prior confirmed cases in the area, known status of neighbouring areas or populations, the risk of introduction through contacts between herds or migratory animals, as well as indirect contacts through carcasses or offal used for baiting, or movement of feedstuffs (e.g. lichen) from areas with known presence of CWD. As mentioned above, an expert group should be involved in the assessment of risk of introduction.

When assessing the risk for introduction, a more general guideline can be used as support for the structure of the assessment, e.g. the WOAH Chapter 2.1 on import risk analysis, although an assessment on the risk of introduction in this case may also apply within country, and not only in relation to import from another country. The assessment needs to be adapted to each region, population and situation and the possible routes to be considered should be based on the specific situation.

C) Minimum background surveillance

Suspicion of CWD in cervids based on clinical signs should be mandatorily notifiable to the relevant authorities in regions or populations where a country wants to assess the probability of CWD presence.

In addition, the background surveillance system in place should allow the testing of cervids from high-risk target groups (SUS, FC and HSNHC).

Systems to alert the authorities to the presence of an animal/carcass eligible for sampling are beneficial, e.g. systems to report fallen cervids can contribute to surveillance if this information is passed on and used by the authority responsible for the CWD surveillance.

D) Training and engaging stakeholders

Active involvement of stakeholders is key in successful surveillance. Stakeholders play a role in detecting animals in target risk groups and often contribute to sample collection. Thus, identifying and engaging relevant stakeholder groups in the area is necessary; examples of stakeholders can be owners of farmed or semi-domestic cervids, hunters, persons responsible for wildlife management, wildlife processing plants or persons involved in slaughter of cervids.

Identified stakeholders should be trained about the disease and, especially, the clinical signs of CWD, where/how to report suspect cases, and how to collect and submit samples, as well as the surveillance strategies to be applied in the area.

E) Designing ad hoc surveillance activities

The surveillance design should be 'output based' rather than 'input based' (Cameron, 2012), i.e. the goal of the surveillance is to draw conclusions on the probability of disease occurrence in a certain area or population(s) rather than sampling a set number of animals. The surveillance design needs to consider what is known about the population(s) and the size of the area, the likelihood that cases are clustered, and not assume an even prevalence over a large area or population. Further, known risks (prior cases or cases in neighbouring or contact populations) need to be taken into account. If the area is large, representativeness from different parts of the area needs to be ensured and stratification may need to be applied. If not too large or small compared to the assumption of random mixing between animals (relevant for contact rates and spatial scale of disease clustering), an option may be to let wildlife management units serve as primary sampling units in a two-stage sampling approach. From experience in North America, as well as in Northern Europe (EFSA BIOHAZ Panel, 2017) and as shown also in this Opinion (Section 3.1), the so-called risk animals contribute more surveillance information than healthy hunt and healthy slaughter animals on a per capita basis. However, Ly+ and Ly- cases were detected during the mandate period among apparently healthy hunted cervids (Tables 9–10). Thus, although each sample from healthy hunted or healthy slaughtered animals contributes less information compared to the risk groups, they still provide some information. This difference should be taken into account both when designing the surveillance and when assessing the results, through assigning different weights to samples from different target groups. This approach, assigning different values to different surveillance streams has been used for TSE surveillance in bovines both at EU- and WOAH-level (Prattley et al., 2007) as well as for CWD in North America (Cullingham et al., 2011; Jennelle et al., 2018; Smolko et al., 2021) and Norway (Viljugrein et al., 2021). Based on available data, a 1:10 weight should be used, assigning 1 to HSHC and 10 to high-risk animals (animals with clinical signs of CWD, fallen or otherwise diseased). This parameter could be revised upon availability of new data.

Nonetheless, the importance of focusing on risk animals in surveillance efforts, and not excluding them from surveillance or largely replacing them with healthy animals, needs to be emphasised. Although it is often challenging to find and sample animals in the risk target groups, systems need to be in place for both the reporting and sampling of the risk animals which are detected. Moreover, available data should be used to refine understanding about the relative risk and potential surveillance contributions of different sample streams, as illustrated in this Opinion (Section 3.1.2.5; Figure 5).

Due to the hypothetical possibility of cross-infection between species, at the same time as there may be partial species barriers, each species needs to be considered separately. Surveillance efforts would need to be continuous to sustain the disease status of an area or a population. This would include continued requirements to notify suspicions, education of stakeholders and a baseline surveillance of fallen cervids and cervids which display clinical signs of CWD.

Design prevalence, risk groups and relative risks

The surveillance sensitivity is dependent on the design prevalence. At country/region level, the cluster-level design prevalence will be 1 among the total number of primary sampling units of the population being surveyed. A risk-based approach can also be used when setting the detection level or design prevalence, which may vary depending on whether it is a region with previously confirmed

cases, an adjacent area, or an area without known cases. The results of the risk assessment should, as stated above, inform the surveillance design. In an area without any previous history of, or known exposure to CWD, the design prevalence should be set at below 1%. If the objective is to detect a disease that is present at a very low prevalence (see Section 3.3.3), the design prevalence should be set at an even lower level. Assuming RR = 10, a large number of risk animals would be needed to obtain the required surveillance sensitivity. The design prevalence corresponds to the average probability of a randomly selected individual in the area or population being infected. The effective probability of infection will vary between different risk groups in the population. For example, assuming a proportion of 5% high-risk animals in the population and a relative risk of 10, for a design prevalence of 1%, the effective probability of infection (Martin et al., 2007) is 0.69% for the low-risk group and 6.9% (10 times higher) for the high-risk group. Alternatively, it is possible to specify the design prevalence for the low-risk group, and the design prevalence for the high-risk target group will then be the design prevalence for the low risk group multiplied by the relative risk.

In a situation where the risk of Ly+ in an area or population is deemed to be relatively high, e.g. through previous confirmed cases or contact with known infected populations, and when early detection or high level of confidence that CWD Ly+ is not present in the population is deemed important, it can be documented by using a lower design prevalence. An example of such a situation is CWD in wild reindeer populations in Norway. The wild reindeer are endangered, and undetected presence of CWD in a population may have consequences for the last remaining population of wild reindeer in Europe. As a basis for management decisions, surveillance activities in herds neighbouring confirmed positive populations have been set to a design prevalence lower than 0.5%. To include the possibility of detecting a relatively recent introduction, the design prevalence was set at two to four infected individuals (Viljugrein et al., 2021; Mysterud et al., 2023).

Surveillance data can be accumulated over several years, while accounting for annual risk of introduction. The design prevalence, in combination with the number of animals available for sampling, will determine the time it takes to reach the surveillance target.

The relative risks of different target groups of animals should be set based on the latest scientific knowledge available. As a general rule, animals displaying clinical signs compatible with CWD should be given the highest relative risk and healthy hunted or healthy slaughtered animals the lowest. Both relative risk and detection probability are higher for adults above 2 years old compared to yearlings/animals less than 2 years old (Section 3.2.2.2). One approach that allows the full use of data from different target groups is scenario tree modelling (a description is provided in Appendix D).

3.5. Design of a genotyping protocol (ToR 5)

3.5.1. Objectives of the genotyping (AQ 5.1-SAQ 5.1.1)

Analysis of variation in the *PRNP* gene of samples collected during the 3-year surveillance programme for CWD in cervids would contribute valuable data relevant for the modelling of the potential spread of CWD among European cervids, and for informing future disease management strategies. For example, the identification of *PRNP* alleles associated with reduced susceptibility towards CWD could be taken into consideration in breeding programs.

Genotype analysis would establish *PRNP* allelic profiles for the major European deer species, mapping the frequencies of known *PRNP* alleles and potentially identifying previously unrecognised alleles.

To pursue the above objectives, a multi-aim study is envisaged. Both validity (in terms of representativeness) and precision of the sampling design have to be ensured for each of the species under investigation, and each country involved. A geographically stratified random sampling approach (based on PSU) should be used for each species and country to ensure that each of them will be accounted for without any risk of over- or under-representation.

A multi-aim sample size calculation could be used to detect polymorphism and frequency estimation and to design susceptibility association studies.

A working definition of a polymorphism emerging from casual mutations was suggested by Brookes (1999) based on the minimal abundance of the least frequent allele: '*SNPs [Single nucleotide polymorphisms] are single base pair positions in genomic DNA at which different sequence alternatives (alleles) exist in normal individuals in some population(s), wherein the least frequent allele has an abundance of 1% or greater.*' This 1% frequency of an allele can be used as a threshold (i.e. a design prevalence) to calculate the minimum sample size able to detect a polymorphism in a certain species.

Each individual animal has a pair of alleles at each codon, and the presence of each allele is assumed to be independent. Based on a binomial distribution applied to a finite population (i.e. the negative samples stored), and assuming a perfect ability of the sequencing to identify the correct alleles, the same formula to calculate the sample size for disease freedom can be used to calculate the number of alleles to be tested. In our case, the number of animals to be considered for sampling will be half of the calculated sample size of alleles.

The formula is as follows:

$$n = \left[1-(1-p)^{1/d}\right] \times [N-d/2] + 1$$

where
n = required sample size of alleles (double of animals necessary to test).
N = the negative samples stored.
d = minimum number expected of alleles with the mutation.
p = probability of finding at least one mutated allele in the sample.

Based on the number of animals tested by species in each country during the mandate period, the required sample size is between 76 and 145 per country. Table 26 shows the calculated sample sizes by country and species.

Table 26: Sample size of surveillance samples to be *PRNP* genotyped by country and species

Country	European moose	Red deer	Reindeer	White-tailed deer	Roe deer
Estonia	114	90			91
Finland	122		137	102	43
Latvia	120	127			102
Lithuania	85	129			170
Poland	76	120			179
Sweden	137	112	145		15

Concerning roe deer, a country specific survey may not be necessary. The availability of the national negative stored samples could be exploited to carry out a multicentric study with a large sample size to confirm the absence of any polymorphisms in this species. Among the 8,907 adult roe deer tested in the six EU MS, an overall sample size of 600 animals distributed proportionally among countries would be sufficient to detect mutations if they should be present with a frequency above about 0.25%.

If a *PRNP* allele has been reported previously, and its presence is confirmed among the negative stocks, the same sample size can be adapted to estimate the frequency of polymorphisms in each species. In the worst-case scenario (i.e. a polymorphism with a frequency close to 50%) the above sample size will allow to the estimation of the real frequency in the species with a maximum error between 5% and 10%.

To determine if specific *PRNP* variants or genetic status are associated with susceptibility/resistance to CWD, case–control studies should be carried out. Since the number of cases is limited, all positive animals should be genotyped. The statistical power of a case–control study can be increased by enrolling more controls than cases. However, the additional power gained decreases as the ratio of controls to cases increases, and ratios greater than 4:1 offer no added value. In the case of moose, after considering all 18 cases detected so far in Fennoscandia and applying a control-to-case ratio of 4:1, the required sample size of negative (controls) would be 72, well below the target sample sizes as in Table 26. Assuming a scenario potentially similar to that studied by Güere et al. (2020) in reindeer, that is, with 52.8% of cases and 14.9% of controls carrying the genotype of interest and resulting in an OR of 6.4, the statistical power available from the above sample size (i.e. 72 controls and 18 cases) would be 84.5% (Schlesselman, 1982).

3.5.2. Description of genotyping protocols (AQ 5.1.2)

The *PRNP* of cervids under surveillance investigation i.e. Eurasian tundra reindeer (*Rangifer tarandus tarandus*), Finnish forest reindeer (*Rangifer tarandus fennicus*), moose (*Alces alces*), roe deer (*Capreolus capreolus*), white-tailed deer (*Odocoileus virginianus*), red deer (*Cervus elaphus*)) share a wild-type amino acid sequence with 100% identity. Polymorphisms observed among these species

differ in the positions and variants observed. In sheep and goats, very similar related species, the wild-type alleles also have a 100% amino acid identity, whereas different *PRNP* allele variants have been observed between species although some are the same. In these species, the allele variants resistant to the disease are different, and the susceptibility of specific genotypes to different strains may also differ. This is the case for the ARR allele in sheep and the 146S/D or 222 K variants in goats. The ARR allele has not been described in goats and similarly the 146S/D and the 222 K variants are absent or very rare in sheep, while in both species, the AHQ allele is associated with susceptibility.

PRNP variability at species level has been investigated widely in several American and European regions and it has been demonstrated that polymorphic codons differ between species and sometimes also within the same species.

Although rapid methods for the identification of the variants present at each polymorphic codon can be easily developed (as has been done for sheep and goats), the use of sanger sequencing is preferred for this initial study. In the absence of robust data on the *PRNP* sequence in cervid species, sequencing the entire *PRNP* open reading frame i.e. the protein coding sequence, in both directions should be undertaken since any additional polymorphic site data can be collected using this technique and it represents the gold standard for sequence analysis. Sequencing in both directions is necessary to achieve high-quality reading of the sequence from both ends of the reading frame. This is important because these regions may also harbour important variation. Protocols have been described in the literature that are able to sequence the entire *PRNP* coding sequence of several cervid species (Kaluz et al., 1997; O'Rourke et al., 1999, 2004; Wik et al., 2012; Güere et al., 2020), but no data have been reported on any validation studies.

3.5.3. Genotype data collection (AQ 5.1.3)

It would be advisable to create a centralised data collection system containing the complete coding sequence of the animal *PRNP* in standard format, together with the metadata associated with each genotype to allow the collation and extraction of data for analysis at the EU level.

Metadata for samples included in the genetic data collection system. For each sample genotyped:

- **Species**: The available options are listed below: Deer (Species unspecified), European moose (*Alces alces*), fallow deer (*Dama dama*), mule deer (*Odocoileus hemionus*), musk ox (*Ovibos moschatus*), red deer (*Cervus elaphus*), Reeve's muntjac (*Muntiacus reevesi*), reindeer (*Rangifer tarandus*), roe deer (*Capreolus capreolus*), sika deer (*Cervus nippon*), white-tailed deer (*Odocoileus virginianus*), wild forest reindeer (*Rangifer tarandus fennicus*).
- **Sample/case assessment**: Final status of the CWD case. The available options are: BSE-not excluded (i.e. BSE like), CWD, CWD Ly+, CWD Ly-, inconclusive case, negative sample, other.
- **Sample ID**: Open text field to report the unique identification number for each sample assigned by the reporting country. If the Sample ID is considered confidential by the reporting country, an alternative ID can be provided. However, the reporting country should keep a mapping table of the actual and alternative ID numbers. It is the responsibility of the reporting country to submit data which can be traced at all times.
- **Sample part**: Represents the main anatomical area of the sample. The available options are Blood, Brain, obex, Retropharyngeal lymph node (RPLN), Other head lymph node (other than RPLN), Tonsil.
- **National case ID**: If positive, open text field to report the unique identification number for each sample assigned by the reporting country. If it is considered confidential by the reporting country, an alternative ID can be provided. However, the reporting country should keep a mapping table of the actual and alternative ID numbers. It is responsibility of the reporting country to submit data which can be traced at all times. The syntax of the National case ID should be such that they cannot be confused with cases from other reporting countries. A proposed syntax is to include the ISO code for the reporting country followed by the type, year and the national identifier of the case. For example, a first hypothetical case of CWD in Croatia in 2018 would be HRCWD20180001 or HR/CWD/2018/0001. In the case of CWD, the national case ID should be the same for the two different samples of the same animal.
- **Animal ID**: for captive/farmed and semi-domesticated. Open text field to report the unique identification number for each animal assigned by the reporting country. The field is mandatory for all positive and inconclusive/pending cases and if it is considered confidential by the reporting country, an alternative ID can be provided. However, the reporting country should keep a mapping table of the real and the alternative ID numbers. It is responsibility of the reporting country to submit data which can be traced at all times.

- **Herd ID**: open text field to report the unique identification number for each herd of origin of the cervid assigned by the reporting country. It is only applicable to farmed/captive and semi-domesticated cervids. If the field is considered confidential by the reporting country, an alternative ID can be provided. However, the reporting country should keep a mapping table of the real and the alternative ID numbers. It is responsibility of the reporting country to submit data which can be traced at all times.
- **Sampling Date**: The available options include numbers between 1 and 31 and the blank option.
- **PSU ID:** number or name of the identifier of the primary sampling unit as reported to the EFSA database.
- **Sampling area:** If PSU is not available, the area where the holding of origin is located. The options available in the drop-down menu are those corresponding to the NUTS 2 level areas of the reporting country.
- **Age category**: The available options are: $<$ 12/24 months; $\geq$ 12/24 months; Unknown.
- **Age**: in years, approximately.
- **Target group**: The available options are clinical suspect animals (SUS); road/predator killed – only applicable for wild animals (RK); fallen/culled (FC); hunted/slaughtered not fit for human consumption (HSNHC); hunted/slaughtered fit for human consumption (HSHC).
- **Sex**: Available options are mixed females and males; females; males.

Data for samples included in the genetic data collection system. For each sample genotyped:

- **Type of genotyping technique used:** sanger sequencing, pyrosequencing, etc.
- **Raw data:** DNA sequencing should be collected in the form of an **electropherogram** (i.e. a plot of DNA fragment sizes) **from sanger sequencing.** In particular an **.ab1** file contains the DNA sequence electropherogram as well as raw data and some other information.
- ***PRNP* genotype**: text file including the complete coding sequence of the animal *PRNP*. Sequence should be reported in FASTA format (file using the IUPAC -International Union of Pure and Applied Chemistry – nucleotide code) or FASTQ format (Phred file) text file containing bases with quality values for each base.

3.6. Uncertainty analysis

Table 27: Sources and causes of uncertainty, and impact on the conclusions

Source of uncertainty	Cause of the uncertainty	Impact of the uncertainty on the conclusions
Origin and epidemiology of CWD phenotypes observed in European host species.	The differences in epidemiological patterns observed thus far and the evidence of different strains being involved in the CWD cases encountered in Europe means that direct parallels cannot be confidently drawn between North American data and the current European situation. European 'strains' of CWD could be either more or less contagious (impacting transmissibility, disease spread and prevalence) and have greater or less cross-species transmission potential (influencing spillover and zoonotic potential) than other TSE strains. Ongoing studies show different epidemiological patterns also between wild reindeer and moose within Europe, not only between NA and Europe. Laboratories studies of eight initial European isolates (three reindeer, four moose, one red deer (Pirisinu et al., 2018; Nonno et al., 2020; Bian et al., 2021; Pritzkow et al., 2022; Wadsworth et al., 2022; Sun et al., 2023) indicate that at least five distinct biological and biochemical patterns can be identified, potentially representing five different strains, none of which matches the North American controls. Not all European isolates, and very few North American isolates have been fully characterised.	It is unclear how much strain variation exists within naturally occurring CWD in Europe, and how phenotypes (including the potential for cross-species transmission) may vary when these strains infect different hosts. Interpretation of the results is currently limited to the two overarching cervid phenotypes Ly+ and Ly-. Variability within phenotypes is unaccounted for.
Evidence to rule out the possibility of the presence of CWD in roe deer in Europe	Roe deer are understood to be monomorphic for the cervid wild-type *PRNP*. This genotype is considered to be susceptible to CWD, so this species is hypothetically uniformly susceptible to CWD, although there have been no reported cases. A significant proportion of the European surveillance effort involved roe deer.	If roe deer are not susceptible, this will lower the achieved surveillance sensitivity where roe deer data were included in the analysis.
Diagnostic sensitivity of the rapid test for CWD used in Norway	PrP rapid detection kits for statutory TSE diagnosis are formally validated and approved before use (EFSA BIOHAZ Panel, 2017) and are designated for either brainstem or lymph node substrates, not mixed. In the case of LN kits, the preparation step is harsher because of the connective tissue that needs to be broken down. Both tissues (LN and brainstem) have focal rather than generalised PrP accumulation, so the possibility that the tissue subsamples used for the analysis will not be positive also increases, even if the source tissue is positive. When the reindeer population from Nordfjella zone 1 was slaughtered, all adult reindeer from this area were tested, using a mixture of brain material and lymph node as the test substrate for routine diagnostic testing using ELISA (TeSeE Bio-Rad). Nineteen positive animals were found among the 2,359 reindeer tested (Mysterud et al., 2019a). Lymph nodes tissues alone from 350 of the negative animals were then re-tested by ELISA tests (TeSeE ELISA from Bio-Rad and HerdChek from IDEXX) and RT QuIC. Two of them gave an initial weak signal of amplification by RT QuIC but these results were not confirmable by Western blot or immunohistochemistry, or repeatable by RtQuIC. None of these lymph nodes tested positive by ELISAs. The separate analysis of lymph nodes tissues gave similar results to the analysis of pooled samples (Benestad, 2023).	Mixing tissues has the potential to reduce both analytical sensitivity and diagnostic sensitivity, overestimating the sensitivity of the surveillance.

Source of uncertainty	Cause of the uncertainty	Impact of the uncertainty on the conclusions
Detection of CWD in different target tissue samples	A disease detection model for how the likelihood of detecting CWD Ly + infection develops during the course of infection, and the type of sample tested, may be utilised (Viljugrein et al., 2019). After the first months (stage 0), disease-associated PrP starts to become detectable in RLN (stage 1), while it takes a longer time before it starts to be detectable also in brainstem (obex) (stage 2). In these earlier stages of disease, it is very important to obtain a sample from the correct part of the brainstem (at the level of the obex). In the last phase of the disease (stage 3), the detection probability is high, both in RLN and brainstem. Current surveillance strategies will only be able to detect Ly- cases once detectable PrP accumulations are present in the brainstem. The whole population cull and screening that was undertaken for Norwegian reindeer provides evidence that the neuropathogenesis of CWD Ly + supports the brainstem at the obex as the most sensitive diagnostic site in the brain. However, similar data are not available from animals with the Ly- phenotype. (Studies in sheep presenting with atypical scrapie (a Ly- phenotype) demonstrate that the obex is not the optimal neuroanatomical level for screening, and no equivalent neuropathogenesis data exists for atypical H- or L-BSE (Ly- cattle phenotypes).) Despite this lack of data, the obex remains the most feasible sampling site at a practical level.	If the obex is not the most sensitive site for PrP detection in the CNS of Ly- cases, then sampling only this site will reduce the sensitivity of the surveillance. If some cases are being missed, the prevalence of Ly- cases may be underestimated.
Misclassification/ missing information in submitted data	It is known that not all samples have been accompanied by detailed and accurate information regarding target group. There were some discrepancies in the reported data in comparison with additional data available, i.e. some moose cases were misclassified as low-risk target group (in reality, it was high risk) and the opposite for wild reindeer. In the data used, the known misclassifications among the cases were corrected, but this information became available late in the process. It is unknown whether negative samples also were misclassified. Other misclassifications may have not been detected and corrected.	Additional misclassification in high-risk target group assignment could have had impact, either increasing or decreasing the relative risk depending on the number and direction of errors in the official data.
Population genetics	It is known that *PRNP* allele frequencies can vary a lot between distinct deer subpopulations. This is the case for both wild populations and semi-domesticated herds. If the *PRNP* allelic modulation of CWD susceptibility is strong, only the subset of the populations carrying 'susceptibility alleles' would be at high risk of acquiring the disease. The proportion of the population carrying such *PRNP* alleles in most instances is unknown or can range widely. Example: in some semi-domestic reindeer herds in Norway, alleles associated with CWD susceptibility (wt and deletion) are at well below 10% of the herd, whereas in some wild populations above 70%. A large difference in the frequency of alleles putatively associated with susceptibility has been observed in reindeer). This could be not the case in other cervids.	Sample sizes calculated may be underestimated when applied to estimate the frequency polymorphisms due to the high variability within the cervid populations. This would reflect in larger errors in the estimates than expected. In the calculation of the power for case–control studies in species other than reindeer (e.g. moose), a large difference in the exposure to alleles between cases and controls has been assumed based on the reindeer. That can have overestimated the estimated power of future studies.

4. Answers to the terms of reference

ToR1

To analyse the results of the monitoring programme carried out in Norway, Sweden, Finland, Iceland, Estonia, Latvia, Lithuania and Poland between 1 September 2017 and 28 February 2022, and in particular, to assess if the two objectives as set in the 2016 EFSA opinion on CWD in cervids have been met.

- Surveillance data for the mandate period reported to EFSA by the eight countries involved included a total of 156,577 cervids sampled during the mandate period, with > 99% (155,660) of them 12 months of age or older and of unknown age, therefore usable in analyses. Five of the six member states tested > 3,000 animals (the target set in the TSE Regulation). Voluntary testing included > 130,000 cervids by Norway and 300 rfeindeer by Iceland.
- The approach applied for the implementation of the European Commission statutory surveillance in the six MS (Finland, Estonia, Latvia, Lithuania, Poland and Sweden) varied in terms of the design (number, size and characteristics of the declared primary sampling units (PSU)), the number of cervids tested in general and per PSU, and the distribution of testing by species and target groups. The hunted and slaughtered fit for human consumption (HSHC) target group – considered to have the lowest risk of CWD – was the most frequently tested group overall (83.6%) and in six of the eight participating countries, with road kills (RK) being the most tested in Finland and Poland.
- Statutory surveillance was effective in detecting CWD in two countries (Finland, Sweden) for the first time. During the mandate period, a total of 31 cases were confirmed: 13 reindeer, 15 moose and 3 red deer. The two objectives of CWD surveillance (detect disease and estimate prevalence) have partially been met, given the high variability in the implementation of surveillance at country, species, management systems and PSU levels. As a result, for countries that conducted surveillance during the mandate period without finding CWD, the evidence is not sufficient to rule out the possibility of CWD being present. For those countries with cases detected, the prevalence is associated with uncertainty due to the sample-based monitoring.
- For all species combined and during the entire mandate period, the detectable prevalence in the general population, including all animals tested older than 12 months of age and also those of unknown age, was close to 0.1% at country level in six countries (Finland, Latvia, Lithuania, Norway, Poland and Sweden). However, considering the sampling at the PSU level and the numbers of animals tested in the high-risk target groups, there was a low proportion of tested PSU (15.3%) in which the minimum detectable prevalence target of 10% or lower was achieved. The size and number of declared PSU affect substantially the interpretation of the calculated minimum detectable prevalence. Larger countries with lower numbers of PSU had better chances to achieve a lower detectable prevalence. However, representativeness of the sampling within large PSU cannot be assessed.
- Apparent (observed) prevalence of CWD, when detected, was relatively low, and below 1%. For CWD Ly- phenotype, prevalence was ~ 0.05% (95% CI 0.06–0.17%) among apparently healthy (HSHC) moose sampled in Finland, Norway and Sweden, and ~ 0.16% (0.02–0.59%) among HSHC red deer in Norway. For CWD Ly+ in Norwegian reindeer, apparent prevalence was < 1% among HSHC animals. Prevalence was 10-fold higher among cervids submitted from high-risk target groups – redefined to include clinical/sick (SUS, i.e. suspects showing abnormal behaviour, locomotor disturbances or otherwise poor health), fallen/culled (FC i.e. individuals found dead or killed for health/age reasons) and hunted/slaughtered but declared unfit for human consumption (HSNHC)) – emphasising the surveillance value of these groups.
- Scenario-tree modelling has been used to estimate the sensitivity of national surveillance activities and to evaluate the species-specific probability of the presence of disease at a prevalence of 5% or 1% (within infected PSU) and a relative risk (high-risk target groups vs. HSHC) (RR) of 2 and 5, all scenarios assuming 3% of PSU infected or, if more than 100 were defined, 3 PSU infected in the country. The overall estimated sensitivity for all cervids except wild reindeer was 95% or greater to detect CWD if present at a minimum 5% prevalence and a RR of 5 in Norway, Sweden and Poland. For 1% prevalence, only Poland (96%) and Norway (94%) reached ~ 95%. In the analysis by species for RR of 5, only the following scenarios reached 95% sensitivity or higher: for semi-domesticated reindeer, Norway and Finland for 5%

or higher prevalence and Norway for 1% or higher prevalence; for moose, Norway for 5% or higher prevalence; for roe deer, Norway and Poland for 5% or higher prevalence and Poland for 1% or higher prevalence. The rest of the assessed combinations (country/species) did not reach 95% sensitivity.

ToR2

To describe any new knowledge on the epidemiology of CWD in EU/EEA countries

- CWD has been detected in new areas within Norway but also in Sweden and Finland, and in a new species (red deer). The geographic distribution in affected countries is patchy, and in areas where the disease has been found, the observed prevalence was low (< 1%).
- Two main phenotypes of CWD have been described in Europe, designated here as 'Ly+' or 'Ly-' depending on the presence (Ly+) or absence (Ly-) of detectable PrP^{Sc} in lymphoid tissues. All cases detected in Finland and Sweden were in moose and were Ly-.
- Additional cases of both phenotypes (Ly+ in reindeer, Ly- in moose and red deer) were detected in Norway, but with no clear geographic overlap between phenotypes. The two phenotypes are different from North American CWD and from each other, and they are also different in terms of geographical distribution and host range.
- In September 2020, CWD Ly+ was confirmed in an 8.5-year-old male wild reindeer in Hardangervidda, the largest population of wild reindeer in Norway, a different geographical area but adjacent to Nordfjella, where all the wild reindeer cases had previously been detected. Another case was confirmed in a 8.5-year-old female wild reindeer in September 2022 in the same area. This may represent an early epidemic stage in a new outbreak of Ly+, and it is regarded as a major concern for CWD management in Norway.
- Age and sex are relevant. The 19 Ly+ cases (2016–2018) were all identified in the Nordfjella Zone 1. Ly+ was detected in six females aged between 3 and 4 years and 13 males aged 1.5–8.5 years. Infection was 2.7 times more likely in adult males compared to adult females. The apparent prevalence was 1.5% in adult males and 0.5% in adult females (above 2 years old). The Ly- cases in moose and red deer were all in females (except one male) and in older individuals (> 10 years of age). Cases were spread over a large geographic area with limited clustering.
- Initial published data revealed genetic variations in Norwegian reindeer: two *PRNP*-alleles (wt and deletion) were more frequently present in CWD Ly+ cases compared to negative animals. However, the frequency of genotypes is different in wild and semi-domesticated reindeer populations for which data are available.
- Current data still support the interpretation that roe deer are monomorphic with the wild-type cervid genotype, which has been associated with susceptibility in other species.
- A third case of CWD Ly- was detected in a moose in Finland in November 2022.

ToR3

To recommend, if considered appropriate, future CWD monitoring activities for the EU based on an assessment of the epidemiological situation

- Several factors have hindered the surveillance efforts in Europe: uncertainty on the distribution and abundance of deer species; difficult access to remote areas to sample semi-domestic and wild cervids; difficulty in focusing sampling efforts on high-risk target groups; conflicting interests of stakeholders; varying levels of public funding and ambiguity of objectives and priorities.
- Future surveillance can draw on a number of strengths and opportunities from past experience: regulatory support, experience in effective surveillance designing, an established European information system and network of experts and diagnostic facilities, outputs of past surveillance on the disease and deer genetics monitoring.
- The recent mandatory surveillance highlights a wider geographical distribution, host range and phenotypic heterogeneity of the disease compared with past knowledge. These differences and the associated uncertainties suggest that some level of further surveillance for CWD in Europe is appropriate.
- A minimum sustained surveillance effort with a dedicated infrastructure and a good system for obtaining samples and testing should be available in every country. This effort should be focused on the testing of samples from relevant cervid species in high-risk target groups (SUS, FC, HSNHC), systematically and/or opportunistically acquired. Surveillance results should be reported annually to a centralised data repository.

- Beyond the minimum surveillance described above, specific surveillance activities can be implemented depending on the objectives set by risk managers. Four main objectives have been identified: generate epidemiological data and knowledge, provide support for statement of disease status, screen for evidence of spillover and generate data and knowledge on population/case genetics.
- Common features of the proposed surveillance activities for the four objectives are: specific surveillance design for countries with/without previously detected cases of CWD, collection and testing of both retropharyngeal lymph node and brainstem samples; testing animals over 2 years of age if possible; maximising the sensitivity by prioritising the sampling of cervids from high-risk target groups within each selected area and management system; consider a sustained rolling time frame for accumulating surveillance data (or, for each year in which new data area added, give slightly less weight to data from previous years); divide the area/region/country into sampling units based on the epidemiological, and management knowledge of cervid populations present; and, in areas where disease is still undetected, set design prevalence based on the findings of this report or on new epidemiological data when available.
- Public health concerns may lead to measures with the aim to prevent/minimise human exposure via the food chain, but without requiring specific surveillance.

ToR4

Based on what is known about the epidemiology of CWD in EU/EEA countries, to describe the criteria relevant for considering an area not to be infected with CWD

- Certain characteristics of animal prion diseases – in particular, their low and slowly increasing prevalence and incidence, long preclinical incubation periods and long disease course, lack of practical ante-mortem screening tests, and multiple phenotypes and strains – makes it difficult to ascertain or declare non-infected areas.
- Consequently, criteria are proposed for assessing the probability of CWD presence rather than for considering an area non-infected with CWD. The criteria include the definition of the geographical area by setting spatial boundaries; the annual assessment of the risk of introduction of CWD into the area to inform the surveillance design; a minimum sustained surveillance with a dedicated infrastructure and a good system for obtaining samples and testing as described in ToR3; training and engagement of stakeholders, and an 'output based' surveillance programme based on data-driven input parameters.
- In the areas of concern, it should be demonstrated that at least a 95% surveillance sensitivity has been achieved by analysing the surveillance data i.e. the number of animals collected and tested during a rolling timeframe, accounting for the design prevalence between and within areas considered, the relative risk of different target groups, their proportional abundance in the adult population, and the test diagnostic sensitivity.
- The prevalence of CWD in Europe, as estimated by analysing the surveillance data of the mandate period, may be very low in certain areas, requiring a very low design prevalence to detect the disease, where present.

ToR5

To provide the design of a genotyping protocol for positive samples, and for the negative samples of the 3-year monitoring programme stored as per point 3.3, section III.A of Annex III of Regulation (EC) No 999/2001, specifying which negative samples should be genotyped, the codons of the *PRNP* gene to be genotyped and recommending genotyping assay/s for the implementation of the requirement by the NRLs

- All positive cases should be genotyped. Negative samples can be used for polymorphism detection and frequency estimation. A polymorphism is recognised when its frequency reaches a minimum of 1%; this was used to calculate sample sizes for polymorphism detection by species (moose, red deer, reindeer, white-tailed deer and roe deer) and country for each of the six MS under mandatory surveillance. These sample sizes will also enable frequency estimation with a maximum error between 5% and 10%, and for susceptibility association studies.
- In the absence of robust data on the *PRNP* sequence in cervid species, double strand sequencing of the entire *PRNP* open reading frame should be undertaken for each sample since any additional polymorphic site data can be collected using this technique and it represents the gold standard for sequence analysis.

- A centralised data collection system at EU level is required, allowing the collation and, extraction of data for analysis, containing the complete coding sequence of the animal *PRNP* in standard format, and metadata associated with each animal.

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Abbreviations

AQ	Assessment questions
BIOHAZ	EFSA Panel on Biological Hazards
cDP	Design prevalence among PSU
CNS	Central nervous system
cSSe	Country level SSe
CWD	Chronic wasting disease
EEA	European economic area
ELISA	Enzyme-linked immunosorbent assay method
EU	European Union
EURL	EU Reference Laboratory
FASTQ format	text-based format for storing both a biological sequence (usually nucleotide sequence) and its corresponding quality scores
FC	Fallen/culled
GMA	Game management association
HSHC	hunted/slaughtered fit for human consumption
HSNHC	hunted/slaughtered unfit for human consumption
ICAHS	International Conference on Animal Health Surveillance
IUPAC	International Union of Pure and Applied Chemistry
MS	Member state/s
NRLs	National Reference Laboratories
PRNP	Prion protein gene
PrP	Normal cellular prion protein
PrP^{Sc}	Abnormal protease-resistant isoform of prion protein
PSU	primary sampling unit
RHC	Reindeer management cooperative
RK	Road/predator killed
RLN	Retropharyngeal lymph node
RR	Relative risk
SAQ	Sub-assessment questions
SFVS	State Food and Veterinary Service (Lithuania)
SSC	Scientific Steering Committee
SSe	Surveillance system sensitivity
SUS	Clinical suspect animals
SVA	National veterinary Institute (Sweden)
SWOT	Strengths, weaknesses, opportunities, and threats analysis
ToR	Terms of Reference
TSE	Transmissible spongiform encephalopathies
uDP	Design prevalence within infected PSU (detectable prevalence within PSU)
uSSe	SSe for PSU
WOAH	World Organization for Animal Health
wt	Wild type

Appendix A – Surveillance provisions for CWD in EFSA opinion and EU legislation

The EFSA surveillance proposal (2017)

The EFSA opinion on chronic wasting disease in cervids (EFSA BIOHAZ panel, 2017) proposed a surveillance system for CWD, following the mandate of the European Commission of May 2016 in which EFSA was requested *'to provide recommendations on surveillance of the cervid populations at the country level aimed at detecting CWD and/or estimating the prevalence of CWD in Norway, Sweden, Finland, Iceland, Estonia, Latvia and Poland, which are the EU and EEA countries with reindeer and/or moose populations, depending on the level of prevalence which is wished to be detected'* (Terms of Reference 1). The proposal consisted of a 3-year surveillance programme based on a two-stage sampling.

- **Countries to be included**: It was agreed to include also Lithuania in the scope of the mandate due to the significant moose population in this country and its geographical location. Thus, the countries considered in this assessment were Estonia, Finland, Iceland, Latvia, Lithuania, Norway, Poland and Sweden.
- **The target species**: The species considered for surveillance were Eurasian tundra reindeer (*Rangifer tarandus tarandus*), Finnish (Eurasian) forest reindeer (*Rangifer tarandus fennicus*), moose (or Eurasian/European elk) (*Alces alces alces*), roe deer (*Capreolus capreolus*), white-tailed deer (*Odocoileus virginianus*) and red deer (*Cervus elaphus*). Fallow deer (*Dama dama*) were excluded. All the species considered should be part of the surveillance system, unless a subset of these species is selected based on the outputs of national RA. Prior to the implementation of the surveillance activities, a preliminary characterisation of the geographical distribution, abundance and biologically based spatial subdivision relative to the selected national cervid population are needed.
- **Sampling units**: For wild cervids and semidomesticated reindeer: As sampling frames are not available, the design is based on the testing of animals (subunits) from geographically based 'primary sampling units' (PSU). PSU are geographical areas, defined by each country, using a geographical criterion that has to be based:

On the population density of the selected species, i.e. areas in which aggregation of animals of a certain species in a certain period of the year is observed, or

On natural barriers and presence/absence of the species if no aggregation is observed for a species, or

On territorial hunting statistics.

For farmed/captive cervids: farms or other captive wildlife facilities.

- **Target groups**: for wild cervids and semidomesticated reindeer, animals more than 12 months of age and any of the following:
  - ✓ animals killed because sick or in poor body condition and not fit for human consumption;
  - ✓ hunted or slaughtered animals considered not fit for human consumption;
  - ✓ road/predator kills;
  - ✓ found dead.
  - ✓ For farmed/captive cervids: Animals more than 12 months of age and any of the following:
  - ✓ animals killed because sick or in poor body condition and not fit for human consumption;
  - ✓ found dead.
- **Sampling design**: Two-stage sampling aiming at testing at a national level a total of 3,000 wild and semidomesticated cervids and 3,000 farmed/captive cervids of all or the subset of selected species over the 3-year period, which corresponds to an overall design prevalence at a population level of 0.1% and a 95% confidence level.

For the first stage, up to 100 PSU/100 PSU (farms) should be selected for surveillance over a 3-year period using a random sampling approach, which corresponds to a design prevalence of 3% and a 95% confidence level. The random sampling will ensure the geographical representativeness.

For the second stage (within each PSU or within each farm), a convenience sample of 30 animals of all or the subset of selected species as defined above should be collected from the target groups (listed above), which corresponds to a design prevalence of 10% and a 95% confidence level.

If a country defines fewer than 100 PSUs in its territory, a compensating increase in the second-stage sample size should be applied, based on all or the subset of selected species, in order to meet the proposed overall design prevalence at a population level of 0.1% and a 95% confidence level, over the 3-year period.

The target sample sizes at PSU and animal levels together allow the estimation of prevalence with high precision considering the target population as the high-risk animals PSU-level and animal-level sensitivity/specificity are assumed to be equal to 100%

- **Tissues to be collected**: Obex and retropharyngeal lymph nodes or tonsils or other head lymph nodes (in this order of preference of lymphatic tissues). Preserved fresh/frozen, and where practical, fixed

The EU surveillance programme 2018–2020

According to the Commission Regulation (EU) 2017/1972, amending Annexes I and III of the TSE Regulation, MS which have a wild and/or farmed and/or semi-domesticated population of moose and/or reindeer (Estonia, Finland, Latvia, Lithuania, Poland and Sweden) shall carry out a 3-year monitoring programme for CWD in cervids, from 1 January 2018 to 31 December 2020, although 'the collection of samples for the monitoring programme may, however, start in 2017' (point 1,1). The 3-year monitoring programme for CWD in cervids is described in detail in Annex III, chapter A, Part III of the TSE Regulation. The other MS may carry out monitoring for CWD in cervids on a voluntary basis. Points 1.2.1.3, 2.1, 2.2,2.3,2.4,2.5 and 3.1 of Section III, Chapter A, Annex III describe the monitoring for CWD, as follows (quoting):

1.2 The 3-year CWD monitoring programme shall cover the following cervid species:

- Eurasian tundra reindeer (Rangifer tarandus tarandus);
- Finnish forest reindeer (Rangifer tarandus fennicus);
- Moose (Alces alces);
- Roe deer (Capreolus capreolus);
- White-tailed deer (Odocoileus virginianus);
- Red deer (Cervus elaphus).

1.3 By way of derogation, a Member State may, based on a documented risk assessment submitted to the European Commission, select for the 3-year CWD monitoring programme a subset of the species listed above.

2 Sampling design.

2.1 The Member States referred to in point 1.1 shall identify primary sampling units (PSU), which shall cover all territories in which cervid populations are present, using at least the following elements:

a) for farmed and captive cervids, each farm and each facility in which cervids are kept in an enclosed territory shall be considered as a PSU.
b) for wild and semi-domesticated cervids, PSU shall be defined geographically based on the following criteria:
c) the areas in which wild and semi-domesticated animals of a species covered by the monitoring programme gather in at least a certain period of the year.
    i) if no gathering takes place for a species, the areas delimited by natural or artificial barriers in which animals of the species covered by the monitoring programme are present.
    ii) the areas in which animals of the species covered by the monitoring programme are hunted and areas connected to other relevant activities related to the species covered by the monitoring programme.

2.2 The Member States involved shall select farmed, captive, wild and semi-domesticated cervids for TSE testing using the following two-stage sampling approach:

a) In the first stage, those Member States shall:
    i) For farmed and captive cervids:

- Select, on a random basis ensuring geographical representativeness, and if relevant taking into account relevant risk factors identified in a documented risk assessment carried out by the Member State, 100 PSU to be covered over the 3-year period of the monitoring programme, or
- if the Member State was unable to identify 100 PSU for farmed and captive cervids, select all PSU identified.

ii) For wild and semi-domesticated cervids:

- Select, on a random basis ensuring geographical representativeness, and if relevant taking into account relevant risk factors identified in a documented risk assessment carried out by the Member State, 100 PSU to be covered over the 3-year period of the monitoring programme, or
- if the Member State was unable to identify 100 PSU for wild and semidomesticated cervids, select all PSU identified.

b) In the second stage:

i) For farmed and captive cervids:

- A Member State having selected 100 PSU shall, within every selected PSU, sample all animals belonging to the target groups listed under point 2.4. (a) over the 3-year period until a target of 30 animals tested per PSU is reached. If however certain PSU are not be able to reach the target of 30 animals tested over the 3-year period due to the limited size of their cervid population, the sampling of animals belonging to the target groups listed under point 2.4. (a) may continue in larger PSU even after having reached the target of 30 animals tested, with the objective of reaching a total number of up to 3 000 farmed and captive cervids, where possible, tested at national level over the 3-year period of the monitoring programme;
- A Member State having identified fewer than 100 PSU shall, within every PSU, sample all animals belonging to the target groups listed under point 2.4. (a) over the 3-year period, with the objective of approaching a total number of up to 3000 farmed and captive cervids, where possible, tested at national level over the 3-year period of the monitoring programme.

ii) For wild and semi-domesticated cervids:

- A Member State having selected 100 PSU shall, within every selected PSU, sample all animals belonging to the target groups listed under point 2.4. (b) Over the 3-year period until a target of 30 animals tested per PSU is reached, with the objective of reaching up to 3 000 wild and semi-domesticated cervids tested at national level over the 3-year period.
- A Member State having identified fewer than 100 PSU shall, within every PSU, sample all animals belonging to the target groups listed under point 2.4. (b) Over the 3-year period, with the objective of approaching a total number of 3 000 wild and semi-domesticated cervids tested at national level over the 3-year period of the monitoring programme.

2.3 All cervids selected must be over 12 months of age. The age shall be estimated on the basis of dentition, obvious signs of maturity or any other reliable information.

2.4 The cervids must be selected from the following target groups:

a) For farmed and captive cervids:

i) Fallen/culled farmed or captive cervids, defined as farmed or captive cervids found dead on the enclosed territory in which they are kept, during transport or at slaughterhouse, as well as farmed or captive cervids killed for health/age reasons.
ii) Clinical/sick farmed or captive cervids, defined as farmed or captive cervids showing abnormal behavioural signs and/or locomotor disturbances and/or as being generally in poor condition.
iii) Slaughtered farmed cervids which have been declared unfit for human consumption.

iv) Slaughtered farmed cervids considered fit for human consumption if a Member State identifies fewer than 3 000 farmed and captive cervids from the groups (i) to (iii).

b) For wild and semi-domesticated cervids:

i) Fallen/culled wild or semi-domesticated cervids, defined as cervids found dead in the wild as well as semi-domesticated cervids found dead or killed for health/age reasons.
ii) Road- or predator-injured or killed cervids, defined as wild or semi-domesticated cervids hit by road vehicles, by trains or attacked by predators.
iii) Clinical/sick wild and semi-domesticated cervids, defined as wild and semidomesticated cervids which are observed as showing abnormal behavioural signs and/or locomotor disturbances and/or as being generally in poor health condition.
iv) Wild hunted cervids and slaughtered semi-domesticated cervids which have been declared unfit for human consumption.
v) Hunted wild game and slaughtered semi-domesticated cervids considered fit for human consumption if a Member State identifies fewer than 3 000 wild and semidomesticated cervids from the groups (i)–(iv).

2.5 In case of a positive finding of TSE in a cervid, the number of samples from cervids collected in the zone where the positive TSE case was found must be increased, based on an assessment carried out by the Member State concerned.

Member states (MS) may, on a voluntary basis, carry out monitoring for TSE in animal species other than bovine, ovine, caprine and cervids according to Annex III, Chapter A, Part IV of the TSE Regulation.

3.1 For each cervid selected in accordance with point 2, a sample of obex shall be collected and tested for TSEs. In addition, where feasible, a sample of one of the following tissues shall be collected in the following order of preference: (a) retropharyngeal lymph nodes; (b) tonsils; (c) other head lymph nodes.

Appendix B – Minimum detectable prevalence by country, species and PSU

The minimum detectable prevalence by PSU was calculated considering only the number of cervids tested older than 12 months of age in the risk group (consisting of FC, HSNHC, RK, SUS) and for the entire mandate period. When the PSU were defined as separate entities for farmed/captive and semi-domesticated/wild, the minimum detectable prevalence was calculated for each of them. When the number of animals tested in a PSU was equal to or less than 10, the minimum detectable prevalence was not calculated. As a reference for each PSU, a 10% minimum detectable prevalence (with a 95% confidence level) was indicated in the EFSA 2017 Opinion as a target to be achieved through the collection and testing of animals (any cervids species) from the risk group.

Table B.1: Minimum detectable prevalence achieved by country and species for the period September 2017–February 2022 based on the number of animals tested older than 12 months of age and of unknown age from all groups and not accounting for the population size. In bold species and countries with cases

Country	Deer	European moose	Fallow deer	Red deer	Reindeer	Roe deer	Sika deer	White-tailed deer	Total
Estonia		0.59%		1.1%		0.23%			0.14%
Finland		**0.4%**			0.17%	0.47%		0.8%	0.08%
Iceland					1%				0.98%
Latvia		0.46%		0.34%		0.2%			0.1%
Lithuania		1.2%		0.29%		0.11%			0.08%
Norway	0.95%	**0.01%**	2%	**0.01%**	**0.008%**	0.04%			0.004%
Poland		2%		0.55%		0.13%			0.1%
Sweden		**0.17%**	18.1%	0.6%	0.05%	1.3%			0.03%

Table B.2: Minimum detectable prevalence by country and species for the period September 2017 to February 2022, older than 12 months of age and of unknown age, from the high-risk surveillance groups (all except HSHC). In bold species and countries with cases

	Deer	European moose	Fallow deer	Red deer	Reindeer	Roe deer	Sika deer	White-tailed deer	Grand total
Estonia		6.1%		15.3%		0.54%			0.47%
Finland		**0.57%**			0.17%	0.49%		0.8%	0.09%
Iceland									25.8%
Latvia		9.5%		6%		2.1%			1.3%
Lithuania		6.1%		2%		0.5%			0.37%
Norway	0.6%	**0.08%**		**0.11%**	**0.19%**	0.04%			0.019%
Poland		**1.5%**		0.96%		0.1%			1%
Sweden		0.4%	17%	3%	0.35%	1.3%			1.6%

Estonia

Out of the 15 PSU declared, 12 PSU had tested animals older than 12 months of age or of unknown age in the risk groups. overall, in six PSU (50% of the tested PSU and 40% of the declared PSU), the minimum detectable prevalence was lower than 10% (Figure B.1).

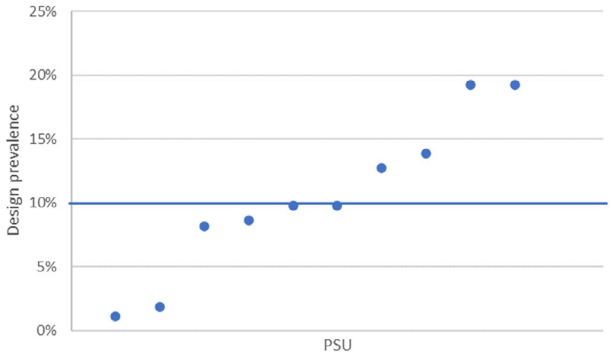

Figure B.1: Minimum detectable prevalence in the 10 PSU (out of 12) in Estonia where 10 or more cervids older than 12 months of age or of unknown age of the high-risk target groups were tested

Finland

In the eight PSU for farmed/captive that had tested animals older than 12 months of age in the risk groups, only one PSU had more than 10 cervids tested (16), with a minimum detectable prevalence of 17.1%.

Despite having initially declared only 54 PSU for semi-domesticated reindeer, results were submitted from 62 PSU that had tested animals older than 12 months of age or of unknown age in the risk groups. While 14 had tested 10 or fewer, in the other 48, the minimum detectable prevalence ranged between 20.5% and 5.1. A total of 37 PSU (59.7% of the tested PSU and 68.5% of the initially declared PSU) had a minimum detectable prevalence of 10% or lower (Figure B.2).

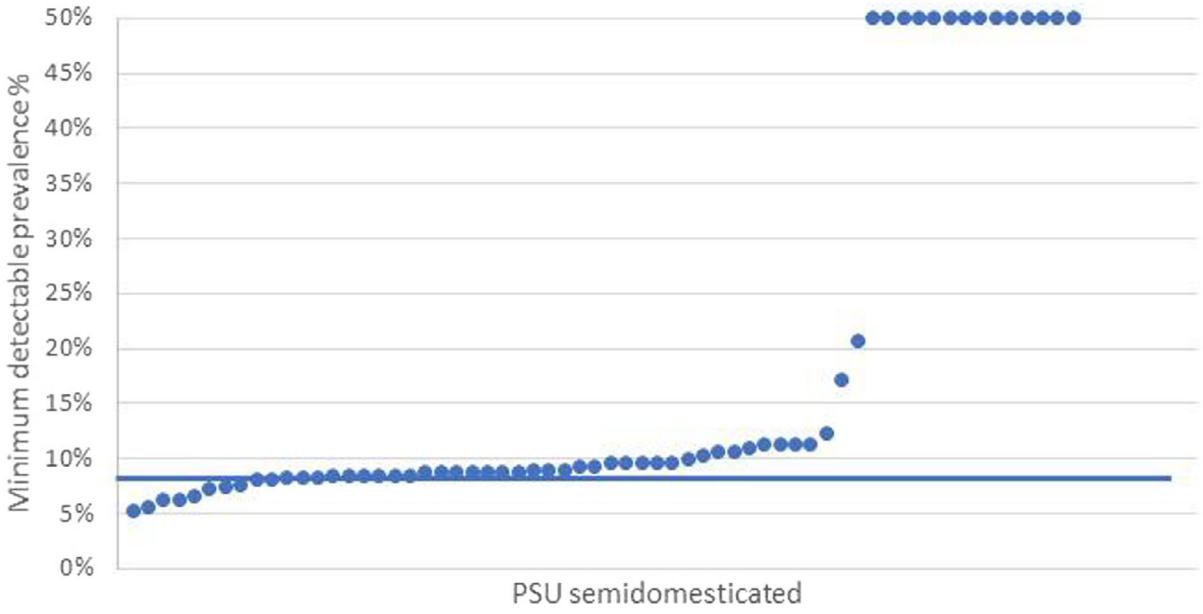

Figure B.2: Minimum detectable prevalence in the 62 PSU semidomesticated in Finland. PSU in which 10 or more cervids older than 12 months of age or of unknown age of the high-risk target groups were not tested appear with 50% minimum detectable prevalence

In the 221 PSU for wild cervids that had tested animals older than 12 months of age (or of unknown age), 213 had tested in the risk groups, and 182 had tested 10 or fewer. In the other 31, the minimum detectable prevalence ranged between 23.8% and 1.8%. Overall, there were 10 wild PSU (25.6% of all PSU) where 10 or more cervids were tested and 4.5% of the PSU that tested animals older than 12 months of age (or of unknown age) in which the minimum detectable prevalence was lower than 10% (Figure B.3).

There were three PSU in which both semi-domesticated (s) and wild (w) animals were tested (38 s + 1w, 30s + 1w and 2 s + 1w). The total number of animals tested in those PSU did not alter the overall results of the minimum detectable prevalence. In one PSU in which the animals in all three categories were tested (1 s + 2w + 16 farmed).

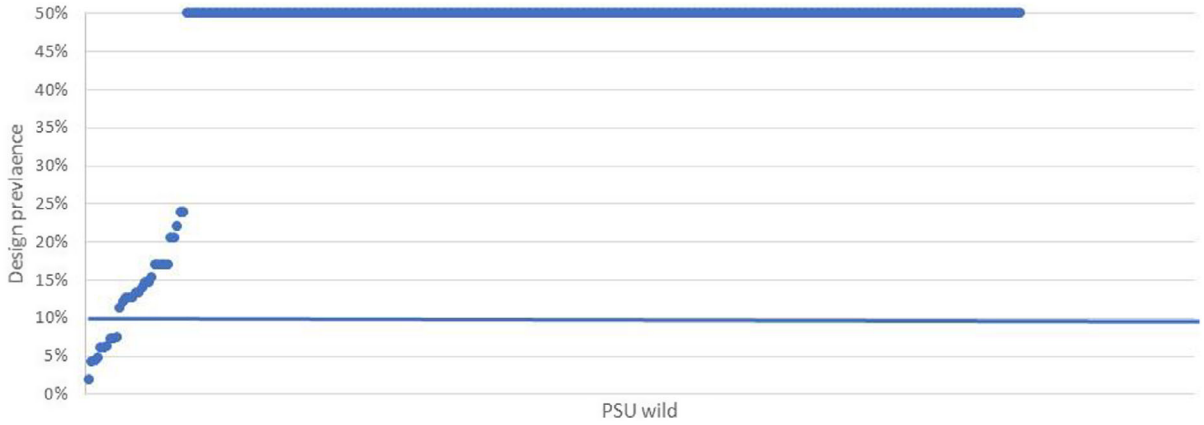

Figure B.3: Minimum detectable prevalence in the 295 PSU for wild cervids in Finland where 31 PSU had tested 10 or more cervids older than 12 months of age or of unknown age of the high-risk target groups. PSU in which cervids were not tested at all or where less than 10 cervids older than 12 months of age or of unknown age of the high-risk target groups were tested appear with 50% minimum detectable prevalence

Latvia

Out of 249 PSU where wild cervids were tested, there were 63 PSU for wild cervids that had animals older than 12 months of age (or of unknown age) tested in the risk groups, and none of them had more than 10 cervids tested (109 animals were tested in unidentified PSU).

Lithuania

Lithuania did not report PSU to the EFSA database.

Poland

In the 16 PSU that had animals older than 12 months of age or of unknown age tested in the risk groups (in 3 of them both farmed and wild were tested), all of them had tested more than 10 cervids, with the minimum detectable prevalence ranging between 17.1% and 1.1%. In all the PSU except one, the minimum detectable prevalence was lower than 10% (Figure B.4). A total of 761 animals were tested in unidentified PSU.

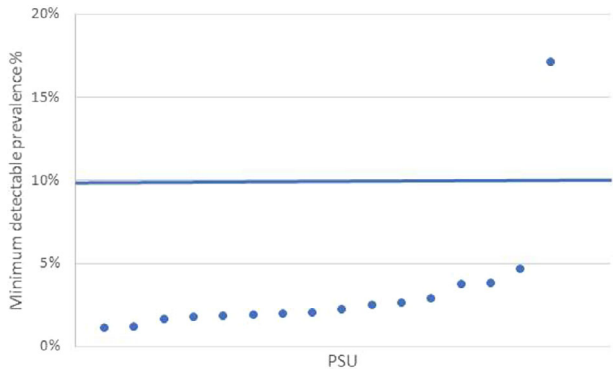

Figure B.4: Minimum detectable prevalence in the 16 PSU in Poland

Sweden

Of the 109 declared PSU for farmed cervids, 71 of them were tested and 30 of them had tested animals older than 12 months of age (or of unknown age) in the risk groups, with only one PSU having

tested more than 10 cervids (16) with a minimum detectable prevalence of 17.1%. None of the PSU for farmed/captive PSU had a minimum detectable prevalence 10% or lower.

Out of the 51 declared PSU for semi-domesticated reindeer, 47 were tested and 41 of them had tested animals older than 12 months of age (or of unknown age) from the risk groups. In 17 PSU, 10 or fewer reindeer had been tested. In the other 24, the minimum detectable prevalence ranged between 22.1% and 4.2%. Overall, in 12 PSU (29.3% of all PSU tested and 23.5% of the declared PSU), the minimum detectable prevalence was 10% or lower (Figure B.5).

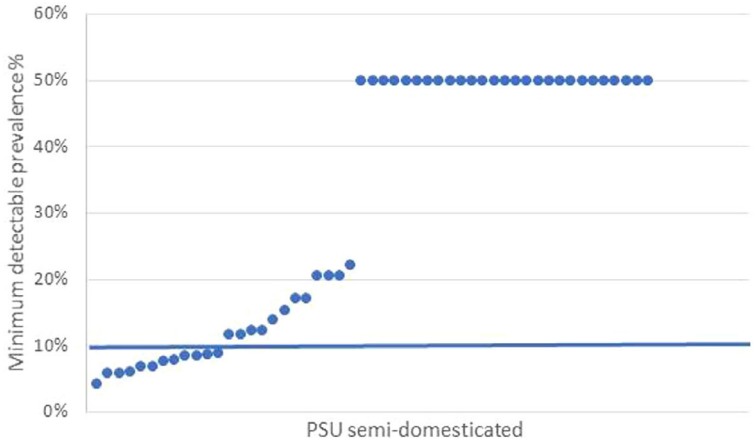

Figure B.5: Minimum detectable prevalence in the 51 PSU for semi-domesticated reindeer in Sweden, where 24 of them had tested 10 or more cervids older than 12 months of age or of unknown age of the high-risk target groups. PSU in which cervids were not tested at all or where less than 10 cervids older than 12 months of age or of unknown age of the high-risk target groups were tested appear with 50% minimum detectable prevalence

All the 50 declared PSU for wild cervids were tested, that had animals older than 12 months of age (or of unknown age) tested in the risk groups, with 22 had tested 10 or fewer. In the other 28, the minimum detectable prevalence ranged between 23.8% and 4.3%. Overall, in 11 PSU (39.3% of all PSU where 10 or more cervids were tested and 22% of the declared PSU), the minimum detectable prevalence was 10% or lower (Figure B.6). A total of 118 animals from unidentified PSU were also tested.

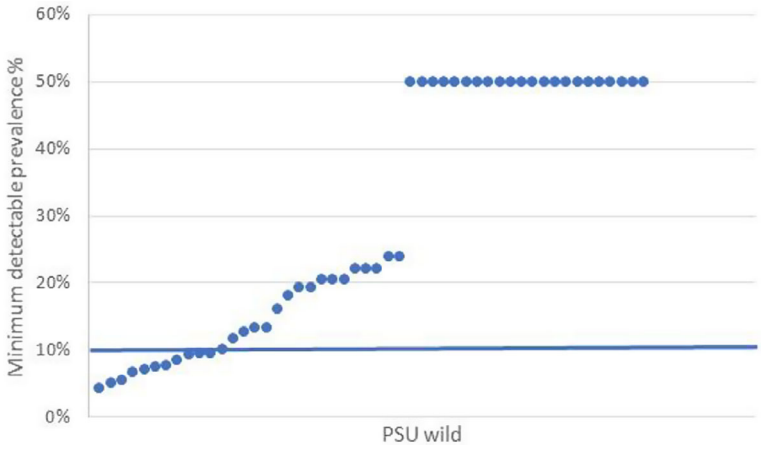

Figure B.6: Minimum detectable prevalence in the 50 PSU for wild reindeer in Sweden, where 28 of them had tested 10 or more cervids older than 12 months of age or of unknown age of the high-risk groups. PSU in which cervids were not tested at all or where less than 10 cervids older than 12 months of age or of unknown age of the high-risk target groups

In the two Norrbotten areas, comprising multiple PSU, where intensified surveillance was conducted, the minimum detectable prevalence considering the semi-domesticated reindeer and wild moose tested in the risk groups (303 and 14, respectively) was 0.9%. These areas include 10 PSU of

semi-domesticated reindeer (Sami villages) and two PSU of wild cervids. In the Västerbotten area, the minimum detectable prevalence considering the wild moose older than 12 months of age (28) tested in the risk groups was 10.1%.

Appendix C – Additional results of the Sensitivity model

All cervids except Norwegian wild reindeer

Table C.1: Outputs of the disease detection model with estimates of the sensitivity of the surveillance by country for wild and semi-domesticated cervids

Country	cDP	uDP	RR	cSSe	Median uSSe	PSU	N PSU Prob 80	N PSU Prob 95
Norway	3/432	1	2	91.9	71.9	402	173	105
Norway	3/432	5	2	99.0	99.8	402	300	258
Sweden	3/101	1	2	75.4	33.6	98	13	9
Sweden	3/101	5	2	98.2	87.9	98	53	36
Finland	3/349	1	2	33.0	5.2	279	1	0
Finland	3/349	5	2	70.2	24.4	279	68	37
Latvia	3/240	1	2	24.3	6.9	240	0	0
Latvia	3/240	5	2	65.5	30.5	240	2	0
Poland	1/16	1	2	87.6	92.0	16	15	5
Poland	1/16	5	2	98.6	100	16	15	15
Estonia	1/15	1	2	52.3	66.3	13	6	3
Estonia	1/15	5	2	71.5	99.6	13	10	8

cDP: % Design prevalence among PSUs; 3 per 100 (or total) PSU. At least 1 per total PSU declared; uDP: % Design prevalence within infected PSU (detectable prevalence within PSU) RR: relative risk of high-risk target groups compared to HSHC; cSSe: % Country level SSe; uSSe: % SSe for PSUs, summarised by the median uSSe for PSUs with samples; PSU: Number of PSU with samples registered; Prob80: Number of PSU with unit SSe reaching at least 80%; Prob95: Number of PSU with unit SSe reaching at least 95%.

Semi-domesticated reindeer

Table C.2: Outputs of the disease detection model with estimates of the sensitivity of the surveillance for semi-domesticated reindeer

Country	cDP	uDP	RR	cSSe	Median uSSe	PSU	N PSU Prob 80	N PSU Prob 95
Norway	3/100	1	2	95.6	96.9	71	48	40
Norway	3/100	5	2	99.0	100	71	64	60
Sweden	3/100	1	2	57.5	44.4	47	9	8
Sweden	3/100	5	2	88.4	95.1	47	33	24
Finland	3/100	1	2	57.0	43.6	58	0	0
Finland	3/100	5	2	96.1	94.9	58	46	27

cDP: % Design prevalence among PSUs; 3 per 100 (or total) PSU. At least 1 per total PSU declared; uDP: %Design prevalence within infected PSU (detectable prevalence within PSU); RR: relative risk of high-risk target groups compared to HSHC.
cSSe: % Country level SSe; uSSe: % SSe for PSU, summarised by the median uSSe for PSU with samples; PSU: Number of PSU with samples registered for more than one (3 for Finland) animal; Prob80: Number of PSU with unit SSe reaching at least 80%; Prob95: Number of PSU with unit SSe reaching at least 95%; Group.

Moose

Table C.3: Outputs of the disease detection model with estimates of the sensitivity of the surveillance for moose

Country	cDP	uDP	RR	cSSe	Median cSSe	PSU	N PSU Prob 80	N PSU Prob 95
Norway	3/274	1	2	77.5	32.5	256	51	19
Norway	3/274	5	2	96.6	86.6	256	139	109
Sweden	3/100	1	2	36.0	19.3	48	4	1
Sweden	3/100	5	2	78.0	67.2	48	19	7
Finland	3/295	1	2	9.7	3.5	182	0	0
Finland	3/295	5	2	36.0	16.9	182	1	0
Latvia	3/100	1	2	15.8	2.6	149	0	0
Latvia	3/100	5	2	52.2	12.7	149	1	1
Estonia	1/15	1	2	24.0	36.5	11	0	0
Estonia	1/15	5	2	52.7	90.3	11	6	3
Poland	1/16	1	2	12.2	13.4	9	0	0
Poland	1/16	5	2	31.9	52.4	9	4	1

cDP: % Design prevalence among PSUs; 3 per 100 (or total) PSU. At least 1 per total PSU declared; uDP: %Design prevalence within infected PSU (detectable prevalence within PSU) RR: relative risk of high-risk target groups compared to HSHC; cSSe % Country level SSe; uSSe % SSe for PSU, summarised by the median uSSe for PSU with samples; PSU: Number of PSU with samples registered; Prob80: Number of PSU with unit SSe reaching at least 80%; Prob95: Number of PSU with unit SSe reaching at least 95%.

Red deer

Table C.4: Outputs of the disease detection model with estimates of the sensitivity of the surveillance for red deer

Country	cDP	uDP	RR	cSSe	Median uSSe	PSU	Prob 80	Prob 95
Norway	3/241	1	2	67.5	21.7	217	34	21
Norway	3/241	5	2	92.1	71.4	217	93	68
Latvia	3/141	1	2	12.4	3.5	141	0	0
Latvia	3/141	5	2	43.9	16.6	141	0	0
Poland	1/16	1	2	24.4	24.2	15	0	0
Poland	1/16	5	2	69.7	75.9	15	5	1
Estonia	1/15	1	2	7.5	10.9	4	1	0
Estonia	1/15	5	2	12.8	44.1	4	1	1

cDP: % Design prevalence among PSUs; 3 per 100 (or total) PSU. At least 1 per total PSU declared; uDP: % Design prevalence within infected PSU (detectable prevalence within PSU); RR: relative risk of high-risk target groups compared to HSHC; cSSe % Country level SSe; uSSe % SSe for PSU, summarised by the median uSSe for PSU with samples; PSU Number of PSU with samples registered; Prob80 Number of PSU with unit SSe reaching at least 80%; Prob95 Number of PSU with unit SSe reaching at least 95%.

Roe deer

Table C.5: Outputs of the disease detection model with estimates of the sensitivity of the surveillance for roe deer

Country	cDP	uDP	RR	cSSe	Median uSSe	PSU	N PSU Prob 80	N PSU Prob 95
Norway	3/236	1	2	62.5	19.1	226	18	7
Norway	3/226	5	2	92.1	66.5	226	88	63
Poland	1/16	1	2	82.8	86.1	16	13	1
Poland	1/16	5	2	98.6	100	16	15	15
Estonia	1/15	1	2	43.8	55.4	13	3	2
Estonia	1/15	5	2	68.0	98.4	13	9	8

Country	cDP	uDP	RR	cSSe	Median uSSe	PSU	N PSU Prob 80	N PSU Prob 95
Latvia	3/198	1	2	16.3	4.8	198	0	0
Latvia	3/198	5	2	53.0	22.2	198	0	0
Finland	3/295	1	2	7.8	1.8	75	1	0
Finland	3/295	5	2	21.1	8.9	75	9	5
Sweden	3/100	1	2	10.2	5.2	32	0	0
Sweden	3/100	5	2	31.7	24.3	32	3	1

cDP: % Design prevalence among PSUs; 3 per 100 (or total) PSU. At least 1 per total PSU declared; uDP: % Design prevalence within infected PSU (detectable prevalence within PSU); RR: relative risk of high-risk target groups compared to HSHC; cSSe: % Country level SSe; uSSe: % SSe for PSU, summarised by the median uSSe for PS with samples; PSU: Number of PSU with samples registered; Prob80: Number of PSU with unit SSe reaching at least 80%; Prob95: Number of PSU with unit SSe reaching at least 95%.

Farmed red deer

Table C.6: Outputs of the disease detection model with estimates of the sensitivity of the surveillance for farmed red deer

Country	cDP	uDP	RR	cSSe	Median uSSe	PSU	N PSU Prob 80	N PSU Prob 95
Sweden	3/109	1	2	12.8	3.5	109	0	0
Sweden	3/109	5	2	43.4	17.0	109	3	0
Poland	1/16	1	2	6.8	12.8	16	0	0
Poland	1/16	5	2	13.6	48.4	16	1	1

cDP: % Design prevalence among PSUs; 3 per 100 (or total) PSU. At least 1 per total PSU declared; uDP: % Design prevalence within infected PSU (detectable prevalence within PSU); RR: relative risk of high-risk target groups compared to HSHC; cSSe: % Country level SSe; uSSe: % SSe for PSU, summarised by the median uSSe for PSU with samples; PSU: Number of PSU with samples registered. For farmed deer in Sweden, PSU refers to individual farms; Prob80: Number of PSU with unit SSe reaching at least 80%; Prob95: Number of PSU with unit SSe reaching at least 95%.

Appendix D – Analytical method for CWD surveillance data. Scenario tree modelling

A suggested approach is scenario tree modelling to enable full use of data from different target groups. This approach enables giving different weight to samples from healthy animals compared to 'risk animals', to collect data cumulative over time, taking into account the yearly risk of introduction (thus reducing confidence) or risk for environmental persistence of prion contamination in an area with previous confirmed cases. By simulation study, the scenario tree modelling may also be used to plan appropriate surveillance for the years to come (Mysterud et al., 2020a; Cowled et al., 2022).

Statistical methods for estimating probability of freedom from infection are well established in veterinary epidemiology (e.g. FAO, 2014). Building on the tools for scenario tree modelling, the methods estimate the probability that a surveillance system will detect at least one infected individual if the number of infections is above the design prevalence (a predefined threshold). This calculation can then be extended to estimate the confidence of freedom from the infection of interest (at the design prevalence) given accumulated absence of detection of cases and according to Bayesian probability theory (Martin et al., 2007). Evidence is accumulated over time to calculate the probability of freedom from infection at the predetermined time-step (year), whereby the probability that the country, region, PSU or flock of interest increases with each negative result. Before surveillance has started, the prior probability of freedom from infection is usually set to 50%, which is a conservative estimate suggesting that presence and absence of infection/disease are both equally likely. For a given design prevalence, the desired system sensitivity can be set according to time to reach a certain level of confidence, for a specific risk of introduction.

In Norway, for example, the probability of CWD-freedom was calculated the wild reindeer populations other than Nordjfella was calculated for design prevalence of 1%, 0.5%, 0.3% and restricted to a lower threshold equivalent to four individuals in the population (Mysterud et al., 2023). A high confidence of freedom-from-CWD was only achieved in a few of the larger populations for a 1% design prevalence. At a design prevalence of 0.5%, the mean probability of freedom-from-infection was 77%, and variable between 60% and 99% (Figure D.1). For stricter design prevalence, the probability of freedom-from-infection was low for most areas. An exception was the Nordfjella management zone 2, where extra-harvesting and marksmen culling were performed to increase sample size.

Below are some examples to illustrate the scenario tree modelling approach showing how surveillance data from several years add to increased probability of freedom.

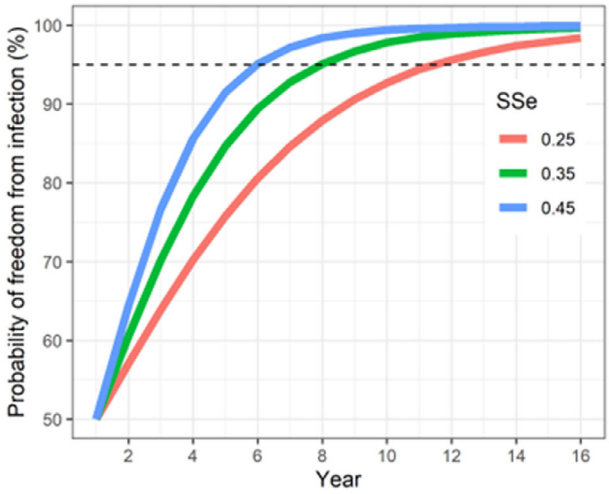

Figure D.1: Accumulating confidence of freedom from infection. With a yearly surveillance sensitivity of 0.45, and a negligible probability of infection (probability of introduction = 0.001), it will take 6 years to achieve a 95% probability of freedom from infection. If the yearly surveillance sensitivity is reduced to 0.35 or 0.25, it will take 8 and 11–12 years, respectively, to achieve a 95% probability of freedom from infection

Risk of CWD introduction

The risk of CWD introduction should be set based on the results of the risk assessment (see above) and dependent on spatial scale of the area of interest, and on species. As a minimum, we can use 0.001, equivalent to 1 introduction per 1,000 years. In other situations, for example with known infection in neighbouring PSU, the risk of introduction can be high (e.g. one or more introductions per 50 years) and the surveillance sensitivity needs to be kept at a high level in order to not reduce the accumulated evidence of freedom from infection (Figure D.2).

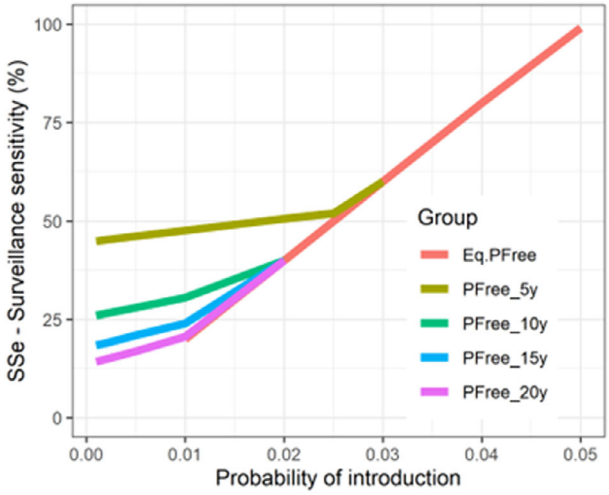

Figure D.2: Yearly surveillance sensitivity required to reach 95% probability of freedom from infection (at design prevalence), within 5, 10, 15 or 20 years, for different level of probability of introduction. In addition, the long-term equilibrium probability of freedom from infection (Eq.PFree) after discounting for the probability of a new introduction was required to be at least 95%. (Assuming the same SSe is achieved from the surveillance each year.)

Surveillance sensitivity

The surveillance can, for example, be the result of a two-stage sampling (country or region) or one-stage sampling in a PSU (or a flock), and samples may vary in terms of relative risk of infection and detection probability. The wanted or required surveillance sensitivity can therefore be achieved in different ways, from different numbers and combinations of samples.

For example, if we use a design prevalence of 1%, assume proportion of adult animals dying from other reasons than hunting/natural slaughtering is 8%, a test sensitivity equal to 96% (Table 15) and the relative risk is five times higher in the high-risk group, the number of samples required to reach a surveillance sensitivity of 40% in a primary sampling unit will be 17 if the proportion of samples from the high-risk group is 80% and 50 if the proportion of samples from the high-risk group is 10%.

The surveillance sensitivity is dependent on the design prevalence. At country/region level, the cluster-level design prevalence will be 1 among the total number of primary sampling units of the population being surveyed. The within-PSU design prevalence may be set dependent on country, species, distance to known infection, importance to detect early (low) levels of infection, etc.

Analysis and interpretation of surveillance results

When assessing the results of the surveillance it would be relevant to consider the latest developments in the field of knowledge of different strains of CWD. Available knowledge already indicates that it would be relevant to analyse Ly- and Ly+ cases separately.

A suggested approach is scenario tree modelling to enable full use of data from different target groups. This approach enables giving different weight to samples from healthy animals compared to 'risk animals', to collect data cumulative over time, taking into account the yearly risk of introduction (thus reducing confidence) or risk for environmental persistence of prion contamination in an area with previous confirmed cases. The design prevalence in combination with the number of animals available for sampling (in relation to population size) will determine the time it takes to reach the surveillance target.

In areas of concern, it should be demonstrated that at least a 95% surveillance sensitivity has been achieved by analysing the surveillance data. Alternative methods can be used to accumulate the surveillance data over several years. One approach is to pool the number of animals collected and tested during a rolling timeframe, another to utilising the whole surveillance period, by giving less weight to data from earlier years. A variant of the last approach is used in statistical methods in veterinary epidemiology for estimating probability of 'freedom from infection' (Martin et al., 2007).

Annex A – Protocol

Annex A is available under the Supporting Information section on the online version of the scientific output.