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Evaluation of the application of Slovenia to be recognised as having a negligible risk of classical scrapie

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Abstract

Slovenia submitted a request to the European Commission to be recognised as a Member State with negligible risk of classical scrapie. EFSA has been asked to assess if Slovenia has demonstrated that, between 2016 and 2022, a sufficient number of ovine and caprine animals over 18 months old, representative of those slaughtered, culled or found dead have been tested, and will continue to be tested annually, to provide a 95% confidence of detecting classical scrapie if it is present at a prevalence rate exceeding 0.1%. A risk-based approach using stochastic scenario tree modelling accounting for surveillance stream and species was applied. Globally, there is still a lack of data on the performance of the approved diagnostic screening tests under field conditions, specifically for sheep. Therefore, alternative scenarios were explored extending the range from the sensitivity (99.6%) provided by the past European Union evaluations to a sensitivity of 50%, more consistent with published data obtained under field conditions in infected goat populations. It was concluded that during the period 2016–2023, Slovenia has tested annually a sufficient number of ovine and caprine animals over 18 months of age, sourced from the NSHC and SHC populations, to ensure a 95% level of confidence of detecting CS if it is present in that population at a prevalence rate exceeding 0.1%, assuming a test sensitivity of 90% or above. The same holds for the years 2016, 2021 and 2023, assuming a test sensitivity of at least 80%. Based on the proposed number of samples for 2024 and future years, Slovenia would continue to meet the testing requirements assuming a test sensitivity of at least 80%.

KEYWORDS

classical, negligible, risk, scrapie, Slovenia, surveillance

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SUMMARY

Since 1 July 2013, Member States (MS) have been able to submit a request to the European Commission (EC) to be recognised as a MS, or zone of a MS, with a negligible risk of classical scrapie (CS). Slovenia submitted this request in June 2023. The EC requested the technical assistance of EFSA, to assess if Slovenia in its application: (a) has demonstrated that, for a period of at least 7 years (2016–2022), a sufficient number of ovine and caprine animals over 18 months of age, representative of slaughtered, culled or found dead on farm, have been tested annually, to provide a 95% level of confidence of detecting CS if it is present in that population at a prevalence rate exceeding 0.1%; (b) and will continue to carry out annually a sufficient number of tests of ovine and caprine animals over 18 months of age, representative of slaughtered, culled or found dead on the farm, to provide a 95% level of confidence of detecting CS if it is present in that population at a prevalence rate exceeding 0.1%, in order to maintain their status.

As in the four previous evaluations conducted for Denmark, Finland, Sweden and the Czech Republic (EFSA, 2015a, 2015b, 2015c, 2023), a risk-based method using scenario tree modelling with stochastic simulation, in order to account for the uncertainty of the estimated parameters, was applied to estimate the overall sensitivity of the surveillance system (SSe) in Slovenia. The model was developed using R and has been made publicly available. Two risk indicators, namely surveillance stream and species, were considered. The estimation of the relative risk of 'not slaughtered for human consumption' (NSHC) versus 'slaughtered for human consumption' (SHC) streams and of sheep vs. goats was done by analysing EU surveillance data from 2010 to 2022 at the member state (MS) level.

Currently, there are no data to quantify, at European Union (EU) level, the overall diagnostic sensitivity, under field conditions, of the screening tests used for the detection of CS in small ruminants over 18 months of age. Given the uncertainty about the test sensitivity under field conditions, alternative scenarios were explored extending the range from the sensitivity provided by the EU evaluations (99.6%) down to a sensitivity of 50%. This lower sensitivity is consistent with published data obtained under field conditions in infected goat populations. No such data is available specifically for sheep.

As agreed in previous evaluations and for consistency purposes, given a design prevalence (DP) (0.1%), *N*, (Total NSHC/SHC sheep/goat population) and *n*, (number of NSHC/SHC sheep/goat tested) for each combination of year and test sensitivity, the 95% confidence level of detecting CS was considered achieved when the SSe was 95% or greater at the 5th percentile of the output distribution of the model.

It was concluded that during the period 2016–2023, Slovenia has tested annually a sufficient number of ovine and caprine animals over 18 months of age, sourced from the NSHC and SHC populations, to ensure a 95% level of confidence of detecting CS if it is present in that population at a prevalence rate exceeding 0.1%, assuming a test sensitivity of 90% or above. The same holds for the years 2016, 2021 and 2023, assuming a test sensitivity of at least 80%. Based on the proposed number of samples to be tested in 2024 and in future years, Slovenia would test annually a sufficient number of ovine and caprine animals over 18 months of age, sourced from the NSHC and SHC, to provide a 95% level of confidence of detecting CS if it is present in that population at a prevalence rate exceeding 0.1%, assuming a test sensitivity of at least 80%.

The diagnostic sensitivity of the screening tests under field conditions is a key parameter when estimating the overall sensitivity of any surveillance system. There is still a lack of data on the actual performance of the approved tests in field conditions, particularly for sheep. It would be advisable to generate such data.

Some of the parameters used in this assessment are dynamic. Prior to the assessment of any subsequent application, parameters relating to risk factors and test sensitivity should be reviewed and, if necessary, updated.

1 | INTRODUCTION

1.1 | Background and Terms of Reference as provided by the requestor

Since 1 July 2013, according to Annex VIII, Chapter A, Section A point 2 to Regulation (EC) No 999/2001, a Member State (MS) can submit a request to the Commission to be recognised as a MS, or zone of a MS, with a negligible risk of classical scrapie (CS). In this case, the Commission (EC) should evaluate this request based on the criteria laid down in point 2.1, and, if the evaluation is positive, the negligible risk status may be approved based on a comitology regulatory procedure with scrutiny. The criteria laid down in point 2.1 are based on those mentioned in Article 14.8.3 of the Terrestrial Animal Health Code of the World Organisation for Animal Health (WOAH).

Slovenia submitted a request to the Commission to be recognised a Member State with negligible risk of classical scrapie on 26 June 2023. The Commission assessed this application positively as regards the criteria in items (a), (b), (d), (e) and (f) of Chapter A, Section A, point 2.1 of Annex VIII to Regulation (EC) No 999/2001, but so far did not conclude its assessment as regards item (c).

Item (c) of Chapter A, Section A, point 2.1 of Annex VIII to Regulation (EC) No 999/2001 reads as follows:

> *'(c) for a period of at least seven years, a sufficient number of ovine and caprine animals over 18 months of age, representative of slaughtered, culled or found dead on farm, have been tested annually, to provide a 95% level of confidence of detecting CS if it is present in that population at a prevalence rate exceeding 0.1% and no case of CS has been reported during that period.'*

Furthermore, point 2.2 of Chapter A, Section A of Annex VIII to Regulation (EC) No 999/2001 specifies that:

> *'2.2 The Member State is to notify the Commission of any change in the information submitted according to point 2.1. relating to the disease. The negligible risk status approved in accordance with point 2.2. may, in the light of such notification, be withdrawn in accordance with the procedure referred to in Article 24(2).'*

This implies that the number of tests required for at least the last 7 years according to item I of point 2.1, should also be maintained in the future for the classical scrapie negligible risk status to be retained.

In the framework of Article 31 of Regulation (EC) No 178/2002, the Commission requests the technical assistance of EFSA to assess if Slovenia:

- has demonstrated that, for a period of 7 years (2016–2022), a sufficient number of ovine and caprine animals over 18 months of age, in the testing streams "slaughtered for human consumption" and "not slaughtered for human consumption", has been tested annually to provide a 95% level of confidence of detecting classical scrapie if it was present in that population at a prevalence rate exceeding 0.1%.
- and will continue to carry out annually a sufficient number of tests of ovine and caprine animals over 18 months of age, in the testing streams "slaughtered for human consumption" and "not slaughtered for human consumption", to provide a 95 percent level of confidence of detecting classical scrapie, should it be present in that population at a prevalence rate exceeding 0.1 percent.

1.2 | Interpretation of the Terms of Reference (if appropriate)

The EFSA Working Group (WG) agreed to clarify the following points:

- Retrospective analysis of surveillance data is conducted on an annual basis, i.e. estimating the confidence of detecting CS if it is present in that population at a prevalence rate exceeding 0.1% in each year separately. EFSA has not considered any method that accounts for the cumulative evidence provided by the analysis of historic surveillance data.
- The period for which surveillance data should be analysed retrospectively is 2016–2022, as in the ToR. However, due to the gap between the submission of the application to the EC and the submission of the mandate to EFSA, full data for 2023 were available at the time of analysis and will be analysed as well.
- The assessment of whether or not Slovenia will continue to carry out a sufficient number of tests will refer to the future in general and not just specifically to 2024, the first year after the retrospective analysis.
- Even though sheep and goats will be considered as a single population (small ruminants) in the assessment, prevalence data will be stratified by species.
- The assessment will be conducted using raw data provided by Slovenia in the dossier and new data that they may provide upon request. The assessment will also consider other data and information contained in the dossier that may help with the assessment, such as demographic data, organisation and implementation of the surveillance system, selection of animals for testing, etc. The aspects of the dossier which are not relevant for the assessment as required in the ToR will not be considered.

- In the 'Guideline for drafting a dossier for the recognition of a Member State or zones of a Member State with a negligible risk of classical scrapie, Version 6', it is stated that '*for the calculation it is recommended to use a scenario tree modelling, similar to that used by EFSA in its 2015 scientific reports on the evaluation of the application of Sweden/Finland to be recognised as having a negligible risk of classical scrapie, assuming that the sensitivity of the surveillance system is equivalent to the diagnostic sensitivity provided by the past evaluations of screening diagnostic tests by the EFSA and the Joint Research Centre Institute for Reference Materials and Measurement (IRMM) (see Appendix A of the EFSA scientific reports)*'. The EFSA Working Group (WG) producing this assessment will apply the same methodology in accordance with this Guideline (EFSA, 2015b, 2015c).
- The regulatory requirements for active surveillance for scrapie in small ruminants in the EU and the minimum requirements for the recognition of the 'negligible risk of classical scrapie status' are different because they are not based on the same assumptions, hence compliance with the former does not mean automatic compliance with the latter.
- Throughout the document, the expression 'test sensitivity' refers to the diagnostic sensitivity of the screening tests in field conditions (see Section 2.1.3), with the exception of the outcomes of the EU evaluation which did not account for field conditions.

1.3 | Additional information (if appropriate)

While reviewing the dossier submitted by Slovenia, and in order to implement the analytical approach agreed by the EFSA WG producing this assessment (see Section 2.2), it was considered necessary to request additional data or re-submission of the data already provided in a different format, or at a different resolution level. In particular, EFSA requested the Slovenian competent authority to:

- To confirm the total number of sheep and goats > 18 months of age slaughtered for human consumption (SHC) and found dead on farm (NSHC) for the period 2016–2022.
- Although the mandate requires an assessment of the data from 2016 to 2022, to provide full data of tested animals and subpopulations for 2023.
- To provide the breakdown of future animals tested (2024 onwards) by species and surveillance group, to clarify the text in the application, where it is stated that *'the estimated number of NSHC ewes and NSHC goats is based on the data on sheep and goats collected in by the VHS service over the past five years. In future years we will include in the annual Decree on the implementation of systematic monitoring of animal health, animal disease eradication programmes and vaccination of animals the same requirement for testing of dead sheep and goats (2500 sheep and 500 goats) and the requirement that all sheep and goats slaughtered in approved slaughterhouses must be tested for TSEs as we had in the previous years*'.

The Slovenian competent authority submitted the additional data and information, as requested.

2 | DATA AND METHODOLOGIES

2.1 | Data

2.1.1 | Population and surveillance data for Slovenia

Demographic and surveillance data, including the number of sheep and goats tested for scrapie, test results and future plans for surveillance were obtained from:

- the original application, plus information included in further communications between the EC and the MS;
- additional data provided by Slovenia upon request, as described in Section 1.3.

2.1.2 | EU surveillance data

EU surveillance data at MS level have been extracted from the EFSA TSE database and from the EU summary reports published by the European Commission prior to 2016. In the previous evaluation (EFSA, 2023), the historical data available covered a period of 13 years from 2009 until 2021. To be consistent, a period of 13 years was used for the current evaluation, covering the period 2010–2022.

Historical data were extracted in a matrix format including the following fields: country (EU member state), species (sheep/goats) surveillance streams (NSHC/SHC), year (2010–2022), number of animals tested and number of classical scrapie cases.

The 2010 EU report includes the total number of TSE cases and the number of atypical cases, but not subdivided into surveillance streams. The number of classical scrapie cases was estimated by subtracting from the total number of scrapie

cases the number of AS proportional to the number of total cases in the two surveillance streams: SHC and NSHC. Otherwise, there would be atypical scrapie cases incorrectly classified as classical scrapie by surveillance stream.

The surveillance stream 'eradication measures' (EM) was excluded to be consistent with previous evaluations. To maximise the number of cases for the calculation of relative risk, the number of tested animals and number of cases in SHC and NSHC were extracted from both infected and non-infected flocks.

The dataset built for the calculation of the relative risk contains the number of tested sheep and goats in the countries that had had at least one case in either species during the period 2010–2022. If a country had not had cases, surveillance data were not included in the dataset. This approach was different from the one applied in previous calculations of the relative risk where only combinations of country, species, year and tested were included if the number of cases was not zero. In this new approach the dataset is more robust, and the estimation of the relative risk is better informed.

The final dataset contained a total of 5,066,175 small ruminants: 3,584,168 sheep and 1,482,007 goats. The number of cases included in the dataset is 6734: 3609 in sheep and 3125 in goats. In total, 2,824,270 were tested in the NSHC group and 2,241,905 in the SHC group. Tables A.1, A.2 of Appendix A show the distribution of animals tested and cases by country, and species (Table A.1) or surveillance stream (Table A.2).

2.1.3 | Sensitivity of diagnostic screening tests

Data and information on the performance of diagnostic screening tests approved for the monitoring of TSE in small ruminants in the EU under laboratory conditions have been sourced from the reports of the Institute for Reference Materials and Measurements (IRMM) and EFSA Opinions (EFSA, 2005a, 2005b; EFSA BIOHAZ Panel, 2010, 2012; IRMM, 2005a, 2005b, 2005c). These data were produced in the framework of the past EU evaluations of post-mortem diagnostic screening tests for the detection of TSE in small ruminants and are used in the present report as estimates of the analytical sensitivity of the EU tests i.e. under laboratory conditions, and therefore represent a 'best case scenario' when applied under field conditions (see Section 2.2.3.2).

2.2 | Methodologies

Scenario tree modelling using a stochastic approach was the analytical method selected for this assessment, to maintain continuity of approach with previous similar evaluations (EFSA, 2015a, 2015b, 2015c, 2023) and in accordance with the Guideline for drafting a dossier for the recognition of a Member State or zones of a Member State with a negligible risk of classical scrapie Version 6, and Regulation (EC) No 999/2001.

2.2.1 | Risk-based surveillance using scenario tree modelling

For a disease as complex as CS, which is characterised by a long incubation period, the absence of any in vivo diagnostic method and the variable susceptibility of individual animals depending on their genetic profile, it is difficult to demonstrate freedom from disease in the territory or part of the territory of an MS. The concept of 'CS-free MS' has therefore been replaced in Annex VIII to Regulation (EC) No 999/2001 by that of 'MS or zone of a MS with a negligible risk of CS' by Commission Regulation (EU) No 630/2013.

Commission Regulation (EU) No 630/2013 amending Annex VIII of Regulation (EC) No 999/2001 also aligned the criteria for a MS to be recognised as having a 'negligible risk of CS' with those laid down in Article 14.8.3 of the WOAH Terrestrial Animal Health code for 'scrapie-free country or zone'.

Owing to the constraints of the nature of the disease, the application of sampling strategies and the limitations of diagnostic test performance, it is not possible to achieve absolute proof of freedom from disease. Thus, a probabilistic approach is used based on the accumulation of evidence (Cameron, 2012). The implication of such a strategy is that the level of confidence that an animal population is 'free' from disease is proportional to the sample size, the design prevalence and the accuracy of the diagnostic test in terms of sensitivity and specificity (FAO, 2014):

- *the sample size*, i.e. the number of animals sampled; the larger the number of animals submitted to testing, the greater is the likelihood of detecting the disease.
- *the design prevalence (DP)*; i.e. the assumed prevalence of disease if it is present and also the probability of infection for each animal in the population; the lower the DP is, the larger will be the effort needed to detect the disease. In ToR 1 it is 0.1%.
- *the accuracy of the diagnostic test in terms of sensitivity and specificity*. Sensitivity is a key factor in terms of both the sensitivity of the diagnostic screening test and the sensitivity of the surveillance system, i.e. the probability that the surveillance system would detect disease if it were present. Therefore, maximising the sensitivity strengthens the confidence in freedom, reducing the uncertainty when communicating results. On the other hand, specificity is not a problem when trying to substantiate freedom from disease (Martin et al., 2007). Even if potential false positives can compromise the freedom statement, each initially positive animal should be subject to further confirmatory testing. As highlighted

in a previous EFSA Technical Report, each surveillance system should encompass all the necessary follow-up testing to resolve potential false positive results (EFSA BIOHAZ Panel, 2012).

A surveillance system can be thought of as a type of diagnostic screening test on the entire population: the population does have or does not have a disease, and the surveillance is applied in order to make a decision on the disease status. The ability of a surveillance system to correctly identify a diseased population is analogous to the ability of a diagnostic test to identify a diseased animal (FAO, 2014). It is measured quantitatively by the sensitivity of the surveillance system, i.e. the level of confidence of detecting the disease mentioned in ToR 1.

As discussed in Stärk et al. (2006), it had been argued by Martin and Cameron (2003) that the assumption in traditional surveillance that the probability of disease is constant across all individuals in the reference population is not realistic. A single standard value for the design prevalence (DP) would imply that all animals in the population have, on average, the same probability of being infected. This is never true: animals vary in their probability of becoming infected and in their probability of being recognised/detected as sick, depending on the nature of the disease and on their susceptibility to it. To deal efficiently with such a context, the evaluation of surveillance systems can be achieved using scenario trees, similar to decision tree structures.

The scenario tree is a modelling format for analysis of surveillance systems under a null hypothesis of the country being infected at a level equal to or greater than the specified prevalence. A scenario tree is developed to represent all applicable relevant factors influencing the probability that a unit in an infected population will be detected as infected. The conditional probabilities associated with each branch of the tree are then multiplied together to give the overall probability of each branch outcome, and these are added up for all branches with positive outcomes to give the probability of the whole surveillance process having a positive outcome for a randomly chosen population unit, given that infection is present in the country. The infection and detection nodes of their trees represent factors affecting the probability of disease occurrence in subpopulations that may be targeted by surveillance.

Scenario trees allow the evaluation of the contribution of risk-based surveillance that aims to take into account the differences in risk (probability of detection) among animals in the population. In particular, by selecting animals with a higher probability of being infected or a higher probability of being detected if they are infected, the sensitivity of the surveillance can be increased without increasing the total number of animals being tested (FAO, 2014). If surveillance is targeted towards a group of animals that are at higher risk of being infected, a scenario tree allows us to calculate the sensitivity that we achieve for that particular group. For details of the calculation of the sensitivity of the surveillance system, see Section 2.2.2.

To conduct the estimation of the sensitivity of the surveillance system using scenario tree modelling, a tailor-made model was coded using R software (R Core Team, 2021). One hundred thousand iterations were used for each simulation performed, which ensured convergence of the model. The R code of the scenario tree model, a readme file and an Excel file containing the input data for Slovenia can be accessed in the following link https://doi.org/10.5281/zenodo.13453176. The code of the model is the same as that applied to the analysis of the data for the application of the Czech Republic (EFSA, 2023). The R code of the multilevel negative binomial regression model to estimate the relative risk and an Excel file containing the historical surveillance data can also be accessed in the same link.

2.2.2 | Estimation of the overall sensitivity of the surveillance system (SSe) using scenario tree modelling

Scenario tree modelling effectively divides the population into different risk groups based on known risk indicator(s), in this case species and surveillance stream. By applying relative risk of infection in each of these groups, the DP, i.e. the theoretical overall probability that a random unit is infected, is adjusted in order to estimate the group-level probability of infection, i.e. the 'actual' probability that a random unit from a specific group is infected, based on the available data on the relative risk for the risk indicator/s.

To summarise, a scenario tree is a tool to assist in the calculation of the sensitivity of a component of a surveillance system (FAO, 2014). In contrast to the simple analysis of representative surveys, the purpose of a scenario tree is to take into account the fact that not all animals in the population:

- have the same probability of being infected (some are at greater risk than others);
- have the same probability of being detected (the sensitivity of detection is greater in some animals than others).

Once the risk indicators are identified and the associated risk parameters estimated, it is possible to combine the different levels in order to obtain the risk groups. If two risk indicators are identified with two levels (categories) each, the four different risk groups can be obtained. Table 1 below shows the distribution of risk groups in the case of two risk indicators with two categories each.

TABLE 1 Theoretical distribution of risk groups using two risk indicators with two categories each.

	Risk indicator II	
Risk indicator I	**RI_IIa**	**RI_IIb**
RI_Ia	Group:1	Group:2
	$CombRP_1$	$CombRP_2$
	$PopProp_1$	$PopProp_2$
RI_Ib	Group:3	Group:4
	$CombRP_3$	$CombRP_4$
	$PopProp_3$	$PopProp_4$

For each risk group, the weighted risk (WR_i) is calculated as follows:

$$WR_i = \frac{CombRP_i}{\sum_{i=1}^{r}\left(PopProp_i \bullet CombRP_i\right)}, \quad (1)$$

where $CombRP_i$, is the risk parameter for the *i*th specific risk group (combination of the two risk indicators), $PopProp_i$ is the fraction of the total population allocated in the *i*th specific risk group and *r* is the total number of risk groups, i.e. four in the example.

Using WR_i, it is then possible to calculate the effective probability of infection for each risk group$_i$ (EPI_i) as follows:

$$EPI_i = DP \bullet WR_i, \quad (2)$$

where DP is the overall design prevalence and WR_i is the weighted risk for each group.

Once the EPI_i values are estimated, they can be used as a better estimate **at group level** in order to calculate:

- the **sample size required** in each group in order to have a probability of detecting at least one positive animal, should the actual prevalence be above the EPI_i; or
- the **sensitivity of a round of testing** (RSe), i.e. the probability that at least one animal out of the tested animals will return a positive result, should the actual prevalence be above the EPI_i at group level.

The RSe is calculated for a finite population as follows:

$$RSe = 1 - \left(1 - \frac{n \bullet TSe}{N - 0.5 \bullet (N \bullet DP \bullet TSe - 1)}\right)^{N \bullet DP}, \quad (3)$$

where *n* is the sample size, DP is the design prevalence, TSe is the sensitivity of the test and *N* is the total population size. The group sensitivity for group *i* (GSe_i) can be calculated for each group just by substituting DP for EPI_i, with n_i, being the sample size in each risk group and N_i the total population in each risk group:

$$GSe_i = 1 - \left(1 - \frac{n_i \bullet TSe}{N_i - 0.5 \bullet \left(N_i \bullet EPI_i \bullet TSe - 1\right)}\right)^{N_i \bullet EPI_i} \quad (4)$$

It is now possible to estimate the overall sensitivity of the surveillance system (*SSe*) as follows:

$$SSe = 1 - \prod_{i=1}^{r}\left(1 - GSe_i\right), \quad (5)$$

where SSe is the system (overall) sensitivity, gSe_i is the group sensitivity of each risk group and *r* is the number of risk groups included in the survey. SSe represents the 'confidence' of detecting the disease given DP, TSe, *N* and *n*. The SSe level required by the legislation is 95%.

Input parameters for the calculation of the overall sensitivity of the surveillance system (SSe) using scenario tree modelling.

The methodology described above has been applied for the calculation of the annual SSe to detect scrapie at the designed prevalence of 0.1%. Two risk indicators have been selected: surveillance stream with two risk categories (NSHC, SHC) and species with two risk categories (sheep, goats), as displayed in Figure 1.

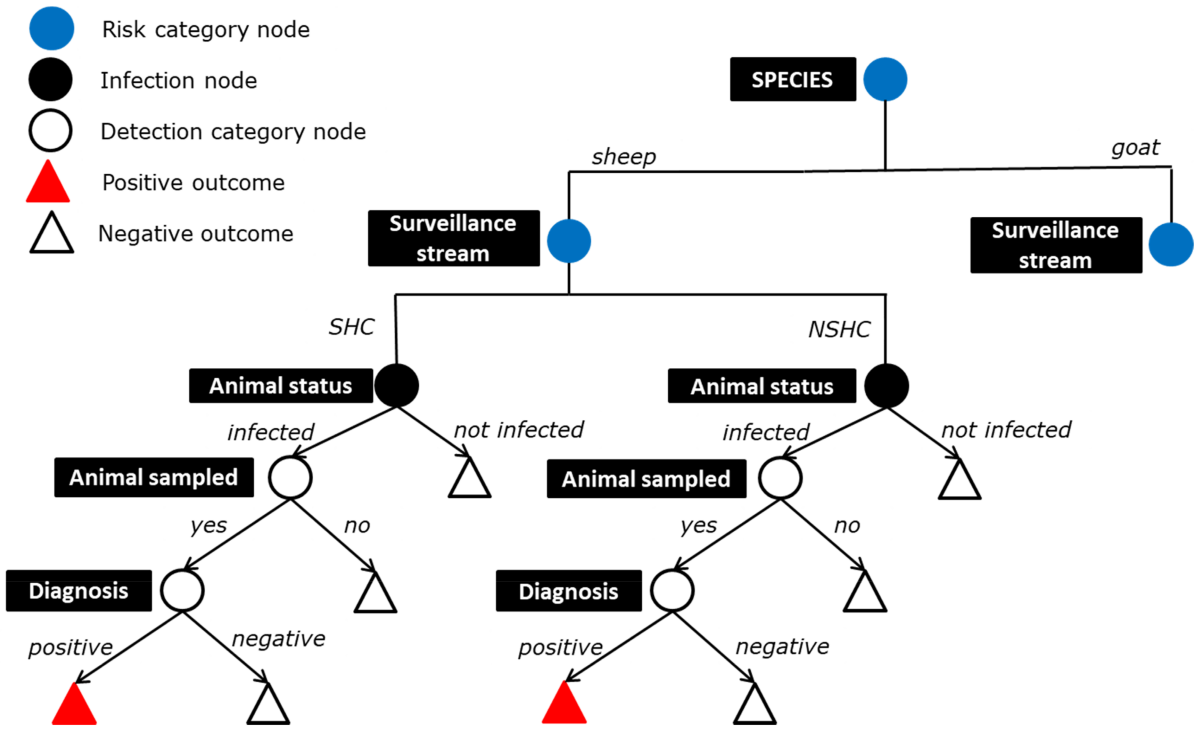

FIGURE 1 Scenario tree flow diagram of the analysis of the active surveillance system for CS. Only the sheep section is shown. The same tree applies to the goat section.

For the calculation of the SSe two different categories of parameters are used: those common to all MS and MS-specific parameters.

2.2.3 | Common parameters of the scenario tree modelling

2.2.3.1 | *Design prevalence (DP)*

Fixed according to the EU legislation: 0.1%.

2.2.3.2 | *Sensitivity of the diagnostic screening tests (rapid tests) (TSe)*

Various prion protein (PrP) detection methods can be applied in the context of statutory surveillance (enzyme-linked immunosorbent assay, Western blot, immunohistochemistry), but active surveillance screening in the EU requires that the method used must be listed in Regulation (EC) No 999/2001.

Initially, evaluation exercises were carried out using brain tissue from clinical cases of bovine spongiform encephalopathy (BSE) in cattle, and tests performing satisfactorily on bovine tissues were provisionally approved for small ruminants and used for surveillance of TSE in sheep and goats (Commission Decision 2000/374/EC;[1] Regulation (EC) No 1053/2003[2]). In 2003, the EC launched a new evaluation of diagnostic and analytical sensitivity, diagnostic specificity and repeatability of *post-mortem* diagnostic screening tests for TSE using natural classical scrapie (CS) samples. Based on the results of these evaluations (EFSA, 2005a, 2005b; IRMM, 2005a, 2005b, 2005c), *post-mortem* diagnostic screening tests were specifically approved for the detection of TSE in small ruminants (Regulation (EC) No 253/2006[3]). Further modifications were made in 2008 and 2009, owing to the withdrawal from the market of some tests, and then in 2010 (Regulation (EC) No 956/2010[4]), with some tests being delisted for performing poorly with regard to atypical scrapie. The approved test list has remained stable since 2010, with the addition of one new test in 2012 as a result of a new EU evaluation procedure that started in 2008.

IRMM and EFSA published reports summarising the results of the 2003 and 2008 evaluations of the *post-mortem* screening tests for the detection of TSE in small ruminants (EFSA, 2005a, 2005b; EFSA BIOHAZ Panel, 2012; IRMM, 2005a, 2005b, 2005c, 2010). When reviewing the results of the laboratory evaluations in relation to the diagnostic sensitivity of the tests recommended for approval and used, at least for some years in MS, the lowest reported value for diagnostic sensitivity was

[1]Commission Decision 2000/374/EC of 27 December 2000 prohibiting the use of certain animal by-products in animal feed. OJ L 6, 11.1.201, p. 16–17.
[2]Regulation (EC) No 1053/2003 of 19 June 2003 amending Regulation (EC) No 999/2001 of the European Parliament and of the Council as regards rapid tests. OJ L 152, 20.6.203, p. 8–9.
[3]Regulation (EC) No 253/2006 of 14 February 2006 amending Regulation (EC) No 999/2001 of the European Parliament and of the Council as regards rapid tests and measures for the eradication of TSEs in ovine and caprine animals. OJ L 44, 15.2.2006, p. 9–12.
[4]Regulation (EC) No 956/2010 of 22 October 2010 amending Annex X to Regulation (EC) No 999/2001 of the European Parliament and of the Council as regards the list of rapid tests. OJ L 279, 23.10.2010, p. 10–12.

99.6% (95% confidence interval (CI) 98.10%–99.99%), based on an evaluation on 246 positive brainstem samples. Summary results of this EU evaluation are available in Appendix B of EFSA (2023).

Additional requirements applied to approved diagnostic screening tests in terms of analytical sensitivity.

All tests were required to fall within an analytical sensitivity of a maximal 2 $\log_{10}$ lower than that of the most sensitive test, based on a $\log_{10}$ dilution series from known positive samples. Despite the potential for apparent differences in analytical sensitivity, the EFSA BIOHAZ Panel (2009) concluded that *'no potential differences in field detection performance can be inferred on the sole basis of the difference in analytical sensitivity reported'*.

In practice, a number of factors other than the analytical sensitivity of a test under laboratory conditions affect the ability of the test to correctly identify sheep and goats affected by CS, and these are discussed below. These factors are difficult to quantify. They contribute to the uncertainty around the value of the parameter for the sensitivity of the test under field conditions and should be taken into account.

While testing laboratories are kept 'under control' by the regulatory requirement to apply tests within recognised quality systems (ISO, 2005) or equivalent (Regulation (EC) No 882/2004[5]), the initial selection of animals and sampling of material falls largely outside of this procedural control.

Regardless of the analytical sensitivity of the test used, anatomical sub-location within a tissue sample is key to good diagnostic sensitivity of the test under field conditions. Current active surveillance screening of the central nervous system looks specifically in the brainstem for evidence of accumulation of the abnormal form (PrP^{Sc}) of the cellular PrP (PrP^{C}). Most of the published data related to PrP^{Sc} dissemination dynamics in sheep naturally affected with CS were obtained in sheep bearing the VRQ/VRQ genotype (for details see EFSA BIOHAZ Panel, 2010). In these animals, lymphoreticular system (LRS) involvement starts in the gut in the first months post exposure, and thereafter spreads to all lymph nodes, reaching a plateau around 6 months post infection. It is not until an age of between 7 and 10 months that PrP^{Sc} becomes detectable in the central nervous system (CNS) (brain and spinal cord), where it accumulates following exponential kinetics. There is a paucity of relevant data related to CS dissemination in sheep of other genotypes. However, the data that do exist indicate that in other genotypes the dissemination kinetics of the PrP^{Sc} is slower, and in some cases, there is also no LRS involvement (EFSA BIOHAZ Panel, 2010, 2014). Any brainstem samples from animals infected for less than a year are therefore likely to test negative. However, this should not affect the overall test sensitivity in VRQ/VRQ animals since the minimum age for testing is 18 months of age, if it is assumed that infection occurs at, or shortly after, birth.

In the case of infected animals over 18 months of age the choice of tissue sampled, genotype, age at testing and the accuracy of sampling will all have a combined effect on the ability of the screening test to detect an infected animal under field conditions. Consistent and accurate sampling of target areas is essential to give confidence in a negative biochemical result. The accuracy of sampling is also critical within the brainstem, since PrP^{Sc} is initially localised to the dorsal nucleus of the vagus nerve, before becoming more widely disseminated as infected animals develop clinical disease (Ryder et al., 2001, 2009; Sisó et al., 2010). Moving away from the target areas at the obex in cattle, for example, has also been shown to result in a drop in detectable PrP^{Sc} by a factor of 3 over 6 mm, potentially compromising detection (Moynagh et al., 1999).

Although the impact of this initially localised PrP^{Sc} deposition on test sensitivity in pre-clinical populations under field conditions has not been systematically assessed in sheep, there are several reports of studies in which whole goat herds have been culled and test performance compared. These all concur that, when PrP^{Sc} accumulation within the brainstem is restricted, sensitivity under field conditions is compromised, with different test sensitivity estimates reported in the literature: 47% (Corbière et al., 2013), 53% (González et al., 2010) and 64% (Ortiz-Pelaez et al., 2014) when compared to the gold standard confirmatory tests.

A further confounding issue when considering test performance in goats is that the formal test evaluations that were undertaken in respect of small ruminant testing were conducted using only sheep scrapie samples. It has been shown subsequently that not all tests perform equally in all genotypes of goats (Konold et al., 2020; Papasavva-Stylianou et al., 2017; Simmons et al., 2020). Not all caprine *PRNP* polymorphisms are synonymous with ovine ones, and some caprine polymorphisms coincide with particular diagnostic antibody binding sites, reducing the sensitivity of individual tests in certain animals. The actual (as opposed to assumed) overall sensitivity of a testing regime would therefore need to consider the genotypes of every screened animal in conjunction with the specific diagnostic screening test being used.

Under field conditions, the sensitivity of a test is likely to be lower than sensitivity estimates obtained under laboratory conditions. Currently, there are no data to quantify at EU level the overall sensitivity of screening tests for the detection of CS in small ruminants above 18 months of age.

Given the above, the following approach is used for the parameterisation of test sensitivity TSe:

- From the results of the past EU evaluations of diagnostic screening tests, the lowest sensitivity obtained with the tests evaluated was selected as the worst case and applied to each MS. A beta distribution was built using 245 successes out of 246 trials (Figure 2), which corresponds to a TSe of 99.6% (95% CI: 98.80–100) (see Appendix B).
- Alternative scenarios using different hypothetical sensitivity values of the diagnostic screening tests, i.e. 90%, 80%, 70%, 60% and 50%, were also applied to reflect the uncertainty of the actual sensitivity of the tests under field conditions.

[5]Regulation (EC) No 882/2004 of the European Parliament and of the Council of 29 April 2004 on official controls performed to ensure the verification of compliance with feed and food law, animal health and animal welfare rules. OJ L 165, 30.4.2004, p. 1–141.

2.2.3.3 | *Relative risk by species (alpha)*

This risk indicator contains two risk categories, namely, sheep/goats.

A preliminary estimation of the specific prevalence of CS by country, year, species and stream has been obtained using the data described in Section 2.1.2.

The excess probability (relative risk) of detecting scrapie in sheep compared with goats was based on the calculation of the prevalence ratio (PR), i.e. the ratio of the prevalence observed in the first group (sheep) to that in the second (goats) declared as baseline. Annual data for each country were used as the unit of analysis. A further restriction was applied: combinations of country, year, species and surveillance stream were excluded if the total number of tested animals was less than 385. This restriction results from the following consideration: assuming a confidence level of 95%, i.e. with a *z*-score of 1.96, in situations where the variance is highest, the sampling error E would exceed 5% when the sample size n is less than 385.[6]

The relative risk sheep/goats was estimated by applying a multilevel negative binomial regression model. The outcome of interest was the number of cases of CS reported by each country in the frame of active surveillance, whereas the total annual number of tested animals was used as an offset of the model. The following independent variables have been included in the model: country, species, year and surveillance stream. The exponentiated coefficient of the final model represents the prevalence rate ratio (PR) of detecting CS in the sheep compared with the baseline category (goats), taking into account the effect of country, surveillance stream and year for the entire EU, for the period 2010–2022, under the testing conditions applied by each country in compliance with the EU legislation.

The results of the final model included 577 observations and showed a risk 1.19 times higher (95% CI 0.8–1.77. $p = 0.385$) in sheep than in goats. The coefficient and associated standard error of the variable 'species' in the final multilevel negative binomial regression model were respectively 0.175 and 0.202. Although currently not significant,[7] it has been left in the scenario tree model for consistency with past evaluations, to ensure reproducibility in future evaluations and to account for the biological plausibility that the relative risk is still greater than 1. A normal distribution matching the results obtained with the multilevel negative binomial regression model was used.

$$\alpha_{\text{Sheep/Goat}} = \exp.(\text{Normal}\ (0.175, 0.202)).$$

2.2.3.4 | *Relative risk by surveillance stream (beta)*

This risk indicator contains two risk categories, namely, not slaughtered for human consumption (NSHC)/slaughtered for human consumption (SHC).

A similar approach was used to calculate the excess probability (relative risk) of detecting scrapie in the NSHC stream compared with the SHC stream. The results of the final model included 577 observations and showed a risk 1.61 times higher (expressed as prevalence rate ratio) (95% CI 1.19–2.18, $p = 0.002$) in the NSHC stream than in the SHC stream. The coefficient and associated standard error of the variable 'surveillance stream' in the final multilevel negative binomial regression model were respectively 0.477 and 0.154. A normal distribution matching the results obtained with the multilevel negative binomial regression model was used, i.e.

$$\beta_{\text{NSHC/SHC}} = \exp.(\text{Normal}(0.477, 0.154)).$$

Summary of the distribution of risk groups using two indicators for the estimation of SSe for CS

Table 2 presents the parameterisation of Table 1 for the estimation of SSe for CS each year. The parameter estimates described above are inserted in Equations (1), (2), (4) and (5) of the scenario tree model. Alpha ($\boldsymbol{\alpha}$) refers to the first risk factor (species) and beta ($\boldsymbol{\beta}$) to the second (surveillance stream).

TABLE 2 Actual distribution of risk groups using two risk indicators with two categories each and associated relative risks for classical scrapie according to the model.

	Risk indicator II	
Risk indicator I	**NSHC**	**SHC**
Sheep	$\text{CombRP}_1 = \boldsymbol{\alpha} \times \boldsymbol{\beta}$ $\text{PopProp}_1 = N_1/N$	$\text{CombRP}_2 = \boldsymbol{\alpha}$ $\text{PopProp}_2 = N_2/N$
Goats	$\text{CombRP}_3 = \boldsymbol{\beta}$ $\text{PopProp}_3 = N_3/N$	$\text{CombRP}_4 = \mathbf{1}$ $\text{PopProp}_4 = N_4/N$

[6] $E = Z \times (p(1-p)/n)^{1/2}$.

[7] Historically, the sheep species has been associated with a higher risk of disease in comparison to goats. This was particularly evident in datasets focusing on the early years of the 2000s. The current PR, based mainly on the second decade, is no longer statistically higher than 1.

2.2.4 | Country-specific parameters for each MS

The model described in Section 2.2.2 is parameterised for each year under consideration, i.e. 2016–2023 and also for future years.

Sheep and goat populations within each surveillance stream (N_i)

The population of sheep and goats will vary between years. The differences in the sheep and goat populations within each surveillance stream are taken into account in the model described in Section 2.2.2. Using the notation described earlier,

N_1 = Total NSHC sheep per year
N_2 = Total SHC sheep per year
N_3 = Total NSHC goats per year
N_4 = Total SHC goats per year
$N = \sum_{i=1}^{r} N_i$ Total population of sheep and goats per year.

The values for N_i used in the analysis are provided in Table 3.

Number of sheep and goats tested within each surveillance stream (n_i)

Finally, the number of sheep and goats tested within each surveillance stream (NSHC, SHC) are defined as:

n_1 = number of NSHC sheep tested per year
n_2 = number of SHC sheep tested per year
n_3 = number of NSHC goats tested per year
n_4 = number of SHC goats tested per year

The values for n_i used in the analysis are provided in Table 3.

In Slovenia, until 2023, sheep and goats over 12 months of age were reported to the Central Register of Small Ruminants once a year as part of the reporting of stocks to the Livestock Register. The calculation with available data regarding sheep and goats over 12 months of age from 2023 shows that the number of sheep and goats over 18 months is about 9% lower than the number of sheep and goats over 12 months (Ambrožič, 2024). The reduction factor was applied to calculate N1 and N3. This approach was proposed by Slovenia and clarified upon request. The correction of 9% of the total number of sheep and goats in the subpopulations N1 and N3 has been accepted and the estimates of the number of animals over 18 months of age have been included in the analysis accordingly.

Regarding the subpopulation N2 and N4, the data were not available in the dossier. Upon request, the Slovenian competent authority reported the number of sheep and goats slaughtered for human consumption coincides with the number tested because all of the animals slaughtered over 18 months of age are tested. The number of animals slaughtered in slaughterhouses is low in Slovenia due to the low prices of meat from older sheep, and the fact that older sheep in poor condition are often euthanised and handed over to the cadaver collection system, which is free is Slovenia. Moreover, there is no tradition in Slovenia of slaughtering old sheep for human consumption and famers are not obliged to report home slaughter of older sheep and goats (Ambrožič, 2024).

The Slovenian competent authority (see Section 2.1.1) was requested to provide information on how the 3000 tests (2500 sheep and 500 goats) stated in the application will be split in future years between the two surveillance streams. They replied that the plan is to test 2500 and 180 sheep in the NSHC and SHC, respectively, and 510 and 80 goats in the NSHC and SHC, respectively (included in Table 3), exceeding the original proposed sample size of 3000 (Ambrožič, 2024).

TABLE 3 Summary of test and population data by surveillance stream (2016–2023) and expected number of sheep and goats to be tested annually in the future by Slovenia.

Year	Total NSHC sheep (N_1)	Total NSHC sheep tested (n_1)	Total SHC sheep (N_2)	Total SHC sheep tested (n_2)	Total NSHC goats (N_3)	Total NSHC goats tested (n_3)	Total SHC goats (N_4)	Total SHC goats tested (n_4)
2016	3011	2192	202	202	2060	656	48	48
2017	6192	2078	197	197	2358	434	74	74
2018	6300	2308	214	214	2506	512	85	85
2019	6818	2500	204	204	2769	476	45	45
2020	6613	2344	171	171	2707	509	47	47

TABLE 3 (Continued)

Year	Total NSHC sheep (N_1)	Total NSHC sheep tested (n_1)	Total SHC sheep (N_2)	Total SHC sheep tested (n_2)	Total NSHC goats (N_3)	Total NSHC goats tested (n_3)	Total SHC goats (N_4)	Total SHC goats tested (n_4)
2021	6076	2548	181	181	2445	528	55	55
2022	7003	2244	198	198	2385	557	102	102
2023[a]	7127	2336	196	196	3058	851	93	93
Future[b]	7000	2500	180	180	2700	510	80	80

[a]2023 is not included in the 7 years of data submitted in support of this application. It is also not included in 'the future' because testing has already occurred, and full data are available.

[b]Specific population size data is not available for future years. The numbers used are the ones provide by the Slovenian competent authority for 2023 (Ambrožič, 2024).

2.2.5 | Interpretation of the results of the model

For every iteration, the model produces for each year and test sensitivity one overall surveillance sensitivity (SSe) value. Out of 100,000 iterations the algorithm builds a distribution. The 5th percentile of the distribution is presented in the results (Table 4) as the value at which there is a 95% confidence of having a SSe equal to or above that value.

As an example, for the combination of 90% diagnostic test sensitivity and 2016, the value 0.985 presented in Table 4 means that in 95% of the iterations, the output SSe (overall sensitivity) is equal to or above 0.985 (Figure 2).

As agreed in previous evaluations and for consistency purposes, given DP (0.1%), *N* and *n*, for each combination of year and test sensitivity, the 95% confidence level of detecting CS was considered achieved when the SSe was 95% or greater at the 5th percentile of the output distribution of the model.

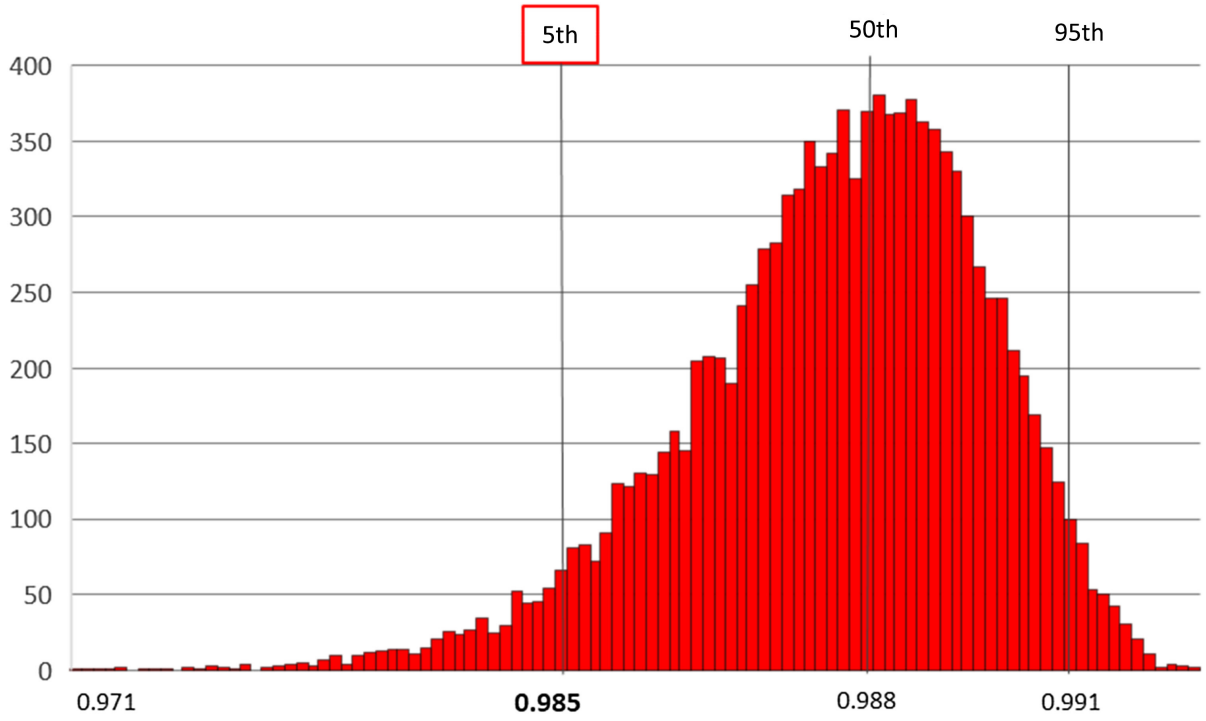

FIGURE 2 Example of a frequency distribution of one output of the scenario tree model.

3 | RESULTS OF THE ASSESSMENT

The summary of the estimation of the overall sensitivity of the surveillance system (i.e. the level of confidence of disease detection mentioned in the ToR) in Slovenia for the different scenarios using historical and future surveillance data is shown in Table 4.

Estimated values of the surveillance system (SSe) of Slovenia are expressed as the 5th percentile of the output distribution of the scenario tree model of 100,000 iterations, obtained for each combination of year (2016–2023 and future surveillance) and values of diagnostic sensitivity. The model also accounts for RR parameters using surveillance data for the period 2010–2022.

TABLE 4 Results of the estimation of the sensitivity of the surveillance system (SSe) in Slovenia, for the period 2015–2022 and proposed future surveillance, for different values of diagnostic sensitivity.

Year/diagnostic sensitivity	EU evaluation	90%	80%	70%	60%	50%
2016	0.994	0.985	0.967	0.940[c]	0.897[c]	0.835[c]
2017	0.973	0.950	0.922[c]	0.885[c]	0.836[c]	0.770[c]
2018	0.984	0.967	0.945[c]	0.914[c]	0.871[c]	0.810[c]
2019	0.984	0.970	0.949[c]	0.920[c]	0.878[c]	0.820[c]
2020	0.980	0.963	0.941[c]	0.909[c]	0.865[c]	0.805[c]
2021	0.988	0.975	0.957	0.930[c]	0.890[c]	0.833[c]
2022	0.981	0.965	0.942[c]	0.911[c]	0.868[c]	0.807[c]
2023[a]	0.988	0.977	0.960	0.935[c]	0.898[c]	0.844[c]
Future[b]	0.985	0.971	0.951	0.923[c]	0.882[c]	0.825[c]

[a]2023 is not included in the 7 years of data submitted in support of this application. It is also not included in 'the future' because testing has already occurred and full data are available.

[b]Specific population size data is not available for future years. The numbers used are the ones provide by the Slovenian competent authority (Ambrožič, 2024).

[c]Values lower than 0.95.

4 | CONCLUSIONS

4.1 | General considerations

- When assessing the overall sensitivity of surveillance systems (the level of confidence of disease detection mentioned in the ToR), it is acknowledged that different approaches to data analysis may produce different results. The application of representative versus risk-based approaches, annual versus cumulative analysis of historic surveillance data or deterministic versus stochastic, requires the use of different input parameters and assumptions specific for each.
- To ensure transparency, consistency and continuity with previous assessments of similar applications, a methodology based on scenario tree modelling has been applied and input data extracted using similar criteria to the past. The parameterisation of variables of the models has been explained and justified accordingly.
- The uncertainties about key parameters for the assessment (the relative risks of sheep versus goats and of NSHC vs. SHC) have been addressed by applying probability distributions, used in the context of a stochastic approach, in order to estimate the overall sensitivity of the surveillance system.
- The test sensitivity established by past EU evaluations is not necessarily representative of the sensitivity achieved under field conditions and may result in an overestimation of the overall surveillance sensitivity. The uncertainty about test sensitivity has been addressed via scenario analysis by exploring an extended range of values from the sensitivity provided by the EU evaluations (99.6%) down to 50%, consistent with published data obtained under field conditions in infected goat populations.
- The calculations of the sensitivity of the surveillance system have been made based on the assumption that the animals tested are representative of the populations from which they were drawn. The assessment of whether this assumption is tenable is beyond the scope of this mandate.
- In the analysis of future surveillance, it has been assumed that the number of small ruminants tested will be as declared by Slovenia in the dossier or in further communications. If the actual number of tests was to be different, the results of the analysis with regard to future surveillance would not be valid and should be re-calculated.

4.2 | Answer to the ToR

The results of the estimation of the overall sensitivity of the surveillance system (i.e. the level of confidence of disease detection mentioned in the ToR) using scenario tree modelling with parameters as described in Sections 2.2.3 and 2.2.4, with data as in Table 3 and applying the criterion described in Section 2.2.5, show that:

- During the period 2016–2023, Slovenia has tested annually a sufficient number of ovine and caprine animals over 18 months of age, sourced from the NSHC and SHC populations, to ensure a 95% level of confidence of detecting CS if it is present in that population at a prevalence rate exceeding 0.1%, assuming a test sensitivity of 90% or above. The same holds for the years 2016, 2021 and 2023, assuming a test sensitivity of at least 80%.
- Based on the proposed number of samples to be tested in 2024 and in future years, Slovenia would test annually a sufficient number of ovine and caprine animals over 18 months of age, sourced from the NSHC and SHC, to provide a 95% level of confidence of detecting CS if it is present in that population at a prevalence rate exceeding 0.1%, assuming a test sensitivity of at least 80%.

5 | RECOMMENDATIONS

- The sensitivity of the screening tests in field conditions is a key parameter when estimating the overall sensitivity of the surveillance system. There is still a lack of data on the actual performance of the approved tests in field conditions, particularly for sheep. It would be advisable to generate such data.
- Some of the parameters used in this assessment are dynamic. Prior to the assessment of any subsequent application, parameters relating to risk factors and test sensitivity should be reviewed and, if necessary, updated.

6 | DOCUMENTATION PROVIDED TO EFSA

1. Application for the recognition of Slovenia as a Member State with a negligible risk of classical scrapie. Ref. Ares(2022)8655055–13/12/2022.
2. Ambrožič, I. RE: Additional data request Slovenia EFSA mandate EFSA-Q-2024-00183. Email to Angel Ortiz-Pelaez. 3 May 2024. 14:06.
3. Ambrožič, I. RE: Additional data request Slovenia EFSA mandate EFSA-Q-2024-00183. Email to Angel Ortiz-Pelaez. 3 May 2024. 16:11.

ABBREVIATIONS

AS	atypical scrapie
BIOHAZ	Biological Hazards
BSE	bovine spongiform encephalopathy
CI	confidence interval
CNS	central nervous system
CS	classical scrapie
DP	design prevalence
EM	eradication measures
EPI	effective probability of infection FAO if this glossary is staying?
GSe	group sensitivity
IRMM	Institute for Reference Materials and Measurements
LRS	lymphoreticular system
MS	Member State
NSHC	not slaughtered for human consumption
PR	prevalence rate ratio
PrP	prion protein
PrP^{C}	cellular prion protein
PrP^{Sc}	abnormal isoform of the cellular prion protein
RiBESS	risk-based estimate of system sensitivity tool
RSe	sensitivity of round of testing
SHC	slaughtered for human consumption
SSe	overall sensitivity of the surveillance system
TSe	test sensitivity
ToR	Terms of Reference
TSE	transmissible spongiform encephalopathy
WG	working Group
WOAH	World Organisation for Animal Health
WR	weighted risk

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CONFLICT OF INTEREST

If you wish to access the declaration of interests of any expert contributing to an EFSA scientific assessment, please contact interestmanagement@efsa.europa.eu.

REQUESTOR

European Commission

QUESTION NUMBER

EFSA-Q-2024-00183

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DECLARATIONS OF INTEREST

The declarations of interest of all scientific experts active in EFSA's work are available at https://ess.efsa.europa.eu/doi/doiweb/doisearch.

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APPENDIX A

Historical scrapie surveillance data 2010–2022

Tables A.1 and A.2 show the distribution of animals tested and cases by country and species (Table A.1) and surveillance stream (Table A.2).

TABLE A.1 Summary of the surveillance data by species for the period 2010–2022 included in the calculation of the relative risks.

	Goats		Sheep		Total	
Country/species	Total tested	Number classical scrapie cases	Total tested	Number classical scrapie cases	Total tested	Number classical scrapie cases
BG	22,576	31	222,411	28	244,987	59
CY	73,181	2675	54,868	85	128,049	2760
DE			263,999	11	263,999	11
EL	47,937	169	127,409	1506	175,346	1675
ES	233,161	57	293,643	234	526,804	291
FI			18,807	3	18,807	3
FR	543,945	8	480,419	9	10,243,64	17
HU	2987	1	231,849	2	234,836	3
IE			277,965	60	277,965	60
IT	293,201	86	309,559	652	602,760	738
NL			96,021	4	96,021	4
PT	51,848	2	330,106	15	381,954	17
RO	201,488	15	428,732	845	630,220	860
SE			56,891	3	56,891	3
SI			24,970	1	24,970	1
SK			127,214	45	127,214	45
UK	11,683	81	239,305	106	250,988	187
Grand Total	1,482,007	3125	3,584,168	3609	5,066,175	6734

TABLE A.2 Summary of the surveillance data by surveillance stream for the period 2010–2022 included in the calculation of the relative risks.

	NHSC		SHC		Total	
Country/surveillance stream	Total tested	Number classical scrapie cases	Total tested	Number classical scrapie cases	Total tested	Number classical scrapie cases
BG	29,510	11	215,477	48	244,987	59
CY	74,365	1692	53,684	1068	128,049	2760
DE	145,960	9	118,039	2	263,999	11
EL	88,879	1361	86,467	314	175,346	1675
ES	292,737	229	234,067	62	526,804	291
FI	18,688	3	119	0	18,807	3
FR	822,861	13	201,503	4	1024,364	17
HU	125,432	0	109,404	3	234,836	3
IE	140,065	56	137,900	4	277,965	60
IT	234,559	407	368,201	331	602,760	738
NL	52,273	1	43,748	3	96,021	4
PT	213,318	11	168,636	6	381,954	17
RO	208,989	293	421,231	567	630,220	860
SE	56,451	3	440	0	56,891	3
SI	23,590	1	1380	0	24,970	1
SK	117,472	23	9742	22	127,214	45
UK	179,121	162	71,867	25	250,988	187
Grand Total	2,824,270	4275	2,241,905	2459	5,066,175	6734