Annex to:
EFSA (European Food Safety Authority), Arnold M, Ru G, Simmons M, Vidal-Diez A, Ortiz-Pelaez A and Stella P, 2021. Scientific report on the analysis of the 2-year compulsory intensified monitoring of atypical scrapie. EFSA Journal 2021;19(7):6686, https://doi.org/10.2903/j.efsa.2021.6686
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Annex A – Protocol

Terms of Reference (ToR)
In the framework of Article 31 of Regulation (EC) No 178/2002, the Commission requests the technical assistance of EFSA to answer the following questions:

1. Do the scientific data on the 2-year intensified monitoring collected by the EC provide any evidence on the contagiousness of atypical scrapie?
2. Do the scientific data on the 2-year intensified monitoring collected by the EC provide any other new knowledge on the epidemiology of atypical scrapie?

Clarification of ToRs
ToRs were clarified during the mandate negotiation stage. No additional clarification of the ToR received with the mandate was needed.
Steps 1 and 2.1: Formulation of the problem and planning of the methods

| ToRs                                                                 | Step 1.1 Assessment questions (reflecting clarification of ToRs) | Step 1.2 Sub-questions (if needed) | Step 1.3 Approach to be followed | Step 2.1 Evidence needs | Step 2.1 Description of method to be used |
|----------------------------------------------------------------------|------------------------------------------------------------------|------------------------------------|----------------------------------|-------------------------|------------------------------------------|
| ToR1 / Do the scientific data on the 2-year intensified monitoring collected by the EC provide any evidence on the contagiousness of atypical scrapie? | AQ 1 / Is the prevalence of atypical scrapie (AS) in the entirety of sheep/goat flocks/herds under intensified monitoring\(^1\) statistically higher than that in the general population of the same EU Member States in the period 2013-2019? | NA | Quantitative | Data from already-available databases: • TSE active surveillance data in the EFSA data warehouse 2013-2019 (general data) • Primary data collated by the EC for this mandate: 2-year intensified monitoring data 2013-2019 (mandate data) | 1. **Descriptive analysis:** Description of general and mandate data in the body of the text and via tables and figures with number of animals tested and cases of CS and AS by species, year and surveillance stream.  
2. **Estimation of prevalence rates:** proportions of cases per number of tests were estimated with 95% confidence intervals (95% CI) using exact binomial method and expressed per ten thousand tests for AS per species, country, surveillance and stream. In general data: prevalence rates of AS in active surveillance (separately in SHC and NSHC) in non-infected flock/herds. In mandate data: prevalence rates of AS secondary cases in infected flocks/herds (separately in SHC and NSHC). Prevalence rates estimated accounting for the cluster sampling design considering as primary sampling unit the country.  
3. **Comparison prevalence rates**  
   **Univariate analysis:** difference in EU-wide and country-specific prevalence rates will be considered statistically significant if there is not overlap of the 95% confidence intervals in the corresponding combination by species and stream.  
   **Multivariable analysis:** A generalised linear regression mixed model fitted with the two datasets (general and mandate) accounting for a potential random effect associated to country and the potential confounding of surveillance stream. In alternative to the mixed model, to avoid convergence problems, a model using standard errors accounting for intragroup (i.e. country) correlation would be also used.  

\(^1\) Commission Regulation (EC) 999/2001 refers to the enhanced surveillance as intensified monitoring, which is the term used throughout this report.
### ToRs

| Step 1.1 | Step 1.2 | Step 1.3 | Step 2.1 |
|----------|----------|----------|----------|
| Assessment questions (reflecting clarification of ToRS) | Sub-questions (if needed) | Approach to be followed | Evidence needs |

#### Step 2.1

**Description of method to be used**

The outputs of the model are prevalence ratios (PR) by comparing the prevalence in infected flocks of mandate data to that in non-infected flocks/herds in general data. Significance of the estimate would be defined by the PR with 95% CI and p values.

#### 4. Design prevalence:

Estimation of the design prevalence of disease at the animal level against which to measure surveillance sensitivity. The given sample size is the number of sheep/goats tested in mandate data, a 95% desired population-level sensitivity, assuming perfect test sensitivity and specificity, and representative sampling. Only flocks with >10 sheep/goats in the flock/ herd and >=3 sheep/goats tested during the 2-year period will be included in the analysis.

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### AQ 2

Based on a simulation model of the dynamics of AS in a flock, which one of two scenarios (contagious vs. non-contagious) better fits the observed intensified monitoring data?

**NA**

#### Quantitative

- Primary data collated by the EC for this mandate: 2-year intensified monitoring data 2013-2019 (mandate data)
- Data to inform parameters of the simulation model: Age distribution of the sheep population at EU level, mean age of detection of atypical scrapie, incubation period of atypical scrapie, sensitivity of diagnostic test vs time before end of incubation period, age dependent susceptibility

#### Simulation models for within flock transmission of atypical scrapie

Model assumptions: each detected flock has 1 infected sheep – assuming that this is detected at the mean age of detection of atypical scrapie in sheep; only sheep <1 year old are susceptible to infection.

Two are implemented in the within-flock model:

1. **Atypical scrapie is contagious.** Each year the number of new infections is simulated from a binomial distribution, based on n=number of sheep<1 year old, and p=1-(1-β)i, where β is the transmission rate (probability that each infected sheep infected another sheep in a year), and i is the number of infected sheep. Infected sheep are allocated a year of death, and an age of clinical onset (which will determine the likelihood that they test positive). Infected sheep are removed from the flock prior to the intensive surveillance if they die or each clinical onset before the start of the intensive surveillance. For each year of the intensive...
Atypical scrapie intensified monitoring

| ToRs | Step 1.1 | Step 1.2 | Step 1.3 | Step 2.1 |
|------|----------|----------|----------|----------|
|      | Assessment questions (reflecting clarification of ToR5) | Sub-questions (if needed) | Approach to be followed | Evidence needs |

ToR 2
Do the scientific data on the 2-year intensified monitoring collected by the EC provide any other new knowledge on the epidemiology of atypical scrapie?

AQ 3
Can any of the identified gaps in the knowledge of the epidemiology of AS be filled by the analysis of the mandate data?

Qualitative
- Expert knowledge in the WG
- Literature review of the epidemiology of AS
- Knowledge gained from the analysis of the mandate data as in the answer to ToR1

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surveillance, the prevalence of infected sheep exiting the flock is calculated, along with the probability of detection of each of those sheep (based on time before clinical onset).

2. **Atypical scrapie is non-contagious.** The main difference is that each sheep in the 0-1 age group has a fixed probability of becoming infected i.e. it does not depend on how many infected sheep are in the flock.

The model is fitted to the intensive surveillance data through an approximate Bayesian computation (ABC) framework (Toni et al., 2011). Both models are fitted in parallel, with the model simultaneously estimating (i) the best fitting transmission rate (ii) the best fitting occurrence rate and (iii) which model is the more likely. The ABC method works by running repeated simulations, measuring the agreement between the simulation output and the observed data (by a user-specified metric), and rejecting parameter values for which the error is above a specified threshold. The process is repeated with the threshold gradually reducing, resulting in a set of parameter values that produce the best fit to the observed data. The model will be developed in R with the R package EasyABC.

- Describe what we know: refer to section describing the state of the art on epidemiology and surveillance.
- Describe the knowledge gaps identified by reviewing section on epidemiology and surveillance.

Explain why/why not the analysis of mandate data answer/fill the questions/gaps identified in the previous point, which are not answered yet by AQ1 and AQ2.
### Step 2.2: Integration of evidence across sub-questions and remaining overall uncertainty

| ToRs as clarified | Step 2.2 Integration of evidence between sub-questions | Addressing overall uncertainty |
|-------------------|------------------------------------------------------|--------------------------------|
| **ToR 1**         | • No sub-questions identified, but different AQs.    | • The approach towards uncertainty in the BIOHAZ Panel is the inclusion in the opinions/reports of a table containing three aspects: sources of uncertainty, justification for the uncertainty and impact on the conclusions indicating, whenever possible, the direction of the impact (under-over estimation). |
|                   | • Detailed description of the methods, including assumptions and data sources to parameterize the statistical and simulation models. | • The same approach will be followed. Instead of having a unique table, it was agreed to split it into several at the end of the respective sections: one for each of the analytical strategies and another one for the uncertainty not accounted for in the statistical or the simulation models. |
|                   | • Report the results of the analytical approaches in numeric and in the body of the text with interpretation of the results. | • Finally wording of the impact of the uncertainty on the final conclusions will be included in the answer to the ToR in words supported by numeric expression as proposed by the uncertainty guidance checklist for the BIOHAZ Panel. |
|                   | • The results of all analytical approaches will be integrated in a narrative to support the answer to the ToR1 | |
| **ToR 2**         | • Narrative description in a stepwise format of the findings with regards to the three points: current available knowledge, knowledge gaps identified and the capacity of the mandate data to fill those gaps. | • The knowledge gaps identified are intrinsic uncertainties about the answer to ToR2. If the conclusion is that the mandate data cannot fill them, they will remain as part of the answer without further elaboration in the overall uncertainty analysis. If any of the knowledge gaps can be (partially) filled, the remaining uncertainty will be described in the related conclusions. |
|                   | | • There is no impact of the identified uncertainties on the conclusions or answer to ToR2. |