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Scientific report on the analysis of the 2-year compulsory intensified monitoring of atypical scrapie

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Abstract

The European Commission asked EFSA whether the scientific data on the 2-year intensified monitoring in atypical scrapie (AS) outbreaks (2013–2020) provide any evidence on the contagiousness of AS, and whether they added any new knowledge on the epidemiology of AS. An ad hoc data set from intensified monitoring in 22 countries with index case/s of AS in sheep and/or goats (742 flocks from 20 countries, 76 herds from 11 countries) was analysed. No secondary cases were confirmed in goat herds, while 35 secondary cases were confirmed in 28 sheep flocks from eight countries. The results of the calculated design prevalence and of a model simulation indicated that the intensified monitoring had limited ability to detect AS, with no difference between countries with or without secondary cases. A regression model showed an increased, but not statistically significant, prevalence (adjusted by surveillance stream) of secondary cases in infected flocks compared with that of index cases in the non-infected flocks (general population). A simulation model of within-flock transmission, comparing a contagious (i.e. transmissible between animals under natural conditions) with a non-contagious scenario, produced a better fit of the observed data with the non-contagious scenario, in which each sheep in a flock had the same probability of developing AS in the first year of life. Based on the analyses performed, and considering uncertainties and data limitations, it was concluded that there is no new evidence that AS can be transmitted between animals under natural conditions, and it is considered more likely (subjective probability range 50–66%) that AS is a non-contagious, rather than a contagious disease. The analysis of the data of the EU intensified monitoring in atypical scrapie infected flocks/herds confirmed some of the known epidemiological features of AS but identified that major knowledge gaps still remain.

Keywords: atypical, goats, intensified monitoring, scrapie, sheep, surveillance

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Summary

Since 1 July 2013, according to point 2.2.3 of Chapter B of Annex VII to Regulation (EC) No 999/2001, holdings with a confirmed case of atypical scrapie are to be subject to an intensified transmissible spongiform encephalopathies (TSE) monitoring protocol for a period of 2 years, during which all ovine and caprine animals that are over the age of 18 months and slaughtered for human consumption and all ovine and caprine animals over the age of 18 months that have died or been killed on the holding must be tested for the presence of TSE.

Under the framework of Article 31 of Regulation (EC) No 178/2002, the European Commission requested EFSA to answer the following questions (Terms of Reference, ToR): 1) Do the scientific data on the 2-year intensified monitoring collected by the European Commission provide any evidence on the contagiousness of atypical scrapie? 2) Do the scientific data on the 2-year intensified monitoring collected by the European Commission provide any other new knowledge on the epidemiology of atypical scrapie?

The data, from 2013 to 2020, pertaining to the implementation of intensified TSE monitoring protocols by the Member States and a few third countries, were collected by DG SANTE and shared with the Biological Hazards and Contaminants unit in EFSA. These mandate data were not intended to be a full data set allowing comprehensive epidemiological analysis. They were based on a retrospective investigation and restricted to those infected flocks/herds identified through surveillance.

The ToR were translated into the following assessment questions (AQs): AQ1: Is the prevalence of atypical scrapie (AS) in the entirety of sheep/goat flocks/herds under intensified monitoring statistically higher than that in the general population of the same EU Member States in the period 2013–2019? AQ2: 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? AQ3: Can any of the identified gaps in the knowledge of the epidemiology of AS be filled by the analysis of the mandate data?

The first two assessment questions were addressed quantitatively, analysing the data provided with the mandate (mandate data) and data from TSE surveillance (general data) through a descriptive and comparative analysis (AQ1) and through a modelling approach (AQ2). Results from these analyses were then used to answer ToR1. The third assessment question, answering ToR2, was addressed qualitatively, reviewing evidence from the literature based on the knowledge of the experts involved in the Working Group drafting this scientific report. Gaps in relation to current knowledge on the epidemiology of AS were identified as the first step. When a conclusion arising from the analysis carried out within ToR1 led to the clarification of any aspect of the identified knowledge gaps, this was discussed in a narrative way in the report. Knowledge gaps that the ToR1 analysis was unable to address were listed in the report.

The intensified monitoring data from 22 countries resulted, after aggregations and data cleaning, in a final data set containing 742 flock IDs from 20 countries, in which the index case of AS (first case of AS detected in a flock previously registered as non-infected or not under intensified monitoring) was a sheep. A total of 41,860 sheep were tested (median: 616; range: 1–16,460). As a result of this testing, 35 secondary cases of AS were reported in 28 infected flocks (3.8%) from eight countries.

There were 76 herd IDs from 11 countries in which the index case of AS was a goat. The total number of goats tested as part of the 2-year intensified monitoring in infected herds by these 11 countries was 4,865 (median: 12 range: 0–3,682). No secondary cases of AS were detected in these infected herds.

A negative binomial regression model was built using data from the 20 countries that reported AS and limited to the two surveillance streams 'slaughtered for human consumption' (SHC) and 'not slaughtered for human consumption' (NSHC). The regression model showed a non-statistically significant stream-adjusted prevalence ratio (PR: 1.56; 95% CI: 0.96–2.51) when comparing the prevalence of secondary cases in infected flocks (mandate data) with the prevalence of index cases in the non-infected flocks, a proxy for the prevalence in the general population in the period 2013–2019 (general data).

The design prevalence, for the subset of flocks and herds with sufficient data to allow the calculation, was, in general, low with 4% of the flocks and 16% of the herds in the mandate data set with a design prevalence of 1% Year 1, and 5.3% and 8.7% in Year 2, respectively. In general, there was no difference between countries with/without secondary cases, except for the design prevalence < 1% in Year 1.

Using a threshold of a maximum difference of 15 affected animals between the model and observed total number of cases for the intensified surveillance, the simulation of the within-flock

transmission model produced the following estimates: a transmission rate 5×10^{-4} (95% CI: 1.7×10^{-4}–1.1×10^{-3}) for the contagious scenario and 2.8×10^{-3} (95% CI: 1.2×10^{-3}–54.9×10^{-3}) occurrence rate for the non-contagious scenario. The model produced a better fit for the non-contagious scenario than for the contagious scenario: only 132 of 10,000 iterations of the contagious scenario (1.3%) resulted in a simulated total number of detected positives within 15 of the observed, whereas 1,578 of iterations of the non-contagious scenario (15.8%) were within the 15 case threshold. Applying the assumptions of the model, the overall sensitivity of the intensified monitoring was low. Sensitivity analysis considering different age distributions of the sheep population and varying the diagnostic sensitivity of the test did not modify the conclusions. Given the uncertainties in the parameters, the model assumptions and the method(s) applied to estimate the fit of the model to the observed data, the estimated transmission rates/occurrence rates and the conclusion on the preferred scenario (contagious vs. non-contagious) need to be interpreted with caution. It is not possible to conclude definitively that atypical scrapie is non-contagious based on the model results.

The descriptive analysis of the general and mandate data sets has confirmed that AS is geographically widespread in the EU in sheep and goats, primarily affecting countries with medium- to large-scale populations of either sheep or goats. For goats, the pattern is similar, although the number of countries with cases is smaller. It also confirmed that the detection of cases of AS in general, and of multiple cases of AS cases overlapping in time in the same holding, are rare events in the European small ruminant population, with some exceptions in Portugal and Hungary.

The results of the analyses of the data collected by the intensified monitoring do not provide additional information on several aspects of the epidemiology of AS, including the existence of risk factors other than age or genotype at population and individual level; the possible origin of the disease; any variability of AS strains circulating in the EU small ruminant population and respective susceptibility of small ruminants; the natural evolution of the disease; nor do they offer any reasons for the apparent differences in epidemiology of the disease in sheep and goats.

Based on the analyses of the data obtained from the intensified monitoring, and accounting for uncertainties and data limitations, it was concluded that there is no new evidence that AS can be transmitted between animals under natural conditions, and it is considered more likely (subjective probability range 50–66%) that AS is a non-contagious, rather than a contagious, disease.

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1. Introduction

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

Since 1 July 2013, according to point 2.2.3 of Chapter B of Annex VII to Regulation (EC) No 999/2001, holdings with a confirmed case of atypical scrapie are to be subject to an intensified TSE monitoring protocol for a period of two years. All ovine and caprine animals which are over the age of 18 months and slaughtered for human consumption and all ovine and caprine animals over the age of 18 months which have died or been killed on the holding must be tested for the presence of TSE.

As explained in recital 14 of Regulation (EC) No 630/2013[1] the purpose of this 2-year intensified monitoring period was to gather more scientific data on atypical scrapie, while all restriction measures on the movement of ovine and caprine animals where a case of atypical scrapie has been confirmed were lifted by the same Regulation. Following a widely supported request from the Member States, the Commission is now preparing for the repeal of point 2.2.3 of Chapter B of Annex VII to Regulation (EC) No 999/2001.

The data pertaining to the intensified TSE monitoring protocols implemented, from 2013 to 2020, by the Member States and a few third countries, have been collected by DG SANTE over the last months, and have already been shared with the Biological Hazards and Contaminants unit in EFSA.

In a scientific opinion from 2014,[2] EFSA stated: "Atypical scrapie does not present, epidemiologically, like an infectious disease. This has been interpreted as evidence that it may be a spontaneous disease of older animals, and not contagious".

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?

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

The Terms of Reference were translated into the following assessment questions (AQs):
Term of Reference 1:

- **AQ1:** Is the prevalence of atypical scrapie (AS) in the entirety of sheep/goat flocks/herds under intensified monitoring[3] statistically higher than that in the general population of the same EU Member States in the period 2013–2019?
- **AQ2**: 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?

Term of Reference 2:

- **AQ3:** Can any of the identified gaps in the knowledge of the epidemiology of AS be filled by the analysis of the mandate data?

1.3. Approach to answer the Terms of Reference

The approach to answer the terms of reference, including the different assessment questions identified above, was defined from the outset and is described in the protocol (see Annex A). The protocol covers both the problem formulation (i.e. what the assessment aims to answer, see also Section 1.2) and the definition of the method for addressing the problem (i.e. how the assessment will be carried out, see also Section 2). It followed the draft framework for protocol development for EFSA's scientific assessments (EFSA, 2020).

The first two assessment questions were addressed quantitatively, analysing the data provided with the mandate (mandate data) and data from TSE surveillance (general data) through a descriptive and

[1] OJ L 179, 29.6.2013, p. 60.
[2] Scientific Opinion on the scrapie situation in the EU after 10 years of monitoring and control in sheep and goats. EFSA Journal 2014;12(7):3781, 155 pp. https://doi.org/10.2903/j.efsa.2014.3781
[3] Commission Regulation (EC) 999/2001 refers to the enhanced surveillance as intensified monitoring, which is the term used throughout this report.

comparative analysis (AQ1) and through a modelling approach (AQ2), as described in Section 2. Results from such analyses were then used to answer ToR1.

The third assessment question, answering ToR2, was addressed qualitatively, reviewing evidence from the literature based on the knowledge of the experts involved in the Working Group drafting this scientific report. Knowledge gaps in relation to current knowledge on the epidemiology of AS were identified as the first step. When a conclusion arising from the analysis carried out within ToR1 led to the clarification of any aspect of the identified knowledge gaps, this was discussed in a narrative way in the report. Knowledge gaps that the ToR1 analysis was unable to address were listed in the report.

2. Data and methodologies

2.1. Data

2.1.1. The 2020 European Commission questionnaire ('mandate data')

The questionnaire was designed specifically to collate data from the 2-year intensified surveillance carried out in infected flocks/herds after the detection of an atypical index case, i.e. first case of AS detected in a previously registered as non-infected flock or not under intensified monitoring. The data were requested by the European Commission DG SANTE in May 2020 from EU Member States (including the United Kingdom) and European Economic Area (EEA) countries that had reported AS cases since 2013. The following data items were collected from each infected sheep flock or goat herd:

1) Flock ID;
2) Mixed flock (YES/NO);
3) Date of confirmation of the index AS case;
4) Date of start of intensified surveillance;
5) Date of end of intensified surveillance;
6) Number of animals tested during Years 1 and 2 (separate) of the intensified surveillance in the four surveillance routes: not slaughtered for human consumption (NSHC), slaughtered for human consumption (SHC), clinical suspects (SUS) and eradication measures (EM);
7) Number of classical scrapie (CS) and of secondary AS cases (separated) during Years 1 and 2 (separate) of the intensified surveillance in the four surveillance routes: NSHC, SHC, SUS and EM.

For mixed flocks, the species of the index case was identified by including the date of confirmation in the species of the index case. As a result, there are animals tested in flocks or herds under intensified surveillance that are of different species to the index case, as the entire holding was put under restrictions.

For analytical purposes, the numbers of animals tested for both species were aggregated at flock ID level. As a result, two data sets were produced, one per species of the index case. Mixed flocks were identified through a new field. There were some flock IDs reported as mixed, but for which the animals tested were of one single species, either sheep or goats. These flocks were not considered mixed in the new field.

2.1.2. The TSE surveillance data ('general data')

Aggregated data of sheep and goats tested, and number of cases of CS and AS by country and year for the period 2013–2019, and for two surveillance streams; SHC and animals NSHC, were extracted from the EU TSE database. Aggregated data and cases from animals from the other two surveillance streams, i.e. animals suspected of being infected by TSE (SUS) and animals culled under TSE EM have not been considered, as they were not used in the analysis. Historical data were consulted in the 'European Union summary report on surveillance for the presence of transmissible spongiform encephalopathies (TSE) in 2019' (EFSA, 2020), which is based on data retrieved from the EU TSE database that collects standardised surveillance data on all testing activities in all MS and other reporting countries. The reports on the monitoring of ruminants for the presence of TSE in the EU in 2013[4] and 2014[5] were also consulted. For 2013 and 2014, all cases were included in the final data set

[4] https://ec.europa.eu/food/system/files/2016-10/biosafety_food-borne-disease_tse_ms-annual-report_2013.pdf
[5] https://ec.europa.eu/food/system/files/2016-10/biosafety_food-borne-disease_tse_ms-annual-report_2014.pdf

and considered in the analyses as there was no discrimination in the reports between index and secondary cases. For the period 2015–2019, it was possible to identify index cases; therefore, these were included in the final data set, while secondary cases were excluded, to allow comparisons of the prevalence of AS in the general population with that of the infected flocks/herds in the intensified surveillance.

The final data set contained the following fields:

1) Country included in the European Commission questionnaire survey;
2) Year (2013–2019);
3) Species (sheep/goat);
4) Total number of animals tested in NSHC;
5) Total number of animals tested in SHC;
6) Total number of index CS cases in NSHC;
7) Total number of index CS cases in SHC;
8) Total number of index AS cases in NSHC;
9) Total number of index AS cases in SHC.

Data sources and data used for the parameters of the within-flock transmission model are described in Section 3.5.

2.2. Methodologies

2.2.1. Descriptive and comparative analysis

2.2.1.1. Descriptive analysis

Descriptive analysis of both the 'mandate' and 'general' data sets was conducted by reviewing and presenting in tabular or narrative format: the total number of animals tested by flock/herd, surveillance stream, country and year; the total number of index cases by surveillance stream, country, year and type of scrapie; and the total number of secondary cases by surveillance stream, country, year and flock for the mandate data. Prevalence rates of AS, defined as the number of cases per number of tested animals, were estimated with 95% confidence intervals (95% CI) using the exact binomial method and expressed per 10,000. Crude prevalence rates and stream-specific rates were calculated both at country level or combining all the available national data (EU-wide, including Norway).

2.2.1.2. Prevalence comparison

If contagious transmission occurs, it would be expected that a statistically higher prevalence of secondary cases would be present in the infected flocks (mandate data), compared with the prevalence of index cases among animals tested in the context of the active surveillance i.e. from non-infected flock/herds (general data), a proxy for the prevalence in the general population.

The overall prevalence rates of AS in active surveillance (i.e. non-infected flock/herds) and the prevalence rates of AS secondary cases in AS-infected flocks/herds were estimated firstly through a univariate descriptive analysis by species and route, and accounting for the cluster sampling design considering the country as the primary sampling unit. Prevalence rates of secondary cases in infected flocks (mandate data) were considered statistically different from those of index cases in the non-infected flocks (general data) when their 95% CI did not overlap. Comparisons were made between the corresponding combination of species and stream. The same comparisons were carried out at national level accounting for the cluster sampling design in mandate data considering the flock as the primary sampling unit.

After merging the mandate data set with the general data set, differences in the prevalence of AS were assessed through modelling and estimating prevalence ratios (PR) by comparing the prevalence in infected flocks/herds to that in non-infected flocks/herds. This was performed by fitting generalised linear regression mixed models (Poisson and negative binomial) accounting for a potential random effect associated with country and any potential confounding effect due to the surveillance stream (NSHC vs. SHC). Other streams have been excluded based on the absence or the low number of cases among clinical suspects (SUS) and animals culled as part of the EM. To avoid model convergence problems, models using standard errors accounting for intragroup (i.e. country) correlation were also used.

2.2.1.3. Design prevalence

At flock/herd level, data from infected flocks/herds have also been used to calculate the design prevalence detectable on the basis of the number of animals actually tested during the intensified monitoring period (mandate data). The design prevalence values obtained contributed to the interpretation of the data on the potential of the intensified monitoring to detect secondary cases, if the disease spreads within flocks/herds.

Design prevalence is a standard hypothetical prevalence of disease specified at the herd (herd design prevalence) or at the animal population levels against which to measure surveillance sensitivity. 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 (FAO, 2014). So, if the design prevalence is, e.g. 10%, it means there is more than a 95% chance of finding a case if 10% or more of the population is infected. If no cases are detected, up to 10% the population could still be infected.

Based on the number of animals tested per flock and accounting for the flock size, the design prevalence required to achieve the target population sensitivity of 95% was estimated separately for sheep and goats, and, within species, for Year 1, Year 2 and the sum of the animals tested in both Year 1 and Year 2 (Years 1 + 2) and as the number of animals in each flock/herd averaged over Year 1 and Year 2. Flocks/herds with index cases confirmed before 1 January 2013 were disregarded for this analysis. The design prevalence was only calculated for flocks or herds with more than 10 animals at the start of Year 1, Year 2 or Years 1 + 2, and in which more than two animals had been tested in Year 1, Year 2 or Years 1 + 2, respectively. The analysis was performed using the package RSurveillance[6] in R. The number of flocks and herds in which the design prevalence was 1%, 5% and 10% is reported in Section 3.4.

2.2.2. Simulation model

The modelling approach was to simulate surveillance data using models that either assume AS is contagious or non-contagious and assess which of the models produces outputs that more closely match the observed surveillance data. This would then provide evidence of whether one set of models matched the data better than another and would therefore indicate which assumption was more consistent with the data, or else it could show that both were able to equally match the observed data, and therefore demonstrate that no conclusion could be made. Specifically, a technique known as Approximate Bayesian Computation (ABC) (Toni et al., 2009) was used to compare the fit of the models to the intensive surveillance data. This approach enabled both the contagious and non-contagious models to be fitted in parallel to the surveillance data, with the model simultaneously determining the best-fitting transmission/occurrence rates and identifying which of the models provided the best fit to the data. For more details on the simulation model, see Section 3.6.

The model was implemented in R. The R package EasyABC (Efficient Approximate Bayesian Computation Sampling Schemes)[7] and abc (Approximate Bayesian Computation)[8] were used to fit the transmission models to the intensified monitoring data, based on a function with outcome: the number of detected infected sheep in the intensified monitoring. The model was applied to all sheep flocks except those with missing or zero flock size in both years. If the flock size was missing for 1 year, the same number of sheep that occurred in the other year of intense monitoring was imputed. The model was not applied to goat herds as there were no secondary cases and so no evidence to support the preference of a contagious/non-contagious model; the model could equally fit either by choosing sufficiently low transmission/occurrence rates.

2.2.3. Uncertainty analysis

The assessment of uncertainty was undertaken following the EFSA 'Guidance on Uncertainty Analysis in Scientific Assessments' (EFSA Scientific Committee, 2018a) and the EFSA scientific opinion on 'The principles and methods behind EFSA's Guidance on Uncertainty Analysis in Scientific Assessments' (EFSA Scientific Committee, 2018b). Special attention was given to the identification of sources of uncertainty, which were listed, together with their expected impact on the outcome of the assessment. In particular, the overall impact of the identified uncertainties on the final conclusions was

[6] https://www.fp7-risksur.eu/sites/default/files/documents/Deliverables/RISKSUR_%28310806%29_D6.24.pdf
[7] https://CRAN.R-project.org/package=EasyABC
[8] https://CRAN.R-project.org/package=abc

expressed in the overall answer to ToR1 using probability terms supported by subjective probability ranges, following the recommendations of the uncertainty guidance. The subjective probability ranges expressed were not the result of precise calculations, but were agreed by consensus by the members of the working group drafting this scientific report following discussion of the results of the analyses performed, and considering the nature of the data analysed (see Section 3.7.1), the uncertainties associated with the data and methods used in the analysis and their expected impact on the conclusions (see Tables 7 and 9).

3. Assessment

3.1. Introduction

3.1.1. Background

Scrapie is the collective term for the group of naturally occurring TSEs, caused by various prion strains, which affect both sheep and goats. The pathognomonic feature of scrapie cases is the presence of an abnormal host-encoded protein (PrP^{Sc}) in the central nervous system and also, in a proportion of cases, in the lymphoreticular tissues. There are two main subcategories of scrapie recognised: classical and atypical.

CS was first described in sheep in the United Kingdom nearly 300 years ago (McGowan, 1922; Parry and Oppenheimer, 1983), in goats in France in the 1940s (Chelle, 1942), and since then it has been reported globally, although the level of occurrence is not known, in Brazil, Canada, China, Falkland Islands, Iceland, Israel, Japan, Northern Cyprus (Gürel et al., 2013), Norway, Palestinian National Authority (PNA), Russia, Switzerland, Tajikistan and the USA (OIE website; EFSA BIOHAZ Panel, 2015). It transmits naturally under field conditions in both sheep and goat populations in which it causes fatal neurodegenerative disease with clinical signs that include behavioural changes, ataxia, pruritus, weakness, recumbency and loss of body condition (Gavier-Widén et al., 2005; Konold et al., 2006, 2007).

There was renewed attention to scrapie following the emergence of BSE in cattle, and the experimental demonstration that sheep were susceptible to BSE, resulting in a disease in small ruminants that presented with clinical and pathological features overlapping with CS but retaining the zoonotic potential of BSE. The hypothesis that scrapie could be masking an epidemic of naturally occurring BSE in sheep and goats (Ferguson et al., 2002; Kao et al., 2002), and a subsequent surge in research on this new form of an old disease, led to a new flow of data and knowledge. Various mandatory control measures were initially put in place in the EU to control the disease (Commission Regulation (EU) 999/2001[9], as amended), including an active surveillance programme, enforced since 2002 as a targeted sample-based monitoring of adult small ruminants, and which represented a major improvement in TSE surveillance within the EU.

Despite these measures, CS is still a problem in some EU Member States, namely, Spain, Greece, Italy and Portugal, which reported a total of 900 cases in 2019; 98.8% of all CS cases reported in the EU (EFSA, 2020).

AS is different from CS regarding its clinical presentation, but also in its molecular characteristics, the anatomical distribution of PrP^{Sc} within infected sheep, the genotypes affected and its epidemiology (see Greenlee, 2019, for recent review). Whereas more than 20 different strains of CS have been described, only one has been demonstrated in AS so far (Acín et al., 2021). AS was reported for the first time in Norway in 1998 (Benestad et al., 2003, 2008), although later retrospective studies have confirmed its presence in a small number of surveillance cases in the UK from before that date (Bruce et al., 2007; Webb et al., 2009). AS is mostly detected through the active surveillance screening of fallen stock and SHC populations that was put in place for CS, rather than presenting through the clinical suspect stream. Unlike CS, PrP^{Sc} has never been demonstrated in the peripheral tissues in AS cases, but low levels of infectivity can still be present in skeletal muscle, peripheral nerves and lymphoid tissues of affected animals. It has been estimated that the total amount of AS infectivity passing through British abattoirs to the food chain is nearly 3,500,000 Ovine Oral (OO) ID50 per year (Adkin et al., 2018).

CS and AS are found in both sheep and goats, and may occur together, or separately, at the flock or national level. There have even been isolated reports of co-existence of strains within a single animal (Mazza et al., 2010; Chong et al., 2015). Recent scientific evidence has raised the possibility

[9] 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 https://eur-lex.europa.eu/eli/reg/2001/999/oj

that multiple strains may commonly be present in the same animal, and that strains can express/be selected differently depending on the compatibility between the structure of the abnormal protein(s) and that of the host-encoded protein (as determined by species and genotype) (Igel-Egalon et al., 2018, for a review on prion strains and transmission barrier phenomena).

CS can cause severe losses in affected flocks with consequent adverse effects on animal health and welfare. In contrast, the low incidence of AS in affected flocks and herds, together with the lack of impact on productivity and welfare and the absence of any evidence of a link to any human TSE, have resulted in its relative neglect.

3.1.2. Neuroanatomy and pathogenesis of classical scrapie vs. atypical scrapie

The pathogenesis and pathology of CS is well understood, having been established through many studies of both naturally and experimentally infected animals (see below). Natural exposure is thought to occur mostly by the oral route, either through direct contact with infected animals or a contaminated environment and is assumed to occur perinatally in most field situations, although adult animals remain susceptible to infection.

Following natural exposure, the incubation period and pathogenesis of CS are both influenced by the host PrP genotype, with the shortest incubation periods and most PrP^{Sc} accumulation being associated with the most susceptible genotypes (for overviews see EFSA BIOHAZ Panel, 2010; Sisó et al., 2010). Briefly, following ingestion of infected material, prions are taken up through the gut, with PrP^{Sc} accumulating in the gut associated lymphoid tissue (GALT) within a few weeks of exposure. The enteric nervous system is also involved, before retrograde transportation of PrP^{Sc} via the spinal cord to the brainstem at the level of the obex, in which the earliest detectable accumulations of PrP^{Sc} are consistently associated with the dorsal nucleus of the vagus, which is the established target tissue for diagnostic testing. Involvement of this area can occur within 10 months of exposure in the most susceptible animals (EFSA BIOHAZ Panel, 2010), and precedes the onset of clinical disease by many months.

As the disease progresses, there can be widespread accumulation of PrP^{Sc} within the lymphoreticular system (LRS) as a whole, although this aspect of disease pathogenesis is heavily influenced by host genotype, with the more resistant genotypes showing little or no PrP^{Sc} accumulation in the LRS. It is believed that the presence of PrP^{Sc} in the lymphoid tissues is associated with the shedding of infectivity and natural transmission of disease. Before the onset of clinical signs, PrP^{Sc} also accumulates within the brain in very distinctive neuroanatomical patterns that are consistently associated with particular strain and host–genotype combinations (Spiropoulos et al., 2007). Early involvement of the target areas at the obex is a consistent feature of all CS patterns.

The pathogenesis of experimental CS is broadly similar (Ryder et al., 2009), following the natural disease regarding relative incubation period and PrP^{Sc} accumulation patterns, and linkage with genotype, regardless of the route of challenge (oral or intracerebral).

By comparison, very little information is known about the pathogenesis and pathology of AS. Few clinical cases have presented through passive surveillance, and not all of these have had full post-mortem investigations. Most identified naturally occurring cases have been detected through active surveillance, in which only small samples of brainstem have been collected for diagnostic screening purposes. From these samples, it is apparent that PrP^{Sc} accumulation in the brainstem does not occur to the same extent, or in the same neuroanatomical areas, as CS (Benestad et al., 2008). Additionally, studies of the few whole brains available indicated that PrP^{Sc} accumulation in the cerebellum, and more rostral areas of the brain, was much more extensive than in CS, and that the brainstem was a very poor area with regard to diagnostic sensitivity for AS (Benestad et al., 2008; Moore et al., 2008). These observations led to the recommendation that sheep brain sampling should include cerebellum in addition to brainstem whenever possible, but there is a lack of clarity in the case data about whether this has been done. These factors contribute to reduced sensitivity of the surveillance system for the detection of AS, but it is not possible to quantify the impact on case detection.

Involvement of the lymphoreticular system in AS appears to be minimal. Detectable accumulations of PrP^{Sc} are not a reported feature of natural disease, regardless of genotype, although one study has detected low levels of infectivity associated with lymphoid tissues (Andreoletti et al., 2011). The same patterns are found in clinically diseased animals following both oral and intracerebral experimental challenge, with minimal involvement of peripheral tissues, and accumulation of PrP^{Sc} in the brain in a pattern in which the rostral brain areas and cerebellum are much more widely involved than the brainstem (Simmons et al., 2007, 2010, 2011).

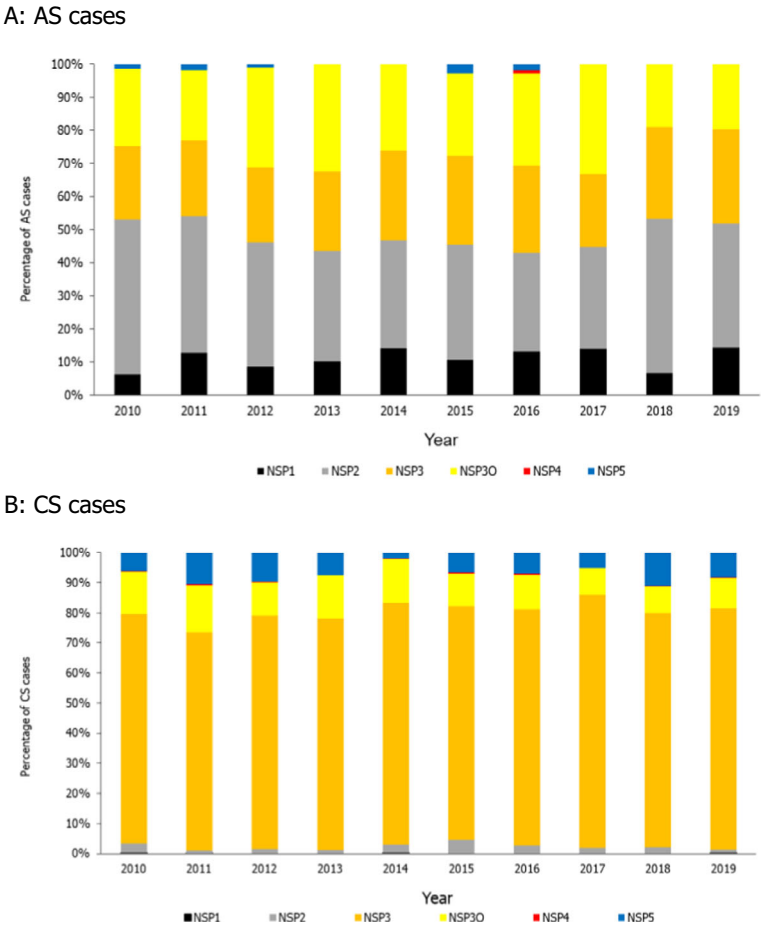
Only one AS time-kill study has been reported (Simmons et al., 2011). In this study, animals were exposed orally at birth, and killed at either 12 or 24 months post-challenge. This study demonstrated that AS could be transmitted by the oral route, and that affected animals developed the same limited pathology with the same PrP^{Sc} accumulation pattern in the brain as natural cases. This study also suggested, albeit with a very small number of cases, that involvement of the brainstem following oral exposure was a late-stage change.

3.1.3. Genetics and susceptibility

In sheep, codons 136 (amino acid alternatives A or V), 154 (R or H) and 171 (Q, R or H) of the *PRNP* gene encoding the prion protein are known to influence susceptibility to CS. At the two extremes of the spectrum, the allele VRQ confers the highest susceptibility while the ARR allele confers the highest resistance (Dawson et al., 1998). Breeding programmes designed to increase the genetic resistance of both high genetic merit flocks and commercial flocks of the sheep population (by minimising the occurrence of animals with valine at codon 136, and maximising those with arginine at codon 171) have successfully contributed to a reduction in the incidence of CS in European countries (EFSA BIOHAZ Panel, 2014). Decision 2003/100/EC established on a voluntary basis from 1 January 2004 and compulsorily from 1 April 2005 a breeding programme based on genetic resistance to scrapie. However, such breeding for resistance schemes have not been mandatory since 1 June 2007 (EFSA BIOHAZ Panel, 2014).

As with CS, polymorphisms in the *PRNP* gene are linked to the occurrence (or not) of AS, with the susceptibility to disease being inverted compared with CS. Polymorphism at codon 141 (L or F) appears to affect the susceptibility; an increased risk of AS has been identified in sheep with the AFRQ haplotype (Saunders et al., 2006). Naturally occurring atypical cases have not been reported in V136 animals, but animals that are R171 can develop disease. However, such R171 animals were not over-represented in the diseased population relative to healthy animals, as confirmed in the genotypes of all cases in Great Britain in the period 2002–2013 (Ortiz-Pelaez and Arnold, 2014). E.g. the same data set showed that the most frequent genotypes associated with AS in Great Britain were ARR/AHQ (32.3%) and AHQ/A(F/L)RQ (20.5%). Animals with an AHQ allele were also much more likely to have AS than CS. Similar findings have been reported in other countries (Moreno et al., 2007). In the EU, the most frequent group of genotypes among the cases of AS reported in the last 10 years is NSP2,[10] which includes all heterozygous ARR except ARR/VRQ, and accounts for over a third of the cases. Figure 1 shows the frequency distribution of genotypes of sheep scrapie cases by case type, year and National Scrapie Plan (NSP) group in the period 2010–2019 in the reporting countries included in the EFSA's EU summary report (EFSA, 2020).

[10] Genotype groups according to the National Scrapie Plan (Great Britain) based on the level of resistance/susceptibility to classical scrapie: NSP1: ARR/ARR (Genetically most resistant); NSP2: ARR/ARQ; ARR/ARH; ARR/AHQ (Genetically resistant); NSP3: ARQ/ARQ (Genetically little resistant); NSP3 Other: AHQ/AHQ; ARH/ARH; ARH/ARQ; AHQ/ARH; AHQ/ARQ (Susceptible); NSP4 : ARR/VRQ (Genetically susceptible); NSP5: ARQ/VRQ; ARH/VRQ; AHQ/VRQ; VRQ/VRQ (Genetically highly susceptible) Susceptible.



(A) Atypical scrapie; (B) Classical scrapie. NSP1: Resistant (black); NSP2: Semi-resistant (grey); NSP3 (orange) + NSP3O (yellow) + NSP4 (red) + SNP5 (blue): Susceptible.

Figure 1: Frequency distribution of genotypes of sheep scrapie cases by case type, year and NSP group in the period 2010–2019 in the reporting countries

It has been speculated that the breeding for resistance programmes for CS, which favour genotypes now known to be susceptible to AS, such as the NSP2 type, may have resulted in an increase in the frequency of AS in the general population. However, the analysis of the EU surveillance data of animals NSHC and SHC for the period 2010–2019 has shown the opposite effect, a significant decreasing trend of AS in sheep, with an annual risk ratio of 0.96 and an average 4% annual decrease in the probability of detecting AS ($p < 0.0001$) adjusted by surveillance stream (EFSA, 2020), and including Member States that did not implement breeding for resistance programmes. This finding could have been influenced by the different ratio in the tested animals between NSHC and SHC over the years, as the probability of detecting AS in sheep in the NSHC was higher than in the SHC (RR = 1.6, $p < 0.0001$). However, the overall ratio NSHC:SHC for the period 2013–2019 in surveillance was 1.5:1, very similar to that of the intensified monitoring for AS (1.6:1) for the same period, favouring the surveillance stream with the highest detection ability.

In goats, codons 146 (amino acid alternatives D, N or S) and 222 (K or Q) of the PRNP gene encoding the prion protein are known to influence susceptibility to CS. K222, D146 and S146 have been shown to be the most relevant for influencing CS susceptibility in goats (for a review, see EFSA BIOHAZ Panel, 2017b). The principles of genetic susceptibility and breeding for resistance to CS in goats have also been confirmed, but the greater number of PRNP polymorphisms in goats, and greater breed diversity requires the design of breeding programmes to be adapted to the genetic characteristics of the specific goat populations. Indeed, when applied locally, it has proven to be successful (EFSA BIOHAZ Panel, 2017b; EFSA, 2020). Knowledge about genetic susceptibility to AS in goats is extremely scarce, with one case–control study identifying an association between histidine at codon 154 and the disease (Colussi et al., 2008).

3.1.4. Transmissibility

CS transmits horizontally, under field conditions, due to the ingestion of PrP^{Sc} either directly from secreta/excreta from infected animals, most notably the presence of PrP^{Sc} in the placenta of infected sheep and goats (Tuo et al., 2002; Schneider et al., 2015), via milk (Konold et al., 2008, 2013; Madsen-Bouterse et al., 2018), or via environmental contamination with such infected materials (Touzeau et al., 2006; Dexter et al., 2009; Konold et al., 2015).

Experimental transmission of CS strains has been achieved both across genotypes within sheep, and between species, with successful transmissions recorded in goats, cattle, cervids and a range of different laboratory rodent models including conventional mice, hamsters, bank voles, primates and transgenic mice, including humanised mice (for a review, see EFSA BIOHAZ Panel, 2015).

Transmission of AS under field conditions is not thought to occur, or to occur at a very low rate, based on studies of flocks containing naturally occurring cases (Simmons et al., 2009; Fediaevsky et al., 2010; Ortiz-Peláez et al., 2016). It has also been speculated that cases may arise spontaneously (McIntyre et al., 2008a). In this regard, it is important to clarify the terminology of spontaneous vs. transmissible disease. As stated in the EFSA's scientific opinion on the BSE BARB cases (EFSA BIOHAZ Panel, 2017a):

'spontaneous cases, interpreted as cases occurring without an apparent cause, are not predictable and may not be detectable either. The classification of a case as spontaneous is circumstantial and may change over time subject to additional information. It does not infer that there is no external cause; just that it could not be ascertained. A case of disease is classified as spontaneous by a process of elimination, excluding all other definable possibilities'.

So, a transmissible disease is not the opposite of a spontaneous disease, i.e. a disease could be at the same time non-transmissible and non-spontaneous.

Successful experimental transmission of AS has been achieved via both the intracerebral and the oral challenge routes, with preservation of the full disease phenotype including clinical signs (Simmons et al., 2007, 2010, 2011). However, another study in ARQ sheep resulted in silent carriers with long incubation times following intracerebral inoculation (Okada et al., 2016). These studies suggest that a failure to transmit under field conditions may be a consequence of low or no shedding of infectivity from affected animals, rather than (or as well as) a high exposure threshold for successful infection.

Attempted transmission of AS to other species has been generally less successful than for CS, although the potential for cross-species transmissions has been established in transgenic mouse models, in which successful transmissions have sometimes resulted in a fundamental shift in the molecular signature of the PrP^{Sc} generated, signalling potential changes in its biological properties (Espinosa et al., 2009; Huor et al., 2019).

The zoonotic potential of scrapie has been explored by testing the species barrier phenomenon. Transmission of CS and AS to mice has produced disease profiles and PrP^{Sc} types different from those seen in BSE and in human prion disease (Tranulis et al., 2011). However, Gibbs et al. (1980) showed that mouse-adapted scrapie could be transmitted to squirrel monkeys via the oral route. At that time, the discrimination between CS and AS did not exist. A recent study reported the replication of c-BSE prions during the *in vivo* propagation of AS isolates in the natural host, suggesting the possibility that AS isolates contain a small amount of BSE infectivity, following the passage of some isolates of AS in sheep and goats through TgBov and Tg sheep expressing VRQ and ARQ alleles (Huor et al., 2019).

An example of the complex interactions between the host and an infecting strain is the study in which intracerebral inoculation of AS into recipients of two different genotypes (both associated with susceptibility to AS) resulted in one sheep displaying an altered phenotype with clinical, pathological, biochemical and murine bioassay characteristics that were all consistent with the CS strain CH1641, and distinct from the AS donor, while the other recipient sheep presented the expected phenotypic parameters for AS (Simmons et al., 2015).

Recently, it has been postulated that such a transformation of AS to contagious CS could occur by means of evolutionary processes defined by the interaction of the disease agent, host and environment (Adams, 2020). Similarly, Orge et al. (2010) explained the unusual presentation of atypical and CS in Portugal by speculating on the possibility that CS emerged from a background of AS, which was the predominant disease form until 2008 when the first outbreak of CS was detected in that country. However, there are other EU countries (Austria, Denmark, Estonia, Finland, Croatia, Poland and Sweden) that have reported AS consistently over the years, albeit at a much lower incidence than that found in Portugal, but have not reported CS cases so far.

3.1.5. Epidemiology and surveillance

While CS appears clustered at flock level (McIntyre et al., 2008b) and with a heterogeneous presentation across countries and surveillance streams (Del Rio Vilas et al., 2007), the presentation of AS is typical of a rare disease with prevalence estimates remarkably homogeneous across countries, surveillance streams and calendar years of surveillance in the EU (Fediaevsky et al., 2008). When detected, AS usually appears in a single sheep per affected flock (De Bosschere et al., 2004; Moum et al., 2005; Rodríguez-Martínez et al., 2010), with some exceptions. In an analysis of the prevalence of AS in 11 European countries, it was concluded that AS is not contagious, or has a very low transmissibility under natural conditions compared with CS (Fediaevsky et al., 2010). AS in sheep can occur both in flocks with CS and in those with no history of classical disease, including a closed, biosecure experimental flock derived from sheep from New Zealand (Simmons et al., 2009).

AS is a non-reportable disease according to the OIE classification, so it is not easy to compile data on the global occurrence of AS. Apart from the publication of the EU summary report by the European Commission between 2001 and 2015 and by EFSA since 2016, the occurrence of AS in non-reporting countries can only be ascertained via national reports, scientific publications or grey literature. As a result, outside the EU, AS cases have been detected in the USA, Canada (Mitchell et al., 2010), the Falkland Islands, and also in Australia and New Zealand (Kittelberger et al., 2010; Cook et al., 2016), where CS was successfully eliminated in the 1950s (Ru, 2017). Japan has been the most recent country to report its first case of AS, in 2016 (Imamura et al., 2017).

Since 2002, when the first case of AS was reported to the European Commission TSE database, over 2,700 cases in sheep have been reported (up to the end of 2019). However, cases of AS occurred at least as early as 1972 amongst scrapie suspects in the UK, unconfirmed by the available techniques at that time, but ascertained in retrospective studies (Webb et al., 2009; Chong et al., 2015).

Twenty-two current or ex-MS have reported AS cases since 2002, with Portugal being the country with the highest number of cases (696) and Estonia the one with the lowest (2). The caseload of the other 20 MS is as follows: Austria (15), Belgium (8), Bulgaria (6), Czechia (8), Germany (132), Denmark (14), Greece (32), Spain (239), Finland (18), France (571), Croatia (2), Hungary (173), Ireland (50), Italy (109), the Netherlands (18), Poland (64), Sweden (49), Slovenia (10), Slovakia (42) and the United Kingdom (368). Two European non-MS have also reported AS cases: Iceland (8) and Norway (169).

In the EU, AS has been consistently reported every year, with over 100 cases per year reported on average in the last 5 years (EFSA, 2020). The peak was achieved in 2006 when 482 cases of AS were reported by 13 MS, coinciding with the extra surveillance in small ruminants conducted in that year due to the finding of the first case of natural BSE in a goat. A 2.73-fold increase in the normal throughput was reached, with more than 888,000 sheep and 247,000 goats tested. The distribution of AS cases per year for the period 2003–2019 is shown in Figure 2.

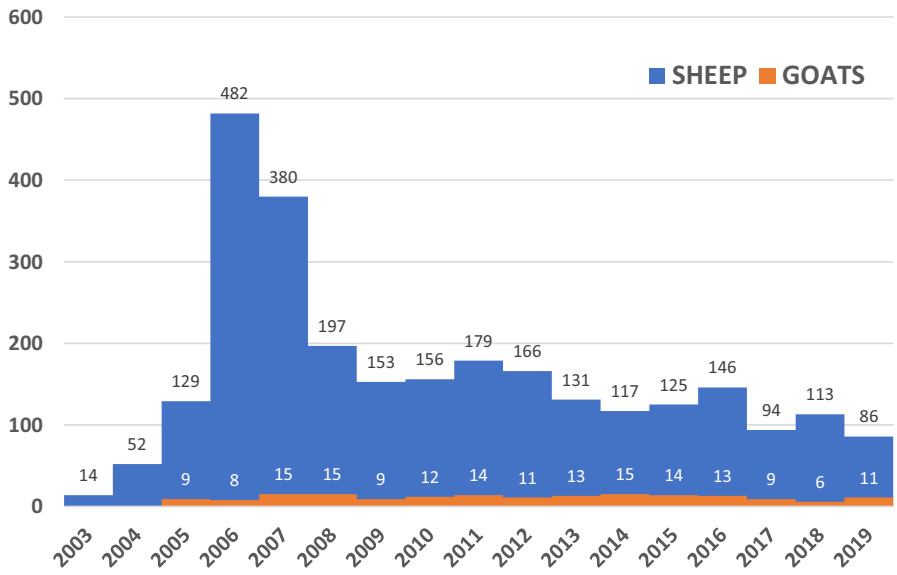


Figure 2: Number of cases of AS reported by EU MS during the period 2003–2019 for sheep and 2005–2019 for goats

Looking at the EU data only, for the period 2002–2019, AS in sheep has been mostly detected in the NSHC category (52.5% of all cases), followed by SHC (43.4%), EM (2.5%) and SUS (1.5%). AS is mostly detected via routine active surveillance, with passive surveillance being a non-relevant source of cases.

Since 2005, when the first case of AS in goats was reported by an EU MS to the EC TSE database, 174 cases have been reported (up to the end of 2019). Eleven current or ex-MS have reported AS cases, with France the country with the largest number of cases (61) and several with only one case reported: Austria, Finland, Poland and Slovenia. The caseload of the other six MS is as follows: Cyprus (6), Germany (2), Greece (5), Spain (55), Italy (29), Portugal (14). One European non-MS has also reported an AS case in a goat: Norway in 2006. In the EU, AS in goats has been consistently reported every year, with on average 10 cases per year reported in the last 15 years (EFSA, 2020). A peak was reached in 2006 when 15 cases of AS were reported by four MS. The distribution of AS case per year for the period 2003–2019 is shown in Figure 2. Looking at the EU data only, for the period 2005–2019, AS in goats is mostly detected in the NSHC (61.7% of all cases), followed by the SHC (36.5%) and EM (1.7%). No clinical cases of AS have been reported in goats in the EU.

AS usually affects older animals. Based on the 20,364 cases of scrapie with known type (clinical and not), species and age between 2009 and 2019, in sheep, the average age of AS cases (85.2 months) is significantly higher ($p < 0.001$) than that of CS cases (51.1 months). Similarly, in goats, the average age of AS cases (87.6 months) is significantly higher ($p < 0.001$) than that of CS cases (52.4 months) (EFSA, 2020).

There is still limited knowledge of the epidemiology of AS. There have been a few efforts to elucidate risk factors for the presence of AS, in the form of case–control studies mostly conducted via questionnaires. The identification of still unknown risk factors could help inform effective preventive measures without 'surrendering to the idea of "bad luck"' (Ru, 2017) i.e. that the disease occurs spontaneously. The results of such studies have shown that when significant associations were identified, they were not consistent with those of a contagious disease. E.g. no risk factors associated with movement of animals between flocks, or direct contact between animals were found (Hopp et al., 2006). Being in a dairy farm and feeding with corn silage were associated with an increased and decreased, respectively, risk of AS (Fediaevsky et al., 2009). Three independent variables were associated with an increase in the odds of AS: the presence on the farm of two sheep breeds (Welsh Mountain and Cheviot) and flock type (store/fattening flocks) (Del Rio Vilas et al., 2010). Farms with AS cases were less likely to implement oestrus synchronisation/superovulation and cases were more likely to occur in large holdings (Marier et al., 2017).

Some attempts have been made to analyse surveillance data to investigate iatrogenic and horizontal transmission in sheep populations at the national level. An early modelling process looking at sheep movement and surveillance data in Great Britain concluded that the incidence of AS was homogeneous between network communities, leading the authors to conclude that should natural transmission of AS be occurring at all, it is doing so slowly (Green et al., 2007). One of the simplest approaches to follow, as a first step to investigate a possible horizontal transmission, is the ascertainment of multiple cases of AS in the same flock via surveillance data. Until the data were gathered for this mandate, this had been substantiated in only a few EU MS: Germany (Lühken et al., 2007), Great Britain (Konold et al., 2007) and Ireland (Onnasch et al., 2004). The next step is the analysis of surveillance data, testing the hypothesis that the probability of the presence of multiple cases of AS in the same holding is consistent with a random event akin to a spontaneous origin, or not. This approach was followed by Ortiz-Peláez et al. (2016) with data from Great Britain. The results showed that the probability of detecting at least two cases of AS in the same holding was much lower in simulated random data than in simulated actual data, and that the sampling bias in surveillance led to multiple sampling from fewer but larger holdings. The authors concluded that the pattern of AS cases observed in GB was unlikely to be explained by a multi-case event in which the cases are epidemiologically linked, but rather by a bias in the selection of the tested flocks and sheep within flocks. Similar attempts have not been made to analyse surveillance data at the European level.

EFSA has not assessed AS in general, or its surveillance in particular, in any scientific opinion since the intensified monitoring was imposed on flocks/herds affected by AS in 2013. The 2014 EFSA Scientific Opinion on the scrapie situation in the EU after 10 years of monitoring and control in sheep and goats (EFSA BIOHAZ Panel, 2014) was the last attempt to analyse the occurrence and trends of AS at EU level. AS detection has never been the main purpose of the scrapie surveillance system in the EU, which was designed before the identification of AS, for the detection of CS, driven by the suspicion

of the potential for BSE to be present in the sheep population, posing a risk to public health (SSC, 1999).

Commission Regulation (EU) No 630/2013 enforced intensified monitoring, through additional compulsory testing of small ruminants in flocks/herds where cases of AS have been confirmed, enabling more scientific data on AS to be gathered. Most of the AS cases reported in the EU are not clinical suspects and are detected by the abattoir and the fallen stock monitoring. Intensified monitoring in infected flock/herds relied mainly on testing animals in these two surveillance streams.

Surveillance data offer the potential for: (i) the detection of further cases and the analysis of the gathered epidemiological data on the cases and the affected flocks/herds to identify risk factors; (ii) the provision of material for retrospective comparative studies of isolates representing different populations, strains and phenotypes, assuming that positive samples have been retained locally; (iii) the improved estimation of the actual frequency of disease in the general population. The latter is otherwise likely to be underestimated, as the diagnostic sensitivity of TSE surveillance screening tests, i.e. considering the sampling and testing of animals that are unlikely to be at disease endpoint and/or relying on brainstem only, as discussed in Section 3.1.2, has been shown to be lower than the analytical sensitivity of the same tests when applied to known positive cases under more controlled conditions (Andreoletti et al., 2011; EFSA, 2015; EFSA BIOHAZ Panel, 2018c).

3.2. Descriptive analysis of the AS data

3.2.1. Data from the European Commission questionnaire (mandate data set)

Twenty-two countries reported data in the questionnaire: 21 MS and Norway. Twenty of them reported flock IDs with sheep as the species of the index cases: 19 MS and Norway. Eleven of them reported herd IDs with goats as the species of the index cases: 10 MS and Norway. For details of the countries included in the mandate data set, see Tables 1 and 2.

Based on the data from 22 countries, made available by the European Commission for this mandate, and after the aggregations explained in Section 2.1, there were 742 flock IDs from 20 countries in which the index case of AS was a sheep, triggering intensified monitoring. The median number of sheep index cases reported by country was 32 (range: 1–127). The period covered by the data provided was not restricted to the 2013–2019 period. One country, Norway, submitted available data dating back to 2004, resulting in it being the country with largest number of index cases in the data set: 127. Other countries also reported index cases detected during the first half of 2020. Full data sets of raw mandate data as submitted by the reporting countries and the aggregated mandate data set for analysis can be accessed in the following weblink: https://doi.org/10.5281/zenodo.4916513

Table 1: Summary of the mandate data of flocks with sheep as index case

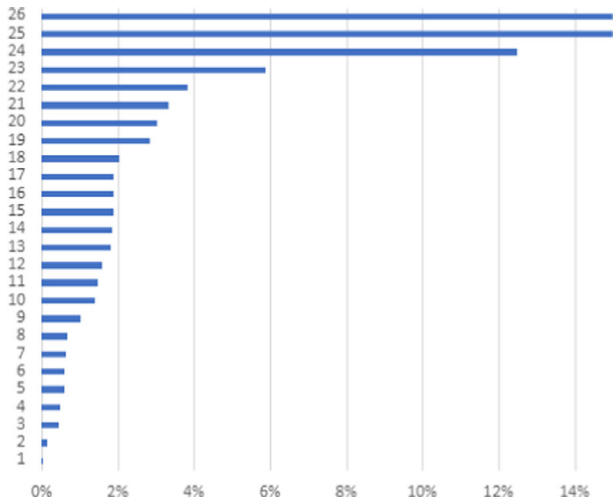
Country	N flocks with sheep AS index case	N tested animals in Year 1	N tested animals in Year 2	Sum of tested animals in Year 1 and Year 2	N secondary AS cases	N flocks with secondary AS cases
AT	11	152	169	321		
BE	1	1		1		
BG	3		234	234		
CZ	7	92	167	259		
DE	47	296	262	558		
DK	2	1		1		
ES	69	2,284	1,798	4,082	2	2
FI	8	132	108	240		
FR	40	841	800	1,641	1	1
HR	1	9	16	25		
HU	88	7,433	9,027	16,460	16	9
IE	37	1,174	758	1,932		
IT	41	488	186	674		
NO	127	2,390	1,547	3,937	4	4

Country	N flocks with sheep AS index case	N tested animals in Year 1	N tested animals in Year 2	Sum of tested animals in Year 1 and Year 2	N secondary AS cases	N flocks with secondary AS cases
PL	38	662	355	1,017	2	2
PT	92	1,434	876	2,310	6	6
SE	20	205	159	364	1	1
SI	10	30	28	58		
SK	27	2,428	2,990	5,418		
UK	73	1,371	957	2,328	3	3
Total	**742**	**21,423**	**20,437**	**41,860**	**35**	**28**

In the 20 countries with flocks in which sheep were the index case, the total number of sheep tested, as part of the intensified monitoring, was 41,860 (median: 616; range: 1–16,460): 21,423 in year 1 and 20,437 in Year 2. The number of secondary cases of AS reported in the 742 identified flocks was 35 in 28 infected flocks (3.8%) from eight countries (Table 1). They were all single secondary cases except two flocks in Hungary that reported two (average size in the 2 years of intensified monitoring: 15,429) and seven secondary cases (average size in the 2 years of intensified monitoring: 12,916), respectively. Hungary is the third largest contributor to the total number of index cases with 88, but it is by far the biggest contributor in terms of animals tested (16,460, 39.2%) and secondary cases (16, 47%). Seven cases of CS were also reported among the infected flocks with sheep index cases of AS: one case in a flock of France and six cases in a flock in Germany, as examples of outbreaks of scrapie in flocks in which classical and atypical scrapie coexist.

Most of the 35 cases were detected in the NSHC stream: 18 in Year 1 and 8 in Year 2, respectively; followed by the SHC stream (five and two, respectively), and only two as clinical suspects (both in Poland in Year 1).

Considering the number of animals tested over the 2-year period and the average size of the flocks, out of the 28 flocks with secondary cases, two of them had no data on animals tested. The crude prevalence (Figure 3) was less than 5% in 22 of the remaining 26 flocks (84.6%) and less than 1% in nine of them (34.6%).



Flocks 25 and 26 had crude prevalence higher than 15% (33.3% and 33.3%, respectively).

Figure 3: Crude prevalence of secondary cases (proportion of positive cases) in 26 of the 28 flocks

As mentioned above, based on the data from 22 countries, made available by the European Commission for this mandate, there were 76 herd IDs from 11 countries in which the index case of AS was a goat, triggering intensified monitoring. No secondary cases of AS were detected in the infected herds. The median number of index cases reported by country was two (range: 1–27). The period covered by the data provided was not restricted to the 2013–2019 period.

Table 2: Summary of the mandate data of herds with goats as the index case

Country	N herds with goat AS index case	N tested animals in Year 1	N tested animals in Year 2	Sum of tested animals in Year 1 and Year 2
AT	1	0	1	1
CY	1	3	9	12
DE	2	0	0	0
EL	4	253	49	302
ES	27	2,192	1,490	3,682
FR	20	138	124	262
IT	16	499	46	545
NO	1	52	4	56
PL	2	0	0	0
PT	1	3	2	5
SI	1	0	0	0
Total	76	3,140	1,725	4,865

The total number of goats tested by the 11 countries in infected herds as part of the intensified monitoring in the 2-year period was 4,865 (median: 12 range: 0–3,682). Spain was the largest contributor to the total number of index cases with 27 (35.5%) and it is by far the biggest contributor in terms of goats tested (3,682, 75.7%). Four cases of CS were reported in two of the infected herds: two cases in one herd in Cyprus and two cases in one herd in Spain.

3.2.2. Data from the EU TSE database (general data set)

The retrieval of data was strictly restricted to the reference period (2013–2019) and the analysis was focused on the active surveillance carried out on non-infected flocks/herds for the period 2015–2019. For 2014–2015, there was no discrimination between tested animals from infected and non-infected flocks/herds. For those 2 years, all the tested sheep were assumed to be sourced from non-infected premises and all the cases were assumed to be index cases.

Even though statutory surveillance according to Regulation (EC) 999/2001 applies to all MS mandatorily and to other non-MS voluntarily, the data extracted from the general surveillance concerns only the 22 countries included in the mandate data, to allow comparisons. Surveillance data from reporting countries that have not reported AS index cases between 2013 and 2019 were not included in the description below. Over the period 2013–2019, a total of 1,904,301 sheep were tested in the 22 countries included in the mandate data: 1,142,017 in the SHC stream (60%) and 762,284 in NSHC (40%). As a result, a similar number of CS and AS cases were detected: 813 (75.8% of cases sourced by SHC) and 812 (89.3% detected in the SHC), respectively. The crude prevalence of AS per 10,000 tested animals was 7.11. Out of the 12 countries that reported CS, three (Greece, Italy and Spain) reported 89.4% of all CS cases (727). Twenty countries reported AS during the same period. Three of them, i.e. Portugal, Hungary and the UK, reported 57.7% of all AS cases (469).

In the mandate data set, the number of index cases reported (excluding cases from Norway for the period 2004–2012: 64) is 678, plus the number of secondary cases (35) making a total of 713 cases included in the mandate data set. If this is compared with the total number of cases included in the general data set (812), it means that 87.8% of all cases appeared in the mandate data set.

Over the same period of time, in total, 726,152 goats were tested (Table 1) in the 22 countries that provided data: 450,801 in the SHC route (62.1%) and 275,351 in the NSHC route (37.9%). As a result, 1,105 cases of CS were reported by eight countries and 77 cases of AS by 10 countries. Compared with sheep, the prevalence of AS per 10,000 tested animals in goats is lower (1.06). The majority of the CS cases were reported by Cyprus (853, 77.2%), whereas the AS cases (77) are more widespread geographically, with Spain being the main contributor with 26 cases (33.8%) followed by France with 21 cases (27.3%).

In the mandate data set, the number of index cases reported (excluding cases from Norway for the period 2004–2012) is 75, plus the number of secondary cases (0) making a total of 75 cases included in the mandate data set. If this is compared with the total number of cases included in the general data set (77), it means that 97.4% of all goat cases appeared in the mandate data set. Full data sets of general data can be accessed in the following weblink: https://doi.org/10.5281/zenodo.4916513.

3.3. Comparative analysis of the prevalence of AS

The absence of observed secondary cases in the infected goat herds prevented the possibility of carrying out the comparison of the prevalence in the mandate and general data. However, the absence of observed secondary cases in goats is a notable epidemiological feature in itself. Possible explanations of this finding could relate to a lack of transmission within infected goat herds or to an insufficient sensitivity of the surveillance at population level in detecting the occurrence of extremely low rates of new cases (as noted above the prevalence of index cases in this species is one-seventh that of sheep).

After merging the data sets for sheep from the two available sources (mandate data set and general data set), in total 822 cases (789 and 33 from non-infected and infected flocks, respectively) and about 1.8 million tested sheep (1,771,490 and 41,804 from non-infected and infected flocks, respectively) were available for analysis.

A preliminary analysis was carried out by comparing stream-specific EU-wide (Table 3, also including Norway) and country-specific (Table 4) prevalence rates obtained from the mandate data set (prevalence of secondary cases in infected flocks subject to intensified monitoring) and the general data set (prevalence of index cases in non-infected flocks involved in active surveillance activities, a proxy for the prevalence in the general population) respectively.

In countries where secondary cases were detected, raw estimates showed a higher prevalence in infected flocks compared with the prevalence of index cases in non-infected flocks. However, this difference was not statistically significant, with the exception of the NSHC-specific rates in France (see Table 4). In the remaining countries, the absence of secondary cases obviously led to the opposite difference, but the 95%CI were always overlapping (see Appendix A). The calculation of the prevalence of AS in the mandate and general data for each county and for SHC and NSHC are displayed in Appendix A (sheep) and Appendix B (goats).

Table 3: EU-wide (including also Norway) prevalence of atypical scrapie (AS) cases by stream category and source (infected flocks vs. non-infected flocks)

Surveillance stream	Populations	Prevalence (cases per 10,000 tested sheep)	95%CI
SHC	Mandate data[(a)]	4.45	1.6–12.23
	General data	3.27	1.95–5.48
NSHC	Mandate data	9.94	6.64–14.88
	General data	4.93	3.26–7.46

(a): Mandate data: prevalence rate of secondary cases in infected flocks. General data: prevalence rate of index cases in the non-infected flocks (a proxy for the prevalence in the general population).

Table 4: Country-specific prevalence of atypical scrapie (AS) cases by stream category and source (infected flocks vs. non-infected flocks) in countries with secondary cases

Surveillance stream	Country	Populations	Prevalence (cases per 10,000 tested sheep)	95%CI
SHC	ES	Mandate data	5.74	0.73–45.19
		General data	4.9	3.47–6.93
	NO	Mandate data	6.66	1.66–26.76
		General data	5.37	3.88–7.45
	PT	Mandate data	9.82	7.42–12.99
		General data	28.68	8.85–92.51
NSHC	ES	Mandate data	4.27	0.44–41.33
		General data	4.49	3.3–6.09
	FR	Mandate data	58.14	8.52–385.61
		General data	1.73	1.23–2.45
	HU	Mandate data	11.42	5.71–22.82
		General data	10.13	7.74–13.25
	NO	Mandate data	21.41	5.2–87.75
		General data	5.92	4.06–8.63

Surveillance stream	Country	Populations	Prevalence (cases per 10,000 tested sheep)	95%CI
	PT	Mandate data	23.73	7.52–74.68
		General data	12.7	10.71–15.06
	UK	General data	12.89	4.47–37.07
		Mandate data	6.29	4.95–7.99

Poisson and negative binomial regression models were used to fit the 'count of cases detected' from the two data sources accounting for the total number of animals tested. In this way, it was possible to estimate PR adjusted for the potential confounding effect of the surveillance stream, i.e. SHC vs. NSHC. A PR larger than 1, i.e. the prevalence in mandate data is higher than in general data, could be considered a proxy for within-flock transmission or higher exposure to unknown risk factors in the infected flocks compared with the general population. A PR is considered statistically significant when its 95% confidence interval (95% CI) does not include 1. The final data set, limited to the SHC and NSHC streams and combining data from the 20 countries that reported AS, included: 789 index cases and 1,771,490 animals tested (general data); 33 secondary cases and 41,804 animals tested (mandate data, only two secondary cases detected in the SUS stream were not included in the model).

As the mixed effects models were not converging when trying to account for a potential random effect associated with country, standard errors of the parameters were calculated to account for intragroup correlation associated with countries. When comparing Poisson and negative binomial regressions, the final model was selected on the basis of the lowest values of the Akaike's information criterion (AIC) and Bayesian information criterion (BIC).

The final model was a negative binomial regression model showing a non-statistically significant stream-adjusted PR (1.56; 95% CI: 0.96–2.51) when comparing the prevalence of secondary cases in infected flocks (mandate data) with the prevalence of index cases in the non-infected flocks (a proxy for the prevalence in the general population) (general data). Therefore, it cannot be concluded that the prevalence of AS in the flocks with sheep as index case under intensified monitoring (mandate data) was statistically higher than that in the surveillance data (general data), a proxy for the prevalence in the general population of the same EU Member States in the period 2013–2019.

Based on the model output, animals from the NSHC stream show a 48% excess risk (PR: 1.48; 95% CI: 1.08–02) of being infected/detected, compared with the SHC stream. One possible explanation for this finding is the potential confounding effect of age, which is a known risk factor for AS. However, the absence of data on age prevented any possibility to adjust for this potential confounder within the analysis.

3.4. Design prevalence

The estimates of the design prevalence for the subset of the flocks and herds included in the final data sets extracted following the criteria described in Section 2.1, for Year 1, Year 2 and Years 1 + 2 are shown in Table 5.

In general, a design prevalence of 10% was achieved by 34.2% and 35.1% of flocks with a sheep as the index case in both year 1 and Year 2, respectively, and by 48.4% and 47.8% of the herds with a goat as the index case, respectively. It is important to note that the design prevalence was only reported for a subset of flocks/herds that represents no more than half of the total population in the data set, but for which the numbers allowed its calculation. If we consider the entire data set, the proportion of holdings in which the design prevalence is 1%, 5% or 10% would be much lower.

If we assume that the prevalence of AS, if transmissible, is expected to be low or very low, the sensitivity of the intensified monitoring to detect a low prevalence is very limited when applied to flocks and herds with index cases of AS; more so in flocks with sheep index cases than in herds with goats as index cases. The sensitivity of the intensified monitoring declines in Year 2 compared with that of Year 1 in terms of the number of flocks/herds with a design prevalence of 1% or lower.

For some of the holdings, the lack of data was explained by the reporting countries, e.g. when the flocks/herds were culled, but for others it is unknown. However, this lack of data for certain holdings does not preclude the conclusion that, whether the testing was conducted in Year 1, Year 2 or both, the sensitivity of the surveillance system to detect cases is very low and is insufficient to detect secondary cases, given the observed prevalence in flocks/herds with secondary cases, with some exceptions (see flocks 23, 24, 25 and 26 in Figure 3) in which the level of testing was large enough to

have a low design prevalence. However, the crude prevalence in most of the flocks is lower than the design prevalence achievable by the testing efforts in most of the flocks.

Table 5: Design prevalence of the subset of the flocks/herds with index cases as atypical scrapie

	Sheep			Goats		
	Year 1	**Year 2**	**Year 1 + Year 2**	**Year 1**	**Year 2**	**Year 1 + Year 2**
Number of flock/herd IDs in the data set	742	742	742	76	76	76
Flocks/herds with index cases before 1 January 2013	64	64	64	1	1	1
Flocks/herds with no size and no animals tested	15	102	13	14	34	14
Flocks/herds with imputed size	20[(d)]	35[(e)]	15[(f)]	1[(a)]	4[(b)]	3[(c)]
Flocks/herds with < 10 animals	60	26	8	12	0	12
Flocks/herds with < 3 sheep/goats tested	179	212	168	18	18	15
Number of flocks/herds for calculation of design prevalence	424 (57.1%)[(g)]	339 (45.7%)	489 (65.9%)	31 (40.8%)	23 (30.3%)	34 (42.1%)
Number of flocks/herds with a design prevalence 1%	17 (4%)[(h)]	18 (5.3%)	35 (7.1%)	5 (16.1%)	2 (8.7%)	6 (17.6%)
Number of flocks/herds with a design prevalence 5%	85 (20%)[(h)]	65 (19.2%)	134 (27.4%)	10 (32.2%)	7 (30.4%)	13 (38.2%)
Number of flocks/herds with a design prevalence 10%	145 (34.2%)[(h)]	119 (35.1%)	232 (47.4%)	15 (48.4%)	11 (47.8%)	16 (47%)

(a): One herd was assigned the herd size equal to the number of goats tested in Year 1.
(b): Four herds were assigned the herd size equal to the herd size in Year 1.
(c): One herd was assigned the herd size equal to the size in Year 1 and three herds equal to the number of animals tested in Years 1 + 2.
(d): Twenty flocks were assigned the flock size equal to the number of animals tested in Year 1.
(e): Nine flocks were assigned the flock size equal to the number or animals tested in Year 2 and 26 flocks equal to the flock size in Year 1.
(f): One flock was assigned the flock size equal to the size in Year 1 and 14 flocks equal to the number of animals tested in Years 1 + 2.
(g): The percentage of all flocks/herds in the data set.
(h): The percentage of all flocks/herds used for the calculation of the design prevalence.

Comparison of design prevalence between countries with/without secondary cases of AS was conducted. The purpose was to assess the ability of the intensified surveillance to detect secondary cases between two groups of countries by testing if there is a statistically significant difference in the proportion of flocks/herds IDs with design prevalence < 1%, < 5% and < 10%. Pairwise comparisons (two-sample test for equality of proportions with continuity correction) were performed testing the hypothesis that the proportion of flocks/herds in each category of the countries with secondary cases in sheep (8) is less than, not equal to or greater than, the proportion of flocks/herds in each category of the countries without secondary cases in sheep (12).

Overall, it can be concluded that the ability of the intensified surveillance to detect secondary cases was not different between countries with/without secondary cases, except for the case of the design prevalence < 1% in Year 1. The results showed that the proportion of flocks with a design prevalence < 1% was significantly higher in the group with secondary cases ($p = 0.02$). The design prevalence for the subset of flocks with/without secondary cases is displayed in Table 6.

Table 6: Design prevalence of the subset of the flocks with sheep index cases as atypical scrapie in countries with/without secondary cases

	Year 1		Year 2		Year 1 + Year 2	
Number of flocks/herds for calculation of design prevalence	424		339		489	
	With secondary case/s	Without secondary case/s	With secondary case/s	Without secondary case/s	With secondary case/s	Without secondary case/s
	316	108	252	87	360	129
Number of flocks/herds with a design prevalence 1%	17 (5.4%)	0	12 (4.8%)	6 (6.9%)	24 (6.7%)	10 (7.7%)
Number of flocks/herds with a design prevalence 5%	58 (18.3%)	27 (25%)	44 (17.5%)	21 (24.1%)	95 (26.4%)	39 (30.2%)
Number of flocks/herds with a design prevalence 10%	108 (34.2%)	37 (34.2%)	81 (32.1%)	38 (43.7%)	170 (47.2%)	62 (48.1%)

3.5. Uncertainty associated with the prevalence comparison

Table 7: Sources of uncertainty associated with the comparative analysis and their possible impact on the estimates obtained

Source of uncertainty	Cause of the uncertainty	Impact of the uncertainty on the conclusions
Mandate data for goats	Due to the lack of secondary cases in the goat data set, there was no possibility to conduct a comparison of the prevalence of AS in the non-infected herds (general data) and infected herds (mandate data)	It is not possible to test whether the prevalence of AS in infected herds is higher than in non-infected herds. The patterns may or may not be similar to sheep flocks
Surveillance (Regulation 999/2001)	The surveillance implemented in small ruminants in the EU is not intended to and does not allow the estimation of the true prevalence of AS at country level, nor at flock/herd level. The implementation of the intensified monitoring is quite heterogeneous across countries and flocks in terms of number of animals tested in the different surveillance streams and years. E.g. based on the number of breeding animals, the percentage of animals tested as fallen stock is very small. The results showed a non-significant higher prevalence of AS in infected flocks.	If the intensified monitoring was implemented in full according to the legislation and more animals were tested in infected flocks and herds, the comparison of prevalence could have the power to detect a significant higher prevalence of AS in infected flocks and herds
Surveillance (Regulation 999/2001)	According to the legislation, 'the competent authority shall collect the appropriate tissues, according to the available scientific advice and the guidelines of the EU reference laboratory, to ensure the detection of all known strains of TSE in small ruminants'. For the detection of AS, it is recommended to sample cerebellum as well as brainstem to maximise the diagnostic sensitivity of the surveillance. It is unknown if this has been consistently carried out, or carried out at all, in all animals tested, surveillance streams and countries.	The sampling of brainstem only could lead to under-detection of index cases of AS in non-infected flocks/herds and in the number of secondary cases detected in infected flocks/herds. This could result in underestimation of the prevalence of the disease in the sampled populations for one or both data sets
Mandate data age and genotype	Both genotype and age are known individual risk factors for the occurrence of AS. In both data sets used for the analysis, there was no genotype or age distribution information available. Therefore, it was not possible to adjust the estimation of the prevalence for their potential confounding effect.	Potentially different genotype and age distributions between the mandate data and the general data could bias the output of the prevalence comparison in either direction
Impact of the mandate data on design prevalence	The proportion of flocks/herds suitable to use for the design prevalence calculation was approximately 50%, due to the missing data on tested animals, number of breeding animals and the limitations of the calculations to flocks/herds with < 10 animals and ≥ 3 tested. The analysis showed that the percentage of flocks able to detect secondary cases is very low. The actual percentage of flocks/herds with a design prevalence $< 1\%$, 5% and 10% could be higher/lower than the estimate.	The sensitivity of the intensified monitoring to detect secondary AS cases could be higher/lower
Impact of the mandate data on design prevalence	The analysis of the data does not support (except in one case) the hypothesis that the flocks in the countries with secondary cases had proportionally more animals tested (measured by the design prevalence) than the flocks in countries without secondary cases.	There could be differences in the ability of the intensified monitoring of flocks with/without secondary cases to detect them

3.6. Within-flock transmission model of AS

3.6.1. Overview of model and key assumptions

Overall, this approach involves building a simulation model for the dynamics of AS in sheep in a closed flock and fitting it to the intensive monitoring data. An overview of the model processes is given in Figure 4, with more details given below. All processes are carried out on a yearly time step. Flocks with 1 year of surveillance only or without a flock size for both years were excluded. The same applied to four flocks with no tested sheep in the other year, and one flock where there was no flock size in either year. Flocks with secondary cases in SHC and NSHC were used in the model.

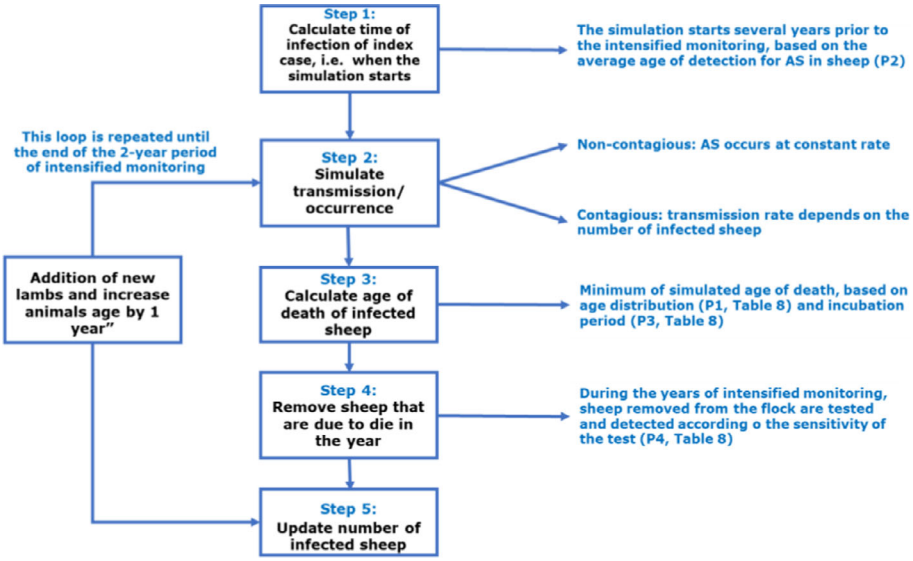


Figure 4: Overview of a simulation model of within-flock transmission of atypical scrapie in sheep for both the scenarios where it is contagious and non-contagious

Step 1: Calculate time of infection of the index case in the flock

The initial step in the model is to determine when to start the simulation of transmission, as it is likely that most flocks will have been infected for several years before an infected sheep being detected. This was based on the distribution of the age at detection of AS in sheep across the EU, and was found to best fit a Weibull distribution (P2, Table 7), and for each iteration of the ABC routine, the start time of each simulated AS outbreak is randomly drawn from the Weibull for each flock.

Step 2: Simulate transmission/occurrence

Once initiated, the within-flock transmission model simulates the processes of lambs being born, potentially becoming infected and, if so, potentially infecting other sheep during their life. In terms of the calculation of the number of infected sheep each year, two approaches have been taken depending on whether AS is assumed to be contagious or non-contagious:

1) AS is contagious.

Each year the number of new infections is simulated via the Reed–Frost model from a binomial distribution. The model assumes that animals are infected in their first year of life. This assumption is based on the higher susceptibility of lambs vs. adults for CS.

2) AS is non-contagious.

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.

Step 3: Calculate age of death of infected sheep

Infected sheep are allocated a year of death, which will be the earlier between: (i) its age of death in the absence of AS, based on the age distribution (P1 Table 8, Figure 6A); and (ii) its age at clinical onset (P3 Table 8), which will also influence the likelihood that they test positive (P4, Table 8).

Step 4: Remove sheep that are due to die in the year

Infected sheep are removed from the flock before the intensified monitoring if they die or reach clinical onset before it starts. For each year of the intensified monitoring, the prevalence of infected sheep exiting the flock is calculated, along with the probability of detection of each of those cases (based on time before clinical onset).

During the years of intensified monitoring, the model simulates the number of positives observed. This is calculated using a binomial distribution where p = the proportion of positive sheep exiting the flock and n = the number tested (sum of healthy slaughter and fallen stock). Calculating the proportion of positive sheep is performed in the following steps:

1) For each positive sheep exiting the flock that year, calculate the test sensitivity (depends on years before onset) and then whether it is detected or not.
2) Calculate the number of sheep exiting the flock (replacement rate × flock size).
3) Calculate the proportion of positive sheep exiting the flock, which is (1)/(2).

Step 5: Update number of infected sheep

The number of infected sheep for the next step will be the number of infected sheep at the beginning of the time step, minus those that are removed in the year, plus new infections that year.

Example of the within-flock transmission model

The simulation starts by calculating how long the infection has been in the flock before the detection of an infected sheep that triggers the intensified monitoring, assuming there was only one initial infected animal. So, Step 1 of the model consists of sampling from the Weibull distribution (P2, age at detection). Supposing this is 6 years, then the simulation will be initiated with one initial infected animal (the index case) 6 years before the start of the intensified monitoring (Figure 5).

This infected animal (as with all infected animals considered in the model) will then have its age of exit from the flock calculated (within Step 3 of the model). This will be the minimum of its age of exit based on its simulated age of death (P1, age distribution) and its incubation period (P3, incubation period of AS). Supposing that the former would be 6 years and the latter 7 years, the age of exit from the flock of this animal would be 6 years.

Within Step 2, the transmission or occurrence of new cases is simulated, for every subsequent year, with the two different scenarios (contagious, non-contagious). The simulation could lead e.g. to 0 new cases in Years 1 and 2 (as the transmission/occurrence rate is low) and an additional case in Year 3.

Therefore, during Year 3 of the model, there will then be two infected animals (the original case and the new case). Supposing that the age at which this second positive animal exits the flock is simulated to be 5 years old, based on its simulated age of death, this means that it will remain in the flock until the second year of intensified monitoring.

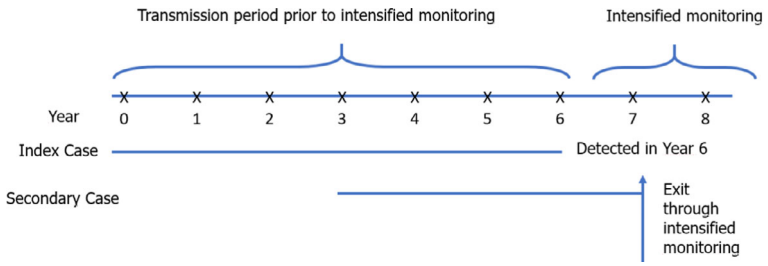


Figure 5: Overview of a timeline of an example of within-flock transmission of atypical scrapie

During the years of the intensified monitoring, the model calculates the proportion of positive sheep exiting the flock. So, in this example, in the first year of the intensified monitoring (Year 6 in Figure 5), as there are no infected sheep exiting the flock, it will be 0%. However, in the second year (Year 7 in Figure 5), there is one infected sheep exiting, as the second infected sheep is due to exit at age 5 in

Year 8 of the simulation. The model first calculates the number of detected positives, so it will look at how long before the end of the incubation period the animal is, in this case it will be 2 years. The sensitivity of the test at 2 years prior to onset (based on CS data, Table 8) is 75%, so there will be a 75% chance the animal has a positive test outcome (a lower probability of detection is considered as part of the sensitivity analysis). If positive, then the proportion of infected sheep exiting the flock will be 1, divided by the number of sheep exiting the flock (replacement rate (0.202) multiplied by flock size). The actual number of detected positives will then be drawn from a binomial distribution with n = number tested in intensified surveillance, and p = proportion of positives exiting the flock.

For the model fitting, the model simulates the number of positive sheep in all of the flocks fulfilling the selection criterion for each year when there were animals tested, and it simulates this for both scenarios (contagious, non-contagious). The model then calculates the error between the model output and the observed data; this is based on the sum of the square errors for the difference between the sum of cases in the model and the observed data for each of the 2 years.

The process of generating final parameter estimates consists of (i) setting ranges for the transmission rate/rate of occurrence; (ii) selecting iterations with an error less or equal to that of an established threshold; (iii) summarising the estimates of the transmission/occurrence rate for each scenario; and (iv) comparing the estimates distributions for the contagious and non-contagious scenarios.

The estimation of the error for the j iteration for both the contagious and non-contagious scenarios is calculated as follows:

$$E_{c(j)} = |m_1 + m_2 - o_1 - o_2|,$$

where

m_1, m_2 is the expected detected cases in Years 1 and 2.

o_1, o_2 is the observed detected cases in Years 1 and 2.

N is the number of flocks and j = 1 … 10,000.

Only the iterations less than a chosen threshold of tolerance for error δ (difference between simulated and actual number of cases $\delta \leq 15$) are selected for each scenario

$$E_c(j) \leq \delta,$$

$$E_{Nc}(j) \leq \delta.$$

The mean $\bar{\tau}_c$, 2.5th and 97.5th percentile of the transmission and occurrence rate for the selected iterations are the last estimation of the rates for each scenario.

For the contagious scenario, the calculation of the transmission rate follows the Reed–Frost model:

$$p_\tau = 1 - (1 - \tau)^{\text{infected}},$$

where

p_τ is the probability of a susceptible animal (sheep < 1 year old) becoming infected each yearly time step,

τ is the transmission rate (probability that one animal transmits the disease to another animal in one time step), and

the number of new infected animals each time step follows a binomial distribution (sheep < 1 year old, p_τ).

For the non-contagious scenario, occurrence rate: $p_\mu = \mu$.

Table 8: Key data requirements for model used to simulate atypical scrapie surveillance data and infer whether atypical scrapie is transmissible

Parameter	Description	Value	Source
P1	Age distribution	Sheep flock population of GB (Figure 6A). Results in a replacement rate of 20.2%	Arnold et al. (2002)

Parameter	Description	Value	Source
P2	Years before intensified monitoring when within-flock transmission model starts the simulation	Age at detection (months)~Weibull (95.8, 2.3)	Informed by age of detection of AS in EU Member States (EFSA, 2020)
P3	Incubation period of AS	Lognormal (4.47, 0.3) (years) (Figure 6C)	Estimated from UK atypical positive fallen stock data (Ortiz-Peláez et al., 2016) updated using age distribution P1
P4	Sensitivity of diagnostic test vs. time before end of incubation period	Based on estimated incubation period (Figure 6C) and experimental data for CS. This results in 98% sensitivity for up to 12 months before onset, 75% for 13–24 months, 38% for 25–36 months and 13% for 37–48 months. For sensitivity analysis, an alternative lower sensitivity assumption was used, which was 25% sensitivity for < 1 year before onset, and 0% for > 1 year.	Arnold and Ortiz-Pelaez (2014)

R version 3.6.1 and the package EasyABC was used to implement the simulation model. The R code and the input data set to reproduce the results can be accessed at the following weblink: https://doi.org/10.5281/zenodo.4980002.

The package required a function that simulates the model with a summary outcome. The user specifies the proportion of iterations closest to the observed value that are to be retained for further analysis. The summary outcome was the total number of detected infected sheep in both years of intensified monitoring.

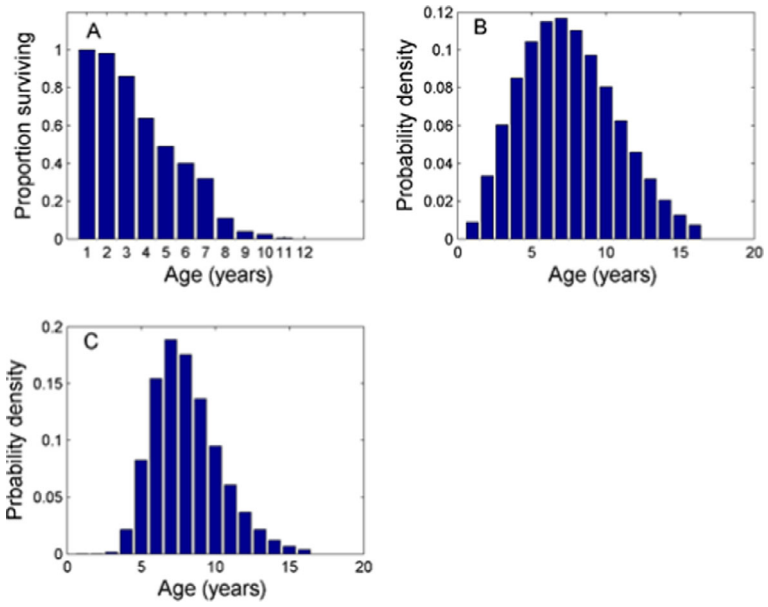


Figure 6: Plot of the distributions used in a within-flock transmission model of atypical scrapie for: (A) the survival probability by age; (B) the age at detection; and (C) the incubation period

3.6.2. Results

There were 28 secondary cases and 375 flocks that fulfilled the criteria described above using a threshold of a maximum difference of 15 between the model and observed total number of cases for the intensified surveillance, the following estimates were obtained: a transmission rate 5×10^{-4} (95%

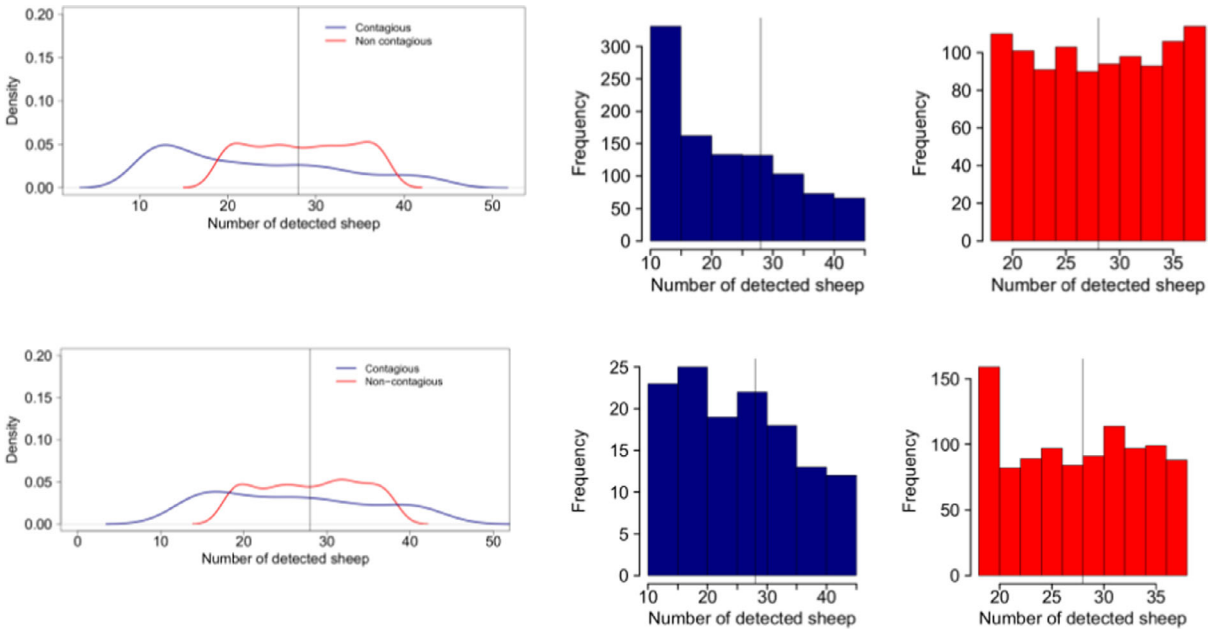
CI: 1.7×10^{-4}–1.1×10^{-3}) for the contagious scenario and 2.8×10^{-3} (95% CI: 1.16×10^{-3}–54.9×10^{-3}) occurrence rate for the non-contagious scenario.

The model produced a better fit for the non-contagious scenario than for the contagious scenario. This can be seen from the top row of Figure 7, which shows results using the closest 1,000 iterations to the observed value in each scenario for Year 1 and for Year each. There was a much greater variability in the number of simulated detected sheep from the contagious scenario than the non-contagious one, which resulted in far fewer iterations being within the specified threshold of the total number of positives. In contrast, all iterations in the non-contagious scenario were within 10 sheep of the observed real value. The bottom row in Figure 7 includes only iterations within 15 sheep of the actual number of detected sheep in the intensified monitoring period.

An indication of the lack of 'goodness of fit' by the contagious scenario is evident from the finding that only 132 iterations out of 10,000 iterations (1.3%) resulted in a simulated total number of detected positives close to the observed number ($\delta = 15$), whereas 1,578 of the non-contagious model iterations (15.8%) were within the 15 threshold. This lack of fit arises from the variability of predicted number of cases under similar transmission rates.

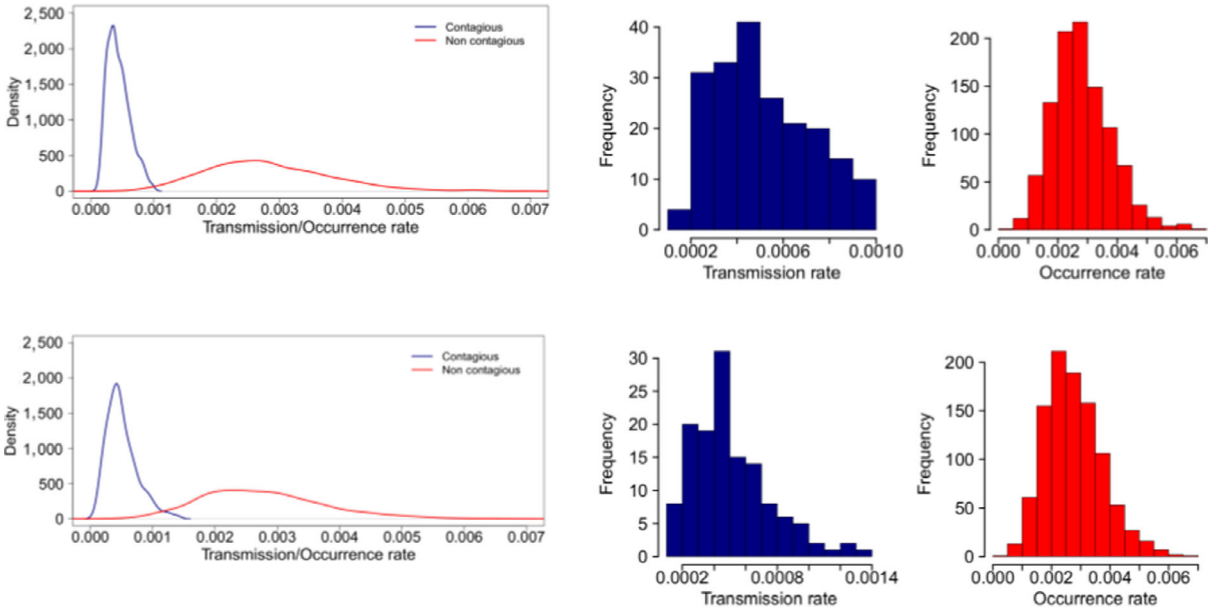
The top and bottom of Figure 7 show that the number of predicted cases is similar to those with absolute numbers of cases (28, vertical line in both graphs). The transmission rates in the contagious scenario that could produce values close to the actual data, presented a skewed and narrow distribution with very low transmission rates. The estimates of the occurrence rate in the non-contagious scenario followed a more spread distribution with long tails and a 5% upper boundary of 0.0054 occurrence rate (Figure 8). The uncertainty in the occurrence rates might be associated with the data quality or the low sensitivity shown by the intensified monitoring. Various estimates could provide similar accuracy.

In summary, the results of the model are more supportive of the non-contagious scenario based on the better fit of the model. However, a small proportion of iterations of the contagious scenario (1.3%) resulted in a simulated number of cases close to the observed ones, which implies that AS may behave as a contagious disease with a very low transmission rate (with a 95% upper boundary of the transmission rate of 1.1×10^{-3}).



The vertical line in the density plot marks the observed number of secondary cases in both Year 1 and Year 2 (28). The top row includes the 1,000 closest iterations to the observed value. The bottom row includes only iterations resulting in a deviation of ± 15 sheep from the observed 28 sheep.

Figure 7: Density (left) and frequency (right) distribution of the number of cases predicted by the model in the contagious scenario (blue) and in the non-contagious scenario (red) in Year 1 and Year 2 of the intensified monitoring



The top row includes the 1,000 closest iterations to the observed value. The bottom row includes only iterations resulting in a deviation of ± 15 cases from the observed 28 cases.

Figure 8: Density (left) and frequency (right) distribution of the transmission rate for the contagious scenario (blue) and the occurrence rate for the non-contagious scenario (red) in Year 1 and Year 2 of the intensified monitoring

The model was also applied to estimate the proportion of predicted cases that could be detected with the implemented intensified monitoring looking at onset of disease in cases at the beginning of the intensified monitoring (Year 1). Two scenarios were performed applying the two rates estimated by the model, and the outcomes were:

- With the estimated average transmission rate for the contagious scenario, at the beginning of the intensified monitoring, 52.2% (95% CI: 27.2–78.4) of the infected sheep would reach onset within a year, 2.1% (95% CI: 0.6–5.2) within 2 years and 4.4% (95% CI: 1.4–8.6) within 3 years, when the sensitivity of the test decreases sharply. On average, we would expect to detect a maximum of 54.4% of the infected sheep because of the limited testing sensitivity. Although the confidence interval for the proportion of sheep within less than 12 months to onset is wide. In the pessimistic scenario, this average upper boundary would decrease to 13.1%.
- With the estimated occurrence rate for the non-contagious scenario, at the beginning of the intensified monitoring, 39.71% (95% CI: 36.8–42.7) of the infected sheep would reach onset within a year, 4.36% (95% CI: 2.89–6.23) within two years and 6.66% (95% CI: 5.02–8.74) within 3 years. At least 55.3% of the infected sheep were expected to be missed in this scenario; 90.1% would be missed in the pessimistic scenario in which only 25% of sheep within 12 months to onset can be detected.

The simulation also estimated the percentage of infected sheep that are expected to die within the two years of intensified monitoring. In fact, 68% (95% CI: 64.8–71.9) of infected sheep were expected to die in the first year of the intensified monitoring and 10.5% (95% CI: 7.9–12.7) in the second year. The overall sensitivity of the intensified monitoring was low because infected sheep may not be selected for testing at the abattoir, not all dead sheep were tested and the sensitivity of the sampling was expected to be low as sheep can only be detected towards the end of the incubation period.

3.6.3. Sensitivity analysis of key parameters

The effect of (i) varying the sensitivity of the diagnostic test relative to the time before clinical onset and (ii) varying the age distribution on both the conclusion that the non-contagious assumption

for AS provided a better match to the data and on the estimates of the transmission rate, was explored.

3.6.3.1. Test sensitivity

The alternative assumption of test sensitivity was used: 25% of infected animals detected in the last 12 months of the incubation period, and none detected > 12 months before clinical onset. This represented a pessimistic estimate in contrast with the one presented in Table 8, which was a very optimistic estimate. Results similarly showed a superior fit of the model assuming that AS was non-contagious. The transmission rate estimate was 9.7×10^{-4} (95% CI: 2.2×10^{-4}–1.9×10^{-3}). The estimated rates for the scenario with an optimistic sensitivity of the diagnostic test are similar, because the distribution of the predicted number of sheep is right-skewed. The rate of occurrence estimate was 2.2×10^{-2} (95% CI: 0.81×10^{-3}–4.1×10^{-2}). The estimates are higher as the sensitivity of the surveillance system decreased significantly.

3.6.3.2. Age distribution

An alternative age distribution was used based on data from a subset of Italian sheep flocks recorded at the end of 2020 (the dairy Sarda breed in the Sardinia region,[12] as follows: total of 72,934 sheep < 12 months; 52,606 between 13 and 24 months; 66,384 between 25 and 36 months; 111,423 between 37 and 60 months; 142,266 between 61 and 96 months; and 77,743 > 96 months. The alternative age distribution used in the sensitivity analysis was generated from this data assuming an equal number of sheep aged 25–36 and 13–24 months, a maximum age of 13 years and exponential decline from age 3 years onwards. This adjustment was necessary because the age group 13–24 months is unlikely to be smaller than the age group of 25–36 months. This feature may reflect either random annual variation or the results of variations in breeding due to contingent economic reasons. The age distribution of the Sarda breed, with a greater proportion of older sheep than that used in the baseline scenarios provided an alternative to explore the impact of the changing of the age distribution.

Results for the fitting of the simulation model to the observed data with the alternative age distribution showed a superior fit of the model when assuming that AS was non-contagious, with an estimate of 2.2×10^{-3} for the rate of occurrence (95% CI: 7×10^{-4}–4.2×10^{-3}).

3.6.4. Concluding remarks

Overall, the model fitting exercise indicates that AS behaving more like a non-contagious disease is more consistent with the observed data than AS behaving like a contagious disease. It also indicates that changes in key parameters (age distribution and diagnostic test sensitivity) do not influence this conclusion but does affect the rates of transmission/rates of occurrence. This is especially the case for the diagnostic test sensitivity, which is itself highly uncertain, and the pessimistic estimate of sensitivity produced estimates of the rate of occurrence approximately 10-fold that of the more optimistic estimates of test sensitivity.

Given the uncertainties in the parameters, the necessity of using a model with simplified assumptions and the method/s applied to estimate the fit of the model to the observed data, the estimated transmission rates/occurrence rates and the conclusion on the preferred scenario (contagious vs. non-contagious) need to be interpreted with caution. It is not possible to conclude definitively that AS is non-contagious on the basis of the ABC model results. It is also not possible with this model to attempt to fit a combination of direct animal transmission and natural occurrence of AS because there is insufficient power in the data.

An example of the effect of a key feature of AS that has not been accounted for in the model is the variable susceptibility by genotype. Inclusion of genotype could significantly influence model behaviour. E.g. if only a small proportion of a flock was of the more susceptible genotypes, then after these became infected, infection would be likely to die out within the flock. However, the current model would predict infection continuing to spread as it assumes all animals are equally susceptible.

[12] Italian Sheep Registration and Identification database https://www.vetinfo.it/j6_statistiche/#/report-pbi/89

3.6.5. Uncertainty associated with the simulation model

Table 9: Sources of uncertainty associated with the simulation model and their possible impact on the estimates obtained

Source of uncertainty	Cause of the uncertainty	Impact of the uncertainty on the conclusions
Number of initial infected sheep in each flock at start of model simulations	No way to determine the historical number of infected animals in each affected flock	It is possible that there would be multiple infected sheep in some flocks at the point at which the model starts simulating transmission. This would mean that a lower transmission rate would be required for the model to fit the observed data. It is unlikely to change the conclusion on the better fit to the data of the non-contagious model, as the presence of more infected sheep would not change the pattern of the transmission model having more positive sheep Year 2 compared with Year 1.
Sheep are only susceptible in their first year of life.	Experimental studies, using CS only, have been able to determine that adults are less susceptible than lambs, but not able to infer any quantitative estimates of relative risk of infection by age. There are no data on age susceptibility to AS challenge/exposure.	Susceptibility of adults would require an adaptation of the incubation period distribution, making it shorter to fit the surveillance data from which it was estimated. This would increase the sensitivity estimates of the test, lowering estimated transmission rates. However, it would be unlikely to influence the conclusion that the non-contagious model fitted the data better than the contagious one.
Impact of genotype	There is no genotype information available for the sheep flocks in the data set. The model assumes all sheep in infected flocks are equally susceptible to AS.	The lack of genotype specific information on the flocks means that the model will overestimate the number of susceptible sheep in each flock. In some flocks, there could be an impact of a reduction in the susceptible population over time if a significant proportion of the susceptible sheep become infected. It is difficult to rule out the possibility that inclusion of genotype would enable the model to produce a considerably better fit to the data than the current model without genotype information. Therefore, conclusions on the non-contagious model fitting better need to be interpreted with caution.
Mandate data for goats	Due to the lack of secondary cases in the goat data set, there was no possibility to fit the simulation model to the observed goat data	It was not possible to test which of the two options (contagious vs. non-contagious) tested by the simulation model fits better the goat data.
Model fitting metric for the approximate Bayesian computation approach	The metric used to calculate the error between the model output and the observed data can have an impact on the findings of the model. It is possible in this case, given the low power of the data, that this could have an effect on the comparison between the contagious and non-contagious models.	This factor makes the conclusion on the superior fit of the non-contagious model more uncertain.

3.7. Knowledge gaps on the epidemiology of AS

Section 3.1 of this report provides an overview of the aspects of the clinical presentation, pathogenesis, pathology, genetics, transmissibility and epidemiology of AS, also in comparison with the features of CS. In particular, Section 3.1.5 reviews aspects related to the epidemiology of this disease. From this section it is clear that some data gaps remain in the knowledge on the occurrence and characteristics of AS and related risk factors.

As requested in the ToRs, this report is focused on the analysis of a specific data set, i.e. the data from the EU intensified surveillance carried out in AS-infected flocks and herds between 2013 and 2019. The analysis of this data set confirms and provides additional knowledge on some aspects of the epidemiology of AS, which are described in Section 3.7.2 below. However, given the nature of the data provided, many other aspects cannot be clarified, and remain as open questions. These are listed in Section 3.7.3.

3.7.1. The nature of the mandate data

Some considerations should be made regarding the data set analysed before discussing to what extent its analysis can lead to filling the current data gaps.

The mandate data are not intended to be a full data set allowing comprehensive analysis. They are based on a retrospective investigation, restricted to those infected flocks/herds identified through surveillance. No data have been obtained from a suitable 'control' group of e.g. non-infected flocks. Moreover, no *ad hoc* questionnaires have been administered to collect high-resolution data at flock level and only data that are known to be held in national databases were requested. By definition, data were suitable for hypotheses formulation but not for hypothesis testing on risk factors of AS. No data on potentially relevant covariates such as genotype or age at individual, flock and population levels were available.

The data set analysed is based on the heterogeneous implementation of intensified monitoring, according to the legislation, and only collected for the mandate over a short time frame and considering only the surveillance data that EU countries usually hold. Moreover, the duration of the intensified monitoring was not long enough to fit a model that simulates the transmission within flocks of AS over a longer period of time. In fact, the 2-year period is the bare minimum to simulate any within-flock transmission: between Year 1 and Year 2.

The data set collated by the European Commission provided robust data that allowed the description of the occurrence of AS in infected flocks and herds. However, the data set is not complete in terms of the requested fields, especially for some of those that have been used in the analyses, such as the number of breeding animals and the number of animals tested during the 2-year period. This required some assumptions to be made in the analysis, as discussed previously.

3.7.2. Answers to knowledge gaps from the analysis of the mandate data

This has been the first attempt to analyse the data from the intensified monitoring undertaken by EU, and other reporting countries implementing small ruminant surveillance as per Commission Regulation (EU) 999/2001, since its enforcement in 2013. This intensified monitoring had limited sensitivity to detect AS cases in infected flocks/herds (see Sections 3.4 and 3.6.4). The analysis undertaken informed some considerations with regard to the epidemiology of AS:

- The descriptive analysis of the general and mandate data sets has confirmed that AS is a type of TSE that is widespread in Europe, affecting most of the EU countries with medium- to large-scale sheep populations. For goats, the pattern is similar, although the number of countries with cases is smaller, probably due to the different distribution and abundance of goats in Europe (goats are bred in fewer countries and the populations are smaller), and the prevalence of index cases is in general lower (seven times lower) than in sheep.
- The results of the analysis confirmed that multiple cases of AS overlapping in time in the same holding is a rarely detected event in the European small ruminant population.
- The mandate data have revealed exceptions to this pattern, such as a Hungarian holding in which seven secondary cases, three in Year 1 and four in Year 2 were detected. The size of this holding (12,970 sheep) and the level of testing Year 1 and Year 2: 1,143 and 3,102 tested, respectively, resulted in a prevalence of 2.6×10^{-3} in Year 1 and 1.3×10^{-3} in Year 2. A second Hungarian holding in which two secondary cases were detected in Year 1 (2,805 tested)

resulted in a prevalence of 7.1×10^{-4}. The prevalence levels in these two holdings with multiple cases (> 1) lead to speculation about the level of occurrence of AS as a sporadic disease and the ability to detect secondary cases: 7–10 cases per 10,000 animals. In flocks/herds of standard size (< 500), it would be extremely unlikely (1–5%) to detect a secondary case in surveillance, should it be present in the flock, even if assuming an equal prevalence in the NSHC and SHC streams.

- In countries with secondary cases, the observed prevalence of AS in infected sheep flocks is consistently higher than in the general surveillance population: after combining the testing of the SHC and NSHC streams, there was a PR of 1.56, higher in infected flocks in all countries considered in the analysis, but despite this trend the difference was not statistically significant. If the PR was significant, there would be a need to consider the hypothesis that in the infected flocks/herds there is/are risk factor/s that increase the probability of AS compared with the non-infected flocks/herds, either at host level (genotype, age) or at flock level (see next section).

3.7.3. Remaining knowledge gaps

A list of questions on aspects of the epidemiology of AS that are unknown, or for which there are not complete answers, has been compiled by the Working Group. After the analysis of the mandate data and the answers to ToR1, the following questions remain unanswered:

- Are there risk factors at population level for AS? Are certain countries/regions/breeds/husbandry systems more likely to be associated with higher incidence/prevalence of AS than others, and if so, why?
- Why does Portugal have such a high prevalence of AS in sheep in the period 2013–2019 in the SHC stream (9.8 cases per 10,000 tests; 95% CI: 7.3–13) and the NSHC stream (12.7 cases per 10,000 tests; 95% CI: 10.6–15), relative to any other EU country?
- Effect of individual risk factors on population risk factors: does a different genotype distribution in the general population make certain countries more likely to have AS cases?
- Are there risk factors at an individual level, other than age and genotype? Are there any individual risk factors that make certain individuals more likely to acquire the infection and develop the disease?
- Is age a confounding factor that explains the higher probability of detecting AS in the NSHC stream?
- Can the within-flock frequency of susceptible genotypes explain the observed multiple cases in the two Hungarian flocks or is the high sampling level in those two flocks the reason for the detection of multiple cases?
- If AS is not contagious, what is/are the origin of, and risk factors for, the disease?
- If the disease is due to an endogenous mutation/conversion of PrP^{C} into PrP^{Sc}, what are the risk factors triggering the conversion (e.g. age, health status, genotype, concomitant diseases, etc.)?
- Are there strains of AS circulating in the small ruminant populations of Europe that affect the epidemiological presentation of AS? If so, what is the species susceptibility to different AS strains?
- The prevalence of AS in goats is apparently lower than that in sheep in the EU. The lack of secondary cases in goat herds included in the mandate data, as opposed to the sheep flocks, seems to corroborate this. Is it a real phenomenon or does it reflect other factors, or combinations of factors such as: a lower surveillance sensitivity, a different genotype susceptibility/distribution, demographics/husbandry differences between sheep and goats, a different susceptibility of sheep and goats to AS?
- Are there any polymorphisms in the *PRNP* gene of goats conferring different levels of susceptibility/resistance to AS?
- What is the natural evolution of AS in sheep and goats in field cases, from infection to death due to the disease: incubation period, incubation period vs. longevity/commercial longevity, mortality rates, pathogenesis and clinical profile?
- Is there any association or causal relationship between CS and AS at an individual or population level, or any association with other naturally-occurring TSEs in other species?

4. Conclusions

Based on data gathered from the intensified monitoring carried out in the EU and EEA countries:

- There were 742 flocks from 19 EU countries and Norway in which the index case of AS was a sheep. A total of 41,860 animals from these flocks were tested within the 2-year intensified monitoring period, allowing the identification of 35 AS secondary cases from eight countries. They were all single secondary cases except for two large flocks reporting two and seven secondary cases, respectively.
- There were 76 herds from 10 EU countries and Norway in which the index case of AS was a goat; 4,865 animals from these herds were tested within the 2-year intensified monitoring period, with no AS secondary cases identified.
- With different levels of completeness, the mandate data set contains data on 87.8% and 97.4% of all AS cases reported in sheep and goats, respectively, during the period 2013–2019.
- The mandate data do not allow testing of hypotheses on the epidemiology of AS. No additional data associated with each individual case/infected flock were available, nor were control data from non-infected flocks.

Term of Reference 1: Do the scientific data on the 2-year intensified monitoring collected by the European Commission provide any evidence on the contagiousness of atypical scrapie?

AQ1: *Is the prevalence of atypical scrapie (AS) in the entirety of sheep/goat flocks/herds under intensified monitoring statistically higher than that in the general population of the same EU Member States in the period 2013–2019?*

- A negative binomial regression model was built with data from the 20 countries that reported AS and limited to the slaughtered for human consumption (SHC) and non-slaughtered for human consumption (NSHC) streams.
- The results showed a higher, but not statistically significant, stream-adjusted prevalence of AS in infected sheep flocks (included in the intensified monitoring, i.e. mandate data), compared with that of non-infected flocks (a proxy for the prevalence in the general population of the same EU Member States in the period 2013–2019, i.e. general data).

AQ2: *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?*

- Overall, the results obtained from the simulation model indicate that the non-contagious scenario is more consistent with the observed data than the contagious one. AS in infected flocks behaves more like a disease in which there is a very low and the same probability of any animal to become infected, akin to a non-contagious disease.
- However, and due to the uncertainties and data limitations, conclusions on the non-contagious model fitting better need to be interpreted with caution. A small proportion of iterations of the contagious scenario (1.3%) resulted in a simulated number of cases close to the observed ones, which implies that AS may behave as a contagious disease with a very low transmission rate.

Overall answer to ToR 1

- The intensified monitoring applied to flocks and herds with index cases of AS has limited ability to detect AS, based on the calculated design prevalence and on the model simulation on the detectable AS cases, with no difference between countries with or without secondary cases.
- Countries without secondary cases in sheep, and the absence of observed secondary cases in goats, precluded the replication of the same analyses for all countries and species, but these are notable epidemiological features themselves. Possible explanations of these findings could relate to the absence of spread within infected flocks/herds or an insufficient sensitivity of the surveillance at population level.
- Based on the analyses of the data obtained from the intensified monitoring, and accounting for uncertainties and data limitations, it was concluded that:
    - There is no new evidence that AS can be transmitted between animals under natural conditions.

- It is considered more likely (subjective probability range 50–66%) that AS is a non-contagious, rather than a contagious, disease.

Term of Reference 2: Do the scientific data on the 2-year intensified monitoring collected by the European Commission provide any other new knowledge on the epidemiology of atypical scrapie?

AQ3: *Can any of the identified gaps in the knowledge of the epidemiology of AS be filled by the analysis of the mandate data?*

- The results of the analysis confirmed that:
  - AS is geographically widespread in the EU in sheep and goats, primarily affecting countries with medium–large populations of either sheep or goats.
  - The confirmation of cases of AS in general, and of multiple cases overlapping in time in the same holding, are rare events in the European small ruminant population, with some exceptions in Portugal and Hungary.
  - The AS prevalence rates in goats are lower than in sheep.
- The results of the analysis revealed that:
  - The pattern observed in the countries where the prevalence comparison was possible, showing higher AS prevalence within infected flocks (although not statistically significant), might be compatible either with some transmissibility, exposure to unknown risk factors or sampling bias in the infected flocks.
  - There is an apparent higher prevalence of AS among animals sourced in the NSHC stream than in the SHC stream, that could be explained by the potential confounding effect of age, which is a known risk factor, or by other unknown factors.
- The results of the analyses of the data collected by the intensified monitoring do not provide additional information on several aspects of the epidemiology of AS, including the existence of risk factors other than age or genotype at population and individual level; the possible origin of the disease; any variability of AS strains circulating in the EU small ruminant population and respective susceptibility of small ruminants; the natural evolution of the disease; nor do they offer any reasons for the apparent differences in epidemiology of the disease in sheep and goats.

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Abbreviations

ABC	Approximate Bayesian Computation
AIC	Akaike's information criterion
AS	Atypical scrapie
BIC	Bayesian information criterion
BSE	Bovine spongiform encephalopathy
CS	Classical scrapie
EM	Eradication measures
GALT	Gut associated lymphoid tissue
NSHC	Not slaughtered for human consumption
NSP	National Scrapie Plan
PNA	Palestinian National Authority
PR	Prevalence ratio
SHC	Slaughtered for human consumption
TSE	Transmissible spongiform encephalopathies

Country codes

Austria	AT	Hungary	HU	Poland	PL
Belgium	BE	Iceland	IS	Portugal	PT
Bulgaria	BG	Ireland	IE	Romania	RO
Croatia	HR	Italy	IT	Serbia	RS
Cyprus	CY	Latvia	LV	Slovakia	SK
Czechia	CZ	Lithuania	LT	Slovenia	SI
Denmark	DK	Luxembourg	LU	Spain	ES
Estonia	EE	Malta	MT	Sweden	SE
Finland	FI	Montenegro	ME	Switzerland	CH
France	FR	The Netherlands	NL	The United Kingdom	UK
Germany	DE	North Macedonia	MK		
Greece	EL	Norway	NO		

MS countries: AT; BE; BG; HR; CY; CZ; DK; EE; FI; FR; DE; EL; HU; IE; IT; LV; LT; LU; MT; NL; PL; PT; RO; SK; SI; ES; SE; UK.
Non-MS countries: CH (including Lichtenstein); IS; ME; MK; NO; RS

Appendix A – Country-specific prevalence of AS in sheep

Table A.1: Country-specific prevalence of atypical scrapie (AS) cases in sheep by stream category and source (infected flocks vs. non-infected flocks) NA: not in the mandate data. –: calculation of the prevalence not possible

Surveillance stream	Country	Populations	Prevalence (cases per 10,000 tested sheep)	95% CI
SHC	AT	Mandate data	0	0–380.4
		General data[(a)]	0	0–56.5
	BE	Mandate data	–	–
		General data	–	–
	BG	Mandate data	0	0–124.4
		General data	0.7	0.3–1.7
	CY	Mandate data	NA	NA
		General data	0	0–3.1
	CZ	Mandate data	0	0–431.9
		General data	21.2	1.1–136.7
	DE	Mandate data	0	0–355
		General data	1.3	0.6–2.8
	DK	Mandate data	–	–
		General data	0	0–9453.7
	EL	Mandate data	NA	NA
		General data	2.2	0.8–5.7
	ES	Mandate data	5.74	0.73–45.19
		General data	4.9	3.47–6.93
	FI	Mandate data	0	0–344.8
		General data	0	0–1501.2
	FR	Mandate data	0	0–32.5
		General data	1.6	0.8–3.3
	HR	Mandate data	0	0–6042.7
		General data	0	0–1043.7
	HU	Mandate data	0	0–19.5
		General data	8.3	6.2–11.2
	IE	Mandate data	0	0–29.2
		General data	1.5	0.7–2.7
	IT	Mandate data	0	0–97
		General data	1.8	1–3.2
	NO	Mandate data	6.66	1.66–26.76
		General data	5.37	3.88–7.45
	PL	Mandate data	0	0–53.2
		General data	0.8	0–1.7
	PT	Mandate data	9.82	7.42–12.99
		General data	28.68	8.85–92.51
	SE	Mandate data	–	–
		General data	0	0–332.8
	SI	Mandate data	–	–
		General data	0	0–58.4
	SK	Mandate data	0	0–25
		General data	8.3	0–33.5
	UK	Mandate data	–	–
		General data	4.7	0–7.2

Surveillance stream	Country	Populations	Prevalence (cases per 10,000 tested sheep)	95% CI
NSHC	AT	Mandate data	0	0–236
		General data	3	1.4–6.2
	BE	Mandate data	0	0–9453.7
		General data	0	0–4.3
	BG	Mandate data	0	0–1847
		General data	1	0.5–7
	CY	Mandate data	–	–
		General data	0	0–1.8
	CZ	Mandate data	0	0–307.2
		General data	3.5	1.4–8.1
	DE	Mandate data	0	0–111.1
		General data	4.4	3.1–6.3
	DK	Mandate data	0	0–9453.7
		General data	5.1	0.9–2
	EL	Mandate data	–	–
		General data	2.2	0.9–4.7
	ES	Mandate data	4.27	0.44–41.33
		General data	4.49	3.3–6.09
	FI	Mandate data	0	0–439.8
		General data	7.8	3.6–16.1
	FR	Mandate data	58.14	8.52–385.61
		General data	1.73	1.23–2.45
	HR	Mandate data	0	0–1924
		General data	1.9	0.3–7.7
	HU	Mandate data	11.42	5.71–22.82
		General data	10.13	7.74–13.25
	IE	Mandate data	0	0–159.1
		General data	3	2–4.7
	IT	Mandate data	0	0–283.5
		General data	2.8	1.8–4.3
	NO	Mandate data	21.41	5.2–87.75
		General data	5.92	4.06–8.63
	PL	Mandate data	–	–
		General data	6.7	4.9–9.1
	PT	Mandate data	23.73	7.52–74.68
		General data	12.7	10.71–15.06
	SE	Mandate data	0	0–280.2
		General data	6.2	3.8–9.9
	SI	Mandate data	0	0–1000.1
		General data	5.9	2.6–12.8
	SK	Mandate data	0	0–13.6
		General data	3.8	2.5–5.7
	UK	Mandate data	12.89	4.47–37.07
		General data	6.29	4.95–7.99

(a): Calculated for the period 2013–2019.

Appendix B – Country-specific prevalence of AS in goats

Table B.1: Country-specific prevalence of atypical scrapie (AS) cases in goats by stream category and source (infected herds vs. non-infected herds). NA: not in the mandate data. –: calculation of the prevalence not possible

Surveillance stream	Country	Populations	Prevalence (cases per 10,000 tested sheep)	95%CI
SHC	AT	Mandate data	–	–
		General data[(a)]	0	0–297.6
	BE	Mandate data	NA	NA
		General data	–	–
	BG	Mandate data	NA	NA
		General data	0	0–5.2
	CY	Mandate data	0	0–4023
		General data	0.48	0.025–0.3.2
	CZ	Mandate data	NA	NA
		General data	0	0–3012.5
	DE	Mandate data	–	–
		General data	3.5	0.18–2.3
	DK	Mandate data	NA	NA
		General data	–	–
	EL	Mandate data	0	0–174.2
		General data	2.3	0.4–9.4
	ES	Mandate data	0	0–36.6
		General data	2	1.1–3.7
	FI	Mandate data	NA	NA
		General data	–	–
	FR	Mandate data	0	0–191.6
		General data	0.2	0.01–1.4
	HR	Mandate data	NA	NA
		General data	0	0–5370.6
	HU	Mandate data	NA	NA
		General data	0	0–72.9
	IE	Mandate data	NA	NA
		General data	–	–
	IT	Mandate data	0	0–96.6
		General data	0.67	0.3–1.4
	NO	Mandate data	0	0–8021.1
		General data	0	0–328.3
	PL	Mandate data	–	–
		General data	3.7	0.2–24.3
	PT	Mandate data	–	–
		General data	1.5	0.08–9.8
	SE	Mandate data	NA	NA
		General data	–	–
	SI	Mandate data	–	–
		General data	0	0–187.1
	SK	Mandate data	–	–
		General data	0	0–3711.9
	UK	Mandate data	–	–
		General data	0	0–60.9

Surveillance stream	Country	Populations	Prevalence (cases per 10,000 tested sheep)	95%CI
NSHC	AT	Mandate data	0	0–9453.7
		General data	1.2	0.06–8
	BE	Mandate data	NA	NA
		General data	0	0–12.8
	BG	Mandate data	NA	NA
		General data	0	0–34.9
	CY	Mandate data	0	0–6042.2
		General data	0	0–2
	CZ	Mandate data	NA	NA
		General data	0	0–17.4
	DE	Mandate data	–	–
		General data	0.8	0.04–5.4
	DK	Mandate data	NA	NA
		General data	0	0–60.7
	EL	Mandate data	0	0–1372.7
		General data	3.8	1–12.1
	ES	Mandate data	0	0–20.1
		General data	2.1	1.2–3.7
	FI	Mandate data	NA	NA
		General data	0	0–32.8
	FR	Mandate data	0	0–2407.4
		General data	0.8	0.5–1.4
	HR	Mandate data	NA	NA
		General data	0	0–17.7
	HU	Mandate data	NA	NA
		General data	0	0–57.6
	IE	Mandate data	NA	NA
		General data	0	0–55.4
	IT	Mandate data	0	0–960
		General data	1.5	0.7–3.3
	NO	Mandate data	0	0–827.3
		General data	0	0–19.4
	PL	Mandate data	–	–
		General data	0	0–2.2
	PT	Mandate data	0	0–5370.6
		General data	2.5	0.6–8.1
	SE	Mandate data	NA	NA
		General data	0	0–67.9
	SI	Mandate data	–	–
		General data	3.5	0.2–22.9
	SK	Mandate data	NA	NA
		General data	0	0–37.3
	UK	Mandate data	NA	NA
		General data	0	0–10.7

(a): Calculated for the period 2013–2019.

Annex A – Protocol

Annex A can be found in the online version of this output ('Supporting information' section): https://doi.org/10.2903/j.efsa.2021.6686