Surveillance of **spongiform encephalopathies in small ruminants** in 2014: no classical scrapie outbreaks detected

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Abstract
In 2014, 60,557 goats and 39,954 sheep were tested at the slaughterhouse and during rendering to screen for transmissible spongiform encephalopathies. Five cases of atypical scrapie were detected in sheep and five in goats. No cases of classical scrapie were detected in any goats or sheep. An overview of surveillance since 2002 shows that classical scrapie prevalence continues to fall in both sheep and goats. A drop in atypical scrapie prevalence was also observed in sheep in 2013 and 2014, most certainly due to a fall in diagnostic test performance.

Keywords
TSE, Small ruminants, Programmed surveillance, Clinical surveillance, Prevalence

Résumé
Surveillance des encéphalopathies spongiformes des petits ruminants en 2014 : aucun foyer de tremblante classique détecté
En 2014, 60 557 caprins et 39 954 ovins ont été testés à l'abattoir et à l'équarrissage pour la recherche d'encéphalopathies spongiformes transmissibles : cinq cas de tremblante atypique chez les ovins et également cinq chez les caprins ont été détectés. Aucun cas de tremblante classique n'a été détecté que ce soit chez les caprins ou chez les ovins. Le bilan de cette surveillance depuis 2002 continue de montrer la diminution de la prévalence de la tremblante classique chez les ovins et les caprins. On constate par ailleurs une diminution de la prévalence de la tremblante atypique chez les ovins en 2013 et 2014, très certainement attribuable à une baisse de performance des tests diagnostiques.

Mots-clés
EST, petits ruminants, surveillance active, surveillance événementielle, prévalence

An overview of the surveillance system, its objectives and implementation methods is presented in Box 1.

Results

Number of tests carried out

A total of 100,511 samples were taken in 2014. The objectives of the surveillance programme were achieved for sheep at the slaughterhouse (10,103 samples). However, a high rate of under-performance was observed at the rendering plant for sheep: the target of 40,000 tested sheep was not achieved; the testing rate was 75% (29,851 tests carried out). The statistics for rendering in 2014 indicate however that testing 10% of the small ruminants that died in 2014 (this sampling value had been established by the DGAL) should have led to the 40,000 expected tests being undertaken.

For goats, the threshold of 10,000 expected samples at the slaughterhouse was not reached (there were 8,681 samples). At the rendering plant, 51,876 samples were taken; the exhaustiveness of this sampling could not be verified in the current conditions of traceability in rendering.

That said, the number of samples taken was compliant with the minimum targets established by the European Commission, i.e. 20,000 samples (including at least 5,000 at the slaughterhouse) for each of the two species (40,000 samples requested *versus* 100,000 taken in France).

Slaughterhouses and rendering plants combined, slightly more than 7,500 goat farms (or about 47% of goat farms counted) and 15,000 sheep farms (or around 32% of sheep farms counted) had at least one animal tested in 2014.

Changes in prevalence for classical and atypical scrapie

The prevalence of atypical and classical scrapie (Figure 1) is calculated as the number of atypical or classical cases relative to the number of tests performed (like in previous years, all the tests used in 2014 were able to detect atypical scrapie).

In 2014, as in the previous year, no cases of classical scrapie were discovered by programmed surveillance in sheep, whether at the slaughterhouse or rendering plant. The prevalence of classical ovine scrapie has been on a downward trend since 2002, both at the slaughterhouse (Mann-Kendall trend test p=1.6*10^{-3}) and the rendering plant (Mann-Kendall trend test p=1.9*10^{-5}).

Similarly, in goats, for which no cases of classical scrapie have been found at the slaughterhouse since 2008, no cases of classical scrapie were detected at the rendering plant, for the first year. Thus the prevalence of classical caprine scrapie remains low and is on a downward trend, both at the slaughterhouse (Mann-Kendall trend test p=0.05) and the rendering plant (Mann-Kendall trend test p=1.4*10^{-3}).

In 2014, a total of ten cases of atypical scrapie (sheep and goats combined) were detected by programmed surveillance: there were five goats detected at the rendering plant, four sheep at the rendering plant, and one sheep at the slaughterhouse. All these cases came from farms with different origins.

The apparent prevalence of atypical ovine scrapie has significantly decreased since 2002, both at the slaughterhouse (Mann-Kendall trend test p=4.1*10^{-3}) and the rendering plant (Mann-Kendall trend test p=2.7*10^{-3}). Trends in this apparent prevalence since 2010 were modelled (Box 2), confirming a significant decrease in 2013 and 2014 compared to 2010.

In 2014, the apparent prevalence of atypical caprine scrapie stabilised at a very low level, both at the slaughterhouse and the rendering plant (non-significant Mann-Kendall trend test).

Genotyping in sheep

There is genetic determinism for sensitivity and resistance to scrapie in small ruminants. Homozygous ARR sheep are almost completely resistant to classical scrapie, while the VRQ, ARQ and AHQ alleles have

Box 1. Surveillance and health control measures for TSEs in small ruminants

Objectives
- To determine estimated prevalence of TSEs in small ruminants.
- To detect, as the case may be, any presence of BSE in small ruminants.

The population monitored
Live sheep and goats, animals at rendering plants or intended for human consumption in mainland France.

Surveillance procedures
Outbreak surveillance
On the basis of clinical signs on farms or on ante-mortem inspection at the slaughterhouse.

If a clinical case is suspected on a production site, the farmer must inform the farm's mandated veterinarian and the suspected case must be reported to the veterinary authorities.

Programmed surveillance
Annual screening was introduced in 2002, providing minimum compliance with the sampling established by European Regulation (EC) No 999/2001.

Slaughterhouse: screening of 10,000 sheep and 10,000 goats aged over 18 months selected at random.

Rendering: screening of 40,000 sheep aged over 18 months selected at random, and systematic screening of all goats aged over 18 months.

Diagnostic procedure
Regardless of the origin of the samples (programmed or outbreak surveillance), brainstem (obex) samples are tested at the competent Departmental Veterinary Laboratory (DVL) corresponding to the sampling site. Each laboratory undertakes rapid diagnostic tests it has selected from those approved at European level (Bio-Rad© or Idexx©). "Non-negative" samples are sent to the NRL (ANSES Lyon Laboratory) for confirmation.

Health control measures
If a case is reported on clinical suspicion or if a non-negative result is obtained on a rapid test, the farms where the suspect animal was born, lived for more than nine months during its first year, or where it gave birth, are considered at risk. These farms are placed under Prefectural monitoring order (APMS), which specifically prohibits the sale of small ruminants, as well as their milk and any derived dairy products.

When screening returns a non-negative result, the sample is sent to the NRL for confirmation by Western blot. The confirmatory analysis serves to i) rule out the presence of a TSE, ii) confirm the presence of atypical scrapie, or iii) confirm the presence of a TSE other than atypical scrapie. A typing analysis is carried out if the confirmatory analysis indicates the presence of a TSE different to atypical scrapie. This typing analysis serves to confirm the presence of classical scrapie, or even BSE.

If the case is confirmed, the herds are subject to health control measures that vary depending on the TSE strain identified:

BSE: total depopulation of the herd of birth and any herds in which the case animal may have given birth;

Classical ovine scrapie: elimination of genetically susceptible animals from the herd of birth. Animals can be sold only to the slaughterhouse and the milk of genetically susceptible animals must be destroyed. These measures are replaced by reinforced follow-up for three years if the affected animal transited through several farms;

Classical caprine scrapie: total depopulation of the herd of birth;

Atypical scrapie: strict monitoring of herds for two years; any animals dying on the farm or slaughtered when aged over 18 months must be screened.

Regulations

Ministerial Order of 2 July 2009 as amended establishing control measures for caprine spongiform encephalopathy.

Ministerial Order of 2 July 2009 establishing control measures for ovine spongiform encephalopathy.

Ministerial Order of 3 December 1990 establishing control measures for bovine spongiform encephalopathy.

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.
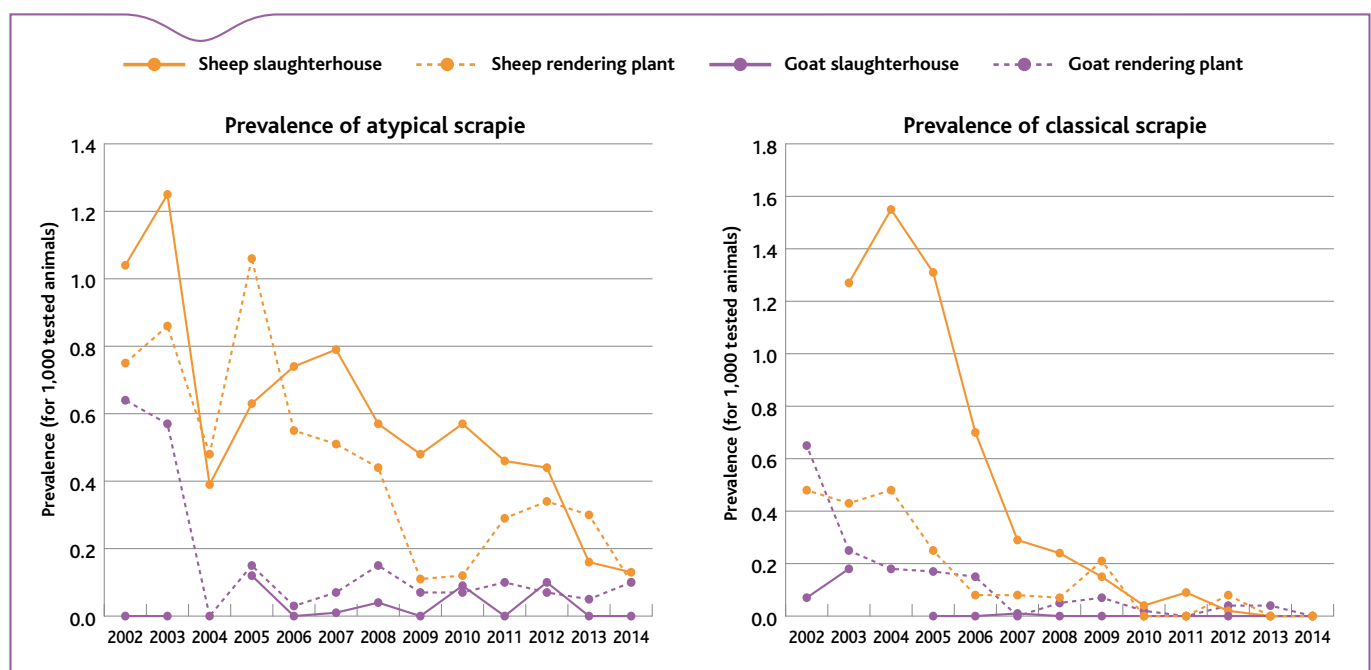

Figure 1. Changes in prevalence of classical and atypical scrapie in sheep and goats at the slaughterhouse and rendering plant

In light of empirical evidence of a decrease in the number of detected atypical scrapie cases in the past few years, trends in the prevalence of atypical scrapie were studied between 2010 (after which only tests capable of detecting atypical scrapie were used) and 2014, using a mathematical model. Given the small number of detected atypical cases in goats, the analysis took into account sheep only, slaughterhouse and rendering programmes combined.

The variable to be explained was the number of detected cases, and since this count variable is proportional to the number of tests undertaken, a decision was made to include this variable in the model. The reference year for the model was 2010. The type of test used (Bio-Rad© or Idexx©)

was not taken into account in the model, since for the five years of the study, a single test was used almost exclusively by the laboratories (depending on the year, 85% to 93% of the analyses undertaken were based on only one test).

The results of the model indicate a non-significant decrease in the number of detected cases for 2011 and 2012 compared to 2010 (respectively p=0.58 and p=0.52). However, there was a significant decrease in the number of detected atypical scrapie cases in 2013 and 2014 compared to 2010 at the 5% threshold (respectively $p=7.8*10^{-3}$ and $p=4.3*10^{-3}$); this could not be linked to poor testing rates.

decreasing susceptibilities. Susceptibility to atypical scrapie in sheep is related to the presence of the AHQ and AF141RQ alleles.

Genetics has now been used for more than ten years to combat classical scrapie in sheep. Genotyping takes place on four levels:

- systematic genotyping of sheep that were non-negative when screened at the slaughterhouse or rendering plant (whether or not the presence of a TSE was subsequently confirmed),
- genotyping of congeners in confirmed outbreaks of classical ovine scrapie, in order to identify the animals to be eliminated,
- genotyping on a random sample of sheep that were negative when screened at the slaughterhouse or rendering plant (a target of 600 genotypes per year nationally) to assess the evolution of allele frequencies,
- genotyping conducted as part of the National plan for genetic improvement of resistance to classical scrapie (PNAGRTc) in order to select resistant breeders. The results of the PNAGRTc and the ram census are given in Box 3.

In 2014, of the 706 genotype tests performed in negative sheep at the slaughterhouse or rendering plant, 665 provided interpreTable results. For all breeds combined, the following frequencies were found in the tested animals: ARR allele 60%, ARQ allele 31%, VRQ allele 5%, and AHQ allele 3%. Since 2002, a slight increase in the frequency of the ARR allele in these surveys has been found in all breeds, with a corresponding decrease in the ARQ allele (Figure 2). The proportions of animals harbouring the VRQ and AHQ alleles estimated by this programme appear to be relatively stable (Cazeau *et al.*, 2011).

Costs (amounts excluding VAT)

Sampling costs

Samples at the slaughterhouse are taken by State employees. This cost in human resources has not been estimated. For samples taken

Figure 2. Distribution of allele frequencies per year for negative sheep (slaughterhouse and rendering plant combined)

at the rendering plant, the State pays a fixed sum of €7.65 to the rendering plants for the cost of removing heads and placing them at the disposal of veterinarians, and a fixed sum corresponding to one veterinary act (AMV) per sample, which was €13.85 in 2014, to the veterinarians responsible for removing the obex. In total, the State spent approximately €650,000 for removing heads and making them available, and €1.2M for obex samples, for a total of €1.85M for sample preparation.

Laboratory costs

Analyses of samples taken at rendering plants and at the slaughterhouse are fully reimbursed by the State, within the limits of the ceilings determined by the volume of analyses carried out by the laboratories (ranging from €32 if the laboratory performs more than 25,000 analyses per quarter to €40 if the laboratory performs less than 6,500 analyses per quarter). The national average unit cost of the small ruminant screening test at the slaughterhouse and rendering plant was respectively €30.80 and €29.10.

In total, the State spent approximately €3M for TSE screening analyses on small ruminants in 2014 (€2.4M for analyses on rendered animals and 600,000 for those on healthy slaughtered animals).

Total cost for the State

In 2014, the State spent approximately €4.85M for samples and analyses as part of TSE screening at slaughterhouses and rendering plants. These sums do not take into account the costs of taking samples at the slaughterhouse, nor the costs of coordination or technical and financial management of the scheme, particularly in terms of the State employees involved.

In addition, management of the scrapie outbreaks identified in 2014, as well as the compensation for animals and products destroyed, amounted to around €420,000 in 2014. The programme for random genotyping cost €17,800 and that for genotyping carried out during outbreaks cost €5,520. The cost of the genotyping carried out as part of the PNAGRTc was €541,000.

The programme for monitoring and combating TSEs in small ruminants is co-financed by the EU, which in 2014 contributed €7.40 for each screening test at the slaughterhouse and rendering plant, and 50% of the amount of compensation, to a maximum of €50 per destroyed animal.

Discussion

Concerning classical scrapie, a significant decrease in prevalence has been observed since 2002, whether in sheep or goats. No cases of classical scrapie were detected by programmed surveillance in 2014, whether in sheep or goats.

The decrease in prevalence of classical scrapie is possibly explained by the control measures implemented for the disease in affected herds, and by selection of genetically resistant animals. However, given the available data, it is difficult to estimate the evolution of the sheep population's genetic status (that of breeding farms is very well known): the survey programme in rendering plants and slaughterhouses, which
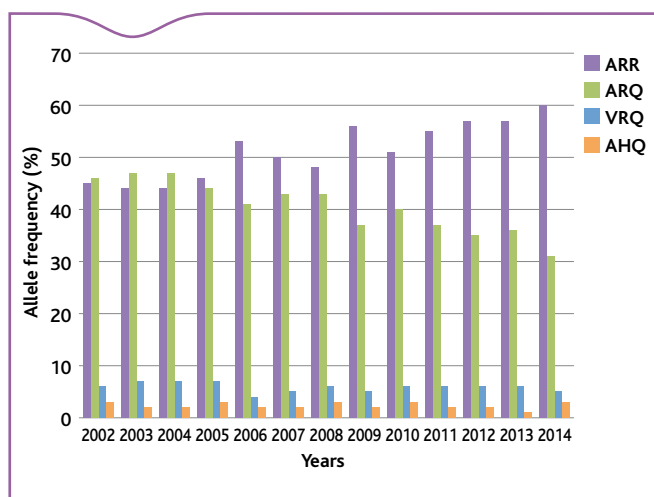
suffers from methodological limitations (number of samples, sampling methods), does not show any major change in genetic structure; in 2013, the ram inventory still included 45% of rams with unknown genotype.

Regarding atypical scrapie, while its apparent prevalence remains stable in goats, it is declining in sheep. The decrease in this form of scrapie considered "sporadic" raises questions as to the survey's ability to actually monitor the prevalence of atypical scrapie. Genetic selection used to reduce classical scrapie has been suggested as a possible cause of the decrease in atypical scrapie, but given the limited impact of genetic selection in the general population, it undoubtedly cannot completely explain this trend.

This decrease may be due to the under-performance of screening tests (for certain batches) and/or a change in tests over time. Since 2010, over 85% of analyses have been undertaken with only one diagnostic kit. The NRL identified several batches of this kit with lower sensitivity for detecting atypical scrapie (A.G. Biacabe, personal communication). To date, batches of diagnostic screening kits are tested only for BSE; this testing is organised by the EURL, which delegates the tests to various European countries. Batches are not tested for scrapie (classical or atypical), whether at European or national level. Furthermore, the test's suppliers do not test their batches for atypical scrapie but only for classical scrapie.

Due to this lack of testing, poor test performance for the detection of atypical scrapie is entirely possible, especially since the molecular characteristics of this type of scrapie are different from those of both classical scrapie and BSE (classical and atypical), making it more difficult to detect.

Overall, both forms of scrapie are rare and remain at very low levels. No suspected cases of BSE were detected in small ruminants in 2014. The system implemented in France exceeds the minimum requirements of the European regulations, which stipulate that 10,000 animals must be tested for each species/plan pair.

ANSES received a formal request in 2014 to examine the possible changes to be made to the surveillance programme for TSEs in small ruminants (Opinion 2014-SA-0032 of 30 September 2014). In light of this opinion, the DGAL decided to keep the current configuration of the surveillance programme in 2015 and 2016. If the number of classical scrapie outbreaks remains as low in these two years as in 2013 (four classical scrapie outbreaks detected) and 2014 (no classical scrapie outbreaks detected), confirming a sustained decrease in the prevalence of this disease, the relevance of scaling down the system will be reconsidered for 2017.

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Box 3. Plan for genetic improvement of resistance to classical scrapie: a few key points

Highly convincing results

The National plan for genetic improvement of resistance to classical scrapie (PNAGRTc) stems from the combined determination of the sheep farming sector and the authorities to use genetics to combat this disease.

This programme, established in October 2001, was specifically set up in dairy- and meat-sheep breeding farms with the following objectives:

- to eliminate the allele with susceptibility to classical scrapie (VRQ) from breeding farms,
- to repopulate scrapie-affected farms with resistant animals,
- to select the allele with resistance to classical scrapie (ARR),
- to disseminate ARR/ARR rams for production farms.

The genotyping carried out as part of PNAGRTc was funded by the Ministry of Agriculture. For several years, this "biological" genotyping has been supplemented by "prediction" genotyping deduced from the genotype of the parents and descendants. Originally intended to run until the end of 2009, the programme was extended to bolster the ability of breeder farms to disseminate resistant breeding males and females.

More than 850,000 genotyping analyses were thus performed between 2002 and 2014. In addition to this, over 7 million informational predictions were carried out.

With respect to the objectives, the programme's results at the end of 2014 can be summarised as follows:

- the VRQ allele has been virtually eliminated from breeding farms (no active ram carriers),
- 98% of active meat rams in breeding farms have the ARR/ARR genotype, compared with only 24% in 2002,
- 98% of AI dairy rams have the ARR/ARR genotype, compared with only 31% in 2002,
- 90% of females from meat breeds and 60% of females from hardy breeds in breeding farms have the ARR/ARR genotype.

These results from breeding farms enable these holdings to supply resistant breeding males and females to all French farmers (including those affected by classical scrapie).

A new tool: the Observatory for Resistance

The genotyping carried out in the framework of the PNAGRTc provides precise knowledge of the frequency of the different genotypes in the selection bases, but cannot provide information on the dissemination of resistance alleles in the rest of the French sheep population.

Since 2012, following an agreement between the authorities and professional sheep organisations, all holders of breeding rams have been invited to provide certain information on these animals (including genotype) during the annual census carried out as part of identification procedures. This inventory of all rams put to use in France responds to two objectives:

- to improve knowledge about the level of resistance to classical scrapie in livestock at national level and in the various production regions,
- to analyse the zootechnical information provided by this census on the origins of the rams used (breed, breeding holding, etc.) to gain a better understanding of the use of the male "pathway", a strategic component in improving herds and for disseminating resistance.

In 2014, the census provided the following results:

- 40,000 sheep farmers declared 168,300 rams. This corresponds to 52% of farmers identified in the BDNI, which contains all known sheep holders, and 80% of farmers identified in the BDNI and owning more than 50 breeders. If rams present on breeding farms are added (which farmers were not required to declare, as their inventory is already managed elsewhere), this makes a total of 168,000 rams analysed.

With regard to resistance, the results are as follows:

- 43% of all rams have the known ARR/ARR genotype,
- 9% have genotypes that are moderately resistant (ARR/AHQ or ARR/ARQ genotypes), susceptible (AHQ/AHQ, AHQ/ARQ or ARQ/ARQ genotypes) or that were incompletely predicted,
- 48% have no known genotypes.

The proportion of unknown rams corresponds to those that were not born on breeding farms (selection bodies). It should be noted that the rams born in selection bodies and disseminated outside the nucleus herd are virtually all resistant rams. This shows that any measure promoting the production and dissemination of resistant animals from breeding farms will help the dissemination of resistance in the remainder of the population and add value to all the efforts of the genetic programme.

A more detailed document containing the 2013 results has been prepared, with data by département and by breed (http://idele.fr/no_cache/recherche/publication/IdeleSolr/recommends/recensement-des-beliers-utilises-dans-les-elevages-ovins-francais-2013.html).