The Director General

Maisons-Alfort, 13 July 2010

OPINION

of the French Agency for Food, Environmental and Occupational Health & Safety regarding the policy on long-term genetic selection of sheep for TSE resistance

1. CONTEXT OF THE REQUEST

On 18 June 2009, the French Food Safety Agency (AFSSA) received a request from the Directorate General for Food for an opinion regarding the policy on long-term genetic selection of sheep for TSE resistance.

AFSSA later received a request on 11 December 2009 regarding a draft ministerial order to increase the frequency of the ARR allele in the commercial sheep population.

2. BACKGROUND

Genetic susceptibility to classical scrapie has been used to control TSEs in sheep through the implementation in 2002 of a breeding programme for scrapie resistance.

In its opinions, AFSSA has frequently underlined the short- and medium-term significance of the breeding programme.

In a solicited request dated 18 June 2009, the DGAL asked AFSSA for an opinion regarding the long-term genetic selection policies that could be pursued regarding the risk posed by TSEs in small ruminants. Among other things, the DGAL referred to atypical scrapie, whose rules of genetic susceptibility appear to differ from those of classical scrapie.

In a new solicited request dated 11 December 2009, the DGAL also asked AFSSA for an opinion on a draft ministerial order imposing the exclusive use of replacement rams with the ARR homozygous genotype (allele associated with resistance to classical scrapie) in all sheep flocks. In fact, to date, the genetic selection programme has had a very limited impact on ARR allele frequencies in the general sheep population.

Given the similarity of these dossiers, the two solicited requests are being addressed in the same opinion.

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Therefore, AFSSA is requested, in light of the complexity of genetic susceptibility phenomena and the existence of several scrapie strains, to:

- *assess the efficacy, in terms of public health and animal health, of a policy that, as is the case presently, would select only the ARR allele and eliminate only the VRQ allele (allele associated with susceptibility to classical scrapie) over the long term, versus a policy that would promote the selection or elimination of other alleles.*

- *issue an opinion about the relevance of targeting a population that is fully homozygous resistant (ARR/ARR) to classical scrapie and propose another genetic selection policy if appropriate.*

- *issue an opinion about the relevance of a ministerial order requiring all farmers to use only ARR/ARR replacement rams or the semen of such rams.*

3. EXPERT ASSESSMENT METHOD

The collective expert assessment, which began in September 2009, was undertaken by the 'TSSE' Scientific Panel (CES). Its opinion was validated by e-mail on 29 June 2010.

To form its opinion, the Panel referred to all existing knowledge of resistance to the various forms of scrapie and to the results of the French breeding programme that was implemented in 2002.

4. DISCUSSION

ANSES's discussion is based on the opinion of the 'TSSE' Scientific Panel, the main points of which are presented below:

1. Background

a. Effect of the *PRNP* gene's polymorphisms at codons 136, 154 and 171.

i. Susceptibility to infection

In sheep, some of the *PRNP* (prion protein) gene's polymorphisms have a major effect on TSE susceptibility/resistance. With classical scrapie and BSE, *PRNP* polymorphisms at codons 136 (A or V), 154 (R or H) and 171 (R, Q or H) have been identified since the mid-1990s as significantly influencing susceptibility to infection in sheep (Clouscard *et al.*, 1995; Hunter *et al.*, 1996).

Animals with the VRQ/VRQ, ARQ/VRQ and ARQ/ARQ genotypes are considered to be the most susceptible to classical scrapie agents, while ARR and AHQ homozygous or heterozygous individuals show only marginal susceptibility. AHQ-carrying animals and ARQ/ARQ animals are considered to be the most susceptible to BSE infection (experimental) whereas VRQ/VRQ animals appear to be less susceptible to this agent. ARR/ARR animals are considered to be highly resistant to infection with these agents (Hunter *et al.*, 1997; Hunter *et al.*, 1996).

However, several natural cases of infection with classical scrapie agents have been reported in ARR/ARR animals (Groschup *et al.,* 2007; Ikeda *et al.* 1995), indicating that some of the agents that cause this disease have the intrinsic capacity to spread in animals hitherto considered resistant.

Moreover, (i) classical BSE in cattle can be transmitted intracerebrally to ARR/ARR animals (Houston *et al.,* 2003) and (ii) classical BSE adapted to sheep effectively infects animals with this genotype when administered orally (Lantier *et al*., Neuroprion Madrid 2008).

With atypical scrapie, the genetic determinism of susceptibility in sheep is radically different from that observed with classical scrapie and classical BSE. A significant increased risk of developing the disease is associated with alleles AHQ and AF141RQ, whereas animals with the ALRQ/ALRQ and VRQ/VRQ genotypes appear to be less at risk. ARR (homozygous or heterozygous) animals can develop the disease and the ARR allele does not appear to be associated with resistance (Arsac *et al*., 2007; Moreno *et al*., 2007; Moum *et al*., 2005).

All of this illustrates that a TSE agent's capacity to spread in a sheep with *PRNP* polymorphisms depends on the interactions between this agent's biological properties (strain characteristics) and these polymorphisms.

In light of the available information, the hypothesis according to which there is or may emerge a TSE agent capable of developing effectively in ARR heterozygous or homozygous sheep should not be disregarded.

According to current knowledge, none of the alleles of the ovine *PRNP* gene can be considered as conferring total resistance to natural infection with TSE agents circulating in ruminant populations.

ii. ARR allele and reduced risk of dietary exposure.

Under natural conditions of exposure to classical scrapie (even with very high probability of infection), prevalence in ARR heterozygous animals remains very low (Elsen *et al*., 1999).
In these animals, when contamination is effective, spread of the infectious agent in the peripheral tissues remains limited. In this context, the application of SRM removal measures effectively limits risks of food chain contamination (Van Keulen *et al.,* 1996 – Andréoletti *et al.,* 2002).
Furthermore, it has been established that infected ARR heterozygous ewes do not spread the scrapie agent through their placenta, which limits risks of environmental and inter-individual contamination (Lacroux *et al.,* 2007).
This phenomenon, together with high resistance to infection in ARR animals, is a plausible explanation for the rapid decrease in new cases of contamination that has been observed in flocks affected by classical scrapie when the ARR allele has been introduced (Corbière *et al.,* 2008; Hagenaars *et al.,* 2010).
Lastly, ARR heterozygous animals appear to be more resistant to infection with the BSE agent than ARR homozygous animals (Lantier *et al*., 2008, Neuroprion, Madrid).

All of this information suggests that in terms of:

(i) controlling classical scrapie in affected flocks,
(ii) preventing classical scrapie or BSE,
(iii) reducing risks of consumer exposure to TSE agents,

the actual differences between ARR heterozygous and ARR homozygous animals are only marginal.

b. Variability of the ovine *PRNP* gene (not including codons 136, 154 and 171) and TSE resistance

The first case-control studies aiming to confirm the effects of the *PRNP* alleles demonstrated that codons 136, 154 and 171 heavily influence susceptibility to scrapie. Most subsequent studies focused on these three codons (Hunter *et al.,* 1996; Hunter *et al*, 1997). More recent work has examined other polymorphisms: for example, the effects of polymorphisms M/T112 and P/L168 have been described. These two polymorphisms appear to be associated with a higher protective effect in heterozygotes than the ARR allele against infection (oral or intracerebral) with the BSE agent (Saunders *et al*., 2009; Goldmann *et al*., 2006). Similarly, several polymorphisms (codons 137 and 176) have been described as conferring significant resistance to natural infection with classical scrapie (Vaccari *et al.,* 2009).
With atypical scrapie, identification of the F141 polymorphism's effects on disease susceptibility once again illustrates the importance of polymorphisms other than those located in positions 136, 154 and 171 of the PrP (Moreno *et al.,* 2007; Moum *et al.,* 2005).

The possibility that some hitherto unexplored polymorphisms may be associated with reduced (or zero) risk of developing scrapie and/or other forms of TSE should not be excluded. Presently, there is a lack of knowledge of how most *PRNP* polymorphisms affect the capacity to spread TSE agents, potentially leaving us without alternatives to ARR allele selection for the genetic control of TSE.

c. Conclusions

ANSES's analysis of genetic selection was limited to classical scrapie for which breeding is justified.

- Selection of the ARR allele still remains the best available approach for the prevention and control of classical scrapie in sheep populations. However, this allele does not prevent atypical scrapie from occurring.
- The hypothesis according to which there is or may emerge a TSE agent capable of spreading effectively in ARR heterozygous or homozygous sheep should not be disregarded.
- In the future, other alleles of the *PRNP* gene (polymorphisms other than those known at positions 136-154 and 171) could serve as alternatives to ARR allele selection for the control and prevention of ovine TSE.

2- Genetic progress in the sheep population.

a. Description and assessment of the national breeding programme for scrapie resistance (PNAGRT)

The PNAGRT and its assessment are described in detail in the Annex.

The programme was created in 2002 and was recently renewed in October 2009 for a three-year period. More than 670,000 animals have been genotyped since the start of the programme (from 2002 to 2009).
Breeding work is performed in the elite nuclei (see Annex) of each breed. From this population, genetic progress is disseminated to general populations (sale of ewe lambs but especially use of rams or semen by artificial insemination).
For information, for dairy ewes, out of a population of 1.2 million, 65% belong to elite nuclei, whereas for meat ewes, out of a population of 4.45 million, this figure is only 6.9%.

In breeding flocks, it can be noted that:
- frequency of the VRQ allele has decreased sharply;

- frequency of the ARR allele has risen sharply. Frequency of this allele is close to 100% in specialized meat breeds and greater than 70% in other meat breeds (hardy meat breeds). In dairy breeds, this frequency currently ranges from 60% (Corsican) to 100% (Lacaune).

We do not have highly precise and reliable data on genotype frequencies in commercial flocks. The sole data come from the annual genotyping of a sheep sample tested in a slaughterhouse (EU programme, see Table 1 below). This sample is small, does not take into account the tested individuals' breeds (a critical parameter given the breeding system's organisation by breed) and its representativeness is questionable.

Table 1: frequencies of alleles ARR, ARQ, AHQ and VRQ by year in sheep, all breeds, all programmes (slaughterhouse and rendering) (analysis of the 2009 active surveillance programme for scrapie in small ruminants, AFSSA Lyon).

	year							
	2002	**2003**	**2004**	**2005**	**2006**	**2007**	**2008**	**2009**
	n = 483	**n = 332**	**n = 857**	**n = 923**	**n = 800**	**n = 319**	**n = 631**	**n = 682**
% ARR	45%	44%	44%	46%	53%	50%	48%	56%
% ARQ	46%	47%	47%	44%	41%	43%	43%	37%
% VRQ	6%	6%	7%	7%	4%	5%	6%	5%
% AHQ	3%	2%	2%	3%	2%	2%	3%	2%

In addition to these reservations, in light of the data obtained by this system, it does not appear that allele frequencies in general populations have thus far been significantly affected by the PNAGRT. This phenomenon may be due to the means chosen to disseminate the ARR allele for the PNAGRT's implementation. In fact, the passive dissemination of a characteristic from the breeding population to general populations is a process that takes 5 to 10 years. However, the increase in the proportion of ARR/ARR rams (from 30% in 2002 to 90% in 2009) sold by breeding farms to commercial farms illustrates the breeding policy's effects.

b. Outlook on genetic progress in the French sheep population.

As was stated above, we do not have precise estimates regarding allele frequencies in commercial flocks. The model that follows aims to give a general idea of times in flocks other than elite nuclei. The governing parameters will be:

- the time after which the ARR allele's frequency will exceed a threshold allowing parameter R_0[1] to fall below 1 (when $R_0 < 1$, the disease cannot spread in a given population) (following the example of Man *et al*, 2009, we will use the value of 0.7 as an illustration),
- the time after which the ARR allele's frequency will exceed 0.95, making backtracking difficult should this allele be counter-selected (if necessary, this backtrack should be quantified depending on the scenarios).

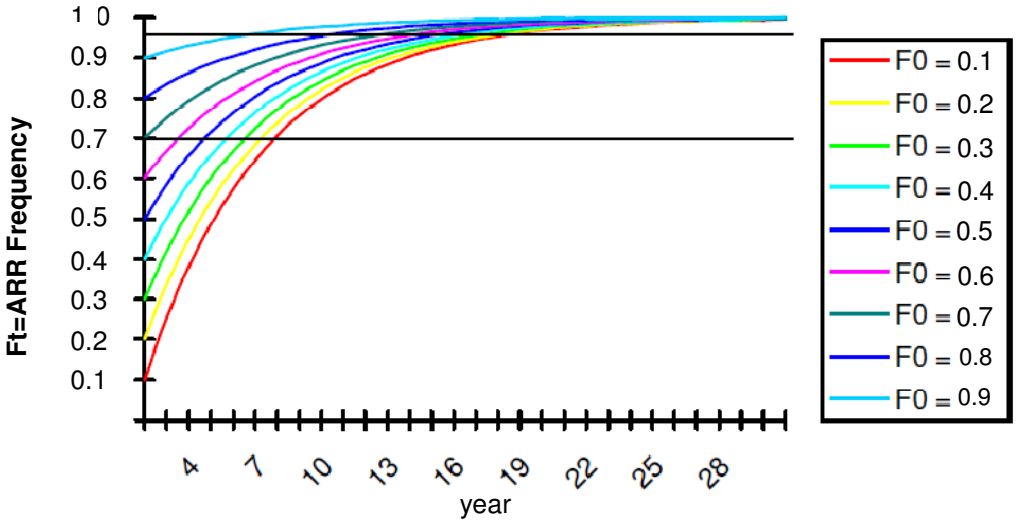
If rf and rm are male and female replacement rates in commercial flocks, and if pm and pf are the percentages of these replacements that occur by purchasing ARR/ARR rams or ewes from breeder flocks, the frequency of the ARR allele in males (fm_t) and females (ff_t) is obtained by iteration:

$Fm_t = (1\text{-}rm)\ fm_{t-1} + rm \cdot [pm \cdot 1 + (1\text{-}pm) \cdot ½\ (fm_{t-1} + ff_{t-1})]$
$Ff_t = (1\text{-}rf)\ ff_{t-1} + rf \cdot [pf \cdot 1 + (1\text{-}pf) \cdot ½\ (fm_{t-1} + ff_{t-1})]$

Figures 5a, b and c give examples of ARR frequencies over time, with an initial frequency of F0. Replacement rates are 1/3 for rams and 1/6 for ewes. The first scenario assumes full replacement from breeding bases, with both males and females.

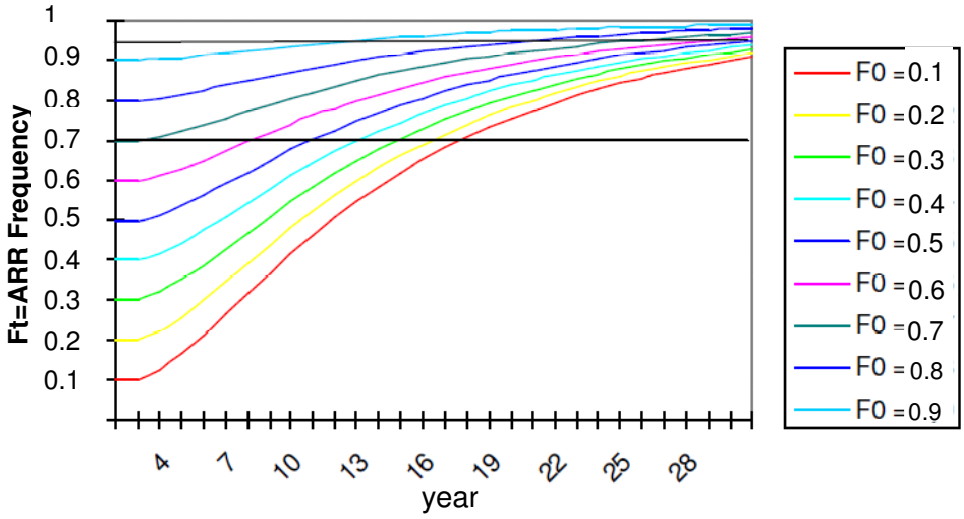
[1] Ro corresponds to the number of new TSE cases generated by an already infected animal.

Figure 5a
ARR allele frequencies in the commercial population
100% of rams from breeder flocks
100% of ewes from breeder flocks



In this extreme case, less than 8 years are needed in order for all the breeds to have an ARR allele frequency greater than 70%, and the 95% threshold is reached between years 7 and 20.
Knowing that the dissemination of females is difficult to organise, the following scenario is limited to the dissemination of rams.

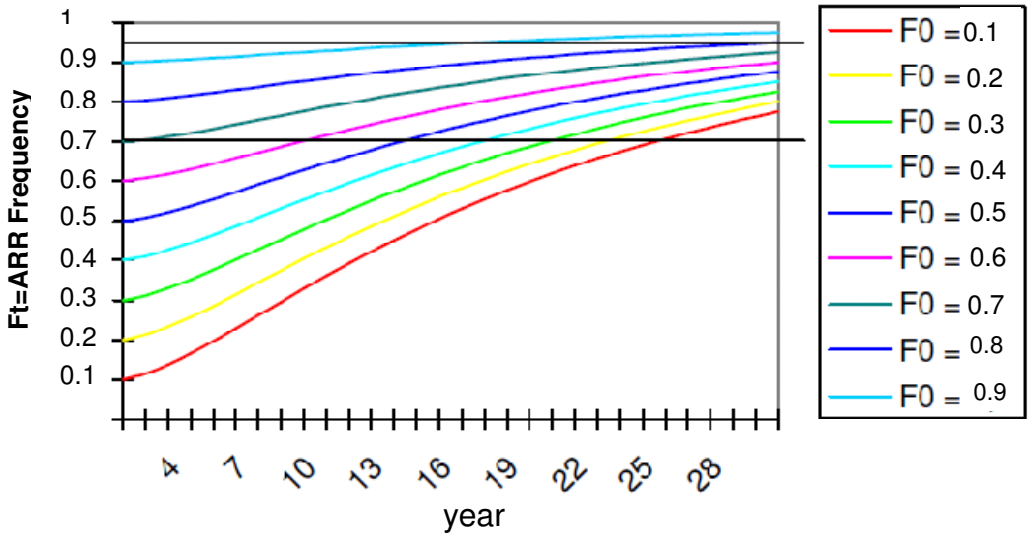
Figure 5b
ARR allele frequencies in the commercial population
100% of rams from breeder flocks
0% of ewes from breeder flocks



In this case, up to 18 years are needed to obtain protection for all breeds (> 70% ARR alleles) and the 95% threshold is reached after thirty years only if the initial frequency was already greater than 50%.

Lastly, Figure 5c shows a more realistic situation (on average) in which 40% of required rams and 10% of required ewe lambs are obtained through purchases from breeders.

Figure 5c
ARR allele frequencies in the commercial population
40% of rams from breeder flocks
10% of ewes from breeder flocks



In this case, at least 25 years are needed to reach the 0.70 threshold and only breeds with a very high initial frequency (0.9) reach 95% after thirty years.

These simple simulations provide estimates of the time needed to obtain a certain percentage of the resistance-associated ARR allele, according to the strategy used (exclusive use of ARR males and females, use of ARR males only, mixed strategy), and according to the starting situation in a given breed.

This simulation underlines:

- the importance of having knowledge of a given animal population's initial genetic structure, without which it is not possible to adapt the breeding strategy to the objective (time taken to reach threshold);
- that any strategy that aims to systematically and continuously introduce a certain percentage of ARR rams (in this case, 40 to 100%) asymptotically leads to a 100% population with the ARR allele.

c. Conclusions

The current ARR allele selection and dissemination scheme:

- risks causing very rapidly, in the elite nuclei of some sheep breeds, the fixation[2] of this allele that will be difficult to reverse.
- should not cause the fixation of this allele in general populations for several years.

3 Genetic selection for classical scrapie resistance: objectives

Selection of the ARR allele appears to be an effective means of:

- preventing and eradicating (in affected flocks) classical scrapie in sheep populations,
- preventing risks of dietary exposure to classical scrapie in sheep.

Therefore, there seems to be no doubt about the importance of implementing a selection programme aiming to increase the ARR allele's frequency in the general sheep population.

However, aside from this principle, several options may be considered depending on the objective:

a. Scrapie prevention and control in the sheep population

Regarding flocks infected with classical scrapie, it has been recognised for several years that it is not necessary to have 100% ARR-carrying sheep to control the disease. According to this concept, above a certain frequency of ARR-carrying animals, the number of secondary cases that an infected animal can cause in its lifetime falls below 1 and the disease cannot spread. Similarly, in a healthy flock with such a genetic structure, the introduction of an animal in incubation or a case of contamination (related to the environment or from contaminated feed) will not cause the disease to spread.
At the present time, there is only one available modelling study (Man *et al.,* 2009) aiming to determine the conditions needed to obtain an R_0 (basic reproduction ratio) lower than 1; this study shows that when 70% of animals have an ARR allele, the disease cannot spread.
However, as this study examined only one infected flock, it would be premature to adopt the frequency of 70% as a general target value.
In the event that such an approach is used, this target value should be estimated beforehand through dedicated prospective studies.

The implementation of a genetic selection programme aiming to reach such a frequency should effectively prevent the spread of scrapie in populations while allowing for a diversity of *PRNP* alleles.

This approach, however, does not guarantee total control over the risk of dietary exposure to scrapie (potential entry in the food chain of positive animals without an ARR allele).

b. Controlling risk of dietary exposure to ovine scrapie

If the objective is to use genetic selection to control dietary exposure to TSE agents from small ruminants with the highest possible level of certainty, limiting consumption to animals with the ARR allele appears to be the most appropriate solution in the current state of knowledge. From this perspective, a population structure where 100% of individuals in commercial flocks would carry this allele appears to be the most pragmatic response, taking into consideration only the genetic selection tool that is the subject of the request[3].

[2] When there is only one remaining allele in the population.

[3] It should be noted that another approach was addressed in a previous opinion issued by the Scientific Panel (AFSSA Opinion of 7 May 2009), which combined genetic knowledge of a flock with the use of rapid tests.

However, over time, such a scenario will entail the fixation of the ARR allele in sheep populations. If an agent adapted to the ARR allele were to emerge and spread, our abilities to modify such a population's genetic structure would be limited.

5. CONCLUSIONS

Regarding the use of genetics to control classical scrapie, ANSES confirms the relevance of a selection programme aiming to increase frequency of the ARR allele and decrease frequency of the VRQ allele with the goal of:

i) controlling classical scrapie in affected flocks,
ii) reducing overall susceptibility to classical scrapie in the French sheep population,
iii) reducing and preventing risks of dietary exposure to classical scrapie agents.

While pursuing these objectives, it is important however not to neglect the risk of other TSEs emerging in sheep, as these TSEs would not be governed by the same genetic determinism.

Irrespective of the dissemination strategy that is adopted and the objectives that are set, in terms of the ARR allele's frequency in the general sheep population, several years will be necessary to achieve them. On the other hand, given the rapid increase of the ARR allele in elite nuclei, a programme should be implemented immediately to preserve the diversity of PrP alleles other than the ARR allele in various sheep breeds.

Therefore, and for the moment, ANSES considers that:

- dissemination of the ARR allele in the general sheep population, to control and prevent TSEs against which this allele is effective, should continue.
- a programme to preserve the diversity of PrP alleles, particularly in the elite nuclei of various sheep breeds, should be implemented immediately.

At the same time, ANSES recommends that an analysis should be undertaken in order to define the medium- and long-term objectives of the breeding programme to control ovine TSEs. In light of the potential consequences that may arise from any choices that are made, this approach appears to be an essential prerequisite to the implementation of an appropriate ARR allele dissemination strategy in sheep populations.
Regarding the exclusive use of males for dissemination of the ARR allele or an alternative strategy (combination of males and females, for example), ANSES considers it premature to make a choice before selection objectives have been set (see Section 5.3).

Lastly, ANSES considers that the high resistance to classical scrapie infection associated with the ARR allele should not rule out the study of effects that other *PRNP* polymorphisms could have in terms of susceptibility to TSE agents, particularly regarding atypical scrapie. Therefore, beyond the simple preservation of allele diversity, a rational exploration of their impact on resistance/susceptibility to TSE agents (classical scrapie/BSE/atypical scrapie) in sheep is recommended.

In response to the DGAL's initial questions:

1) *Assess the efficacy, in terms of public health and animal health, of a policy that, as is the case presently, would select only the ARR allele and eliminate only the VRQ allele over the long term, versus a policy that would promote the selection or elimination of other alleles.*

In the current state of knowledge, genetic selection aiming to increase the ARR allele's frequency and reduce the VRQ allele's frequency appears to be an effective means of limiting the risks (to animal and public health) associated with classical scrapie.

2) *Relevance of targeting a population that is fully homozygous resistant (ARR/ARR) to classical scrapie and proposal of an alternative genetic selection policy if appropriate.*

ANSES considers that the proposal's relevance is directly related to the operational objectives of the policy being pursued. Various scenarios have been described in this opinion (control of consumer exposure/disease control and prevention in sheep populations).
Risk managers are responsible for defining precise objectives in terms of animal health and/or public health.

A genetic selection policy that aimed to obtain a fully ARR homozygous population could expose this same population to TSEs for which this allele does not provide a sufficient level of resistance or has heightened susceptibility. Moreover, it could leave us without the genetic resources we would need to halt this phenomenon.
The ARR allele selection policy that was pursued over the last decade has already significantly increased this allele's frequency in the elite nuclei of sheep breeds and weakened the diversity of other alleles.
It therefore appears necessary to immediately implement a programme to maintain the variability of PrP alleles in the elite nuclei of various sheep breeds.

3) *Relevance of a ministerial order requiring all farmers to use only ARR/ARR replacement rams or the semen of such rams*.

Such an order would accelerate dissemination of the ARR allele in the general population (with the associated benefits and risks). Although if this order is enacted, it would take several years to significantly modify the sheep population's genetic structure, ANSES recommends immediately undertaking an analysis to define the precise medium- and long-term objectives of the breeding programme to control ovine TSE before deciding on a selection strategy.

The Director General

Marc MORTUREUX

KEYWORDS

Keywords: genetic selection, TSE, sheep, scrapie

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ANNEX

Description and assessment of the national breeding programme for scrapie resistance (PNAGRT)

A- Description of the PNAGRT

a. Background - Organisation

Under the supervision of the French Ministry of Agriculture (DGPAAT, DGAL), representatives of the sheep farming sector (FNO, Coop de France, Races de France, FNGDS, ANIO, INTERBEV, the dairy industry, etc.), the Institut de l'Elevage and INRA organised a national breeding programme for scrapie resistance based on knowledge of the *PRNP* gene's effects. Its technical terms were validated in the National Breeding Committee (CNAG) meeting of 21 November 2001.
The programme revolves around two key activities: (1) animals are sorted on the basis of their genotype at the PrP locus identified in a reference laboratory (LABOGENA) or a DGAL-approved laboratory; (2) for each breed, selection bodies (OSs, formerly 'breed promotion units') are made up of breeding farmers who are members of collective pedigree and performance testing systems. All of the data (PrP, Pedigree, performance) from these 'selection bases' are managed in the Genetic Data Processing Centre's national database.
The programme was initially scheduled for the 2002-2006 period. It was extended from 2007 to 2009 and was then renewed for three years in October 2009.

The organisation of breeding in France is governed by the 2006 'Loi d'orientation agricole' (Framework Act for Farming), which succeeded the 1966 'Loi sur l'Elevage' (Farming Act).
The selection plans supported by the Ministry of Agriculture (which opens its regulations and support for discussion in the CNAG (joint consultative body)) are designed by selection bodies and enterprises (OSs, ESs) that bring breeding farmers together into a cooperative. Selection bodies are in charge of regulated missions such as the definition of selection objectives and breed characteristics, the keeping of genealogical records and the organisation of the selection plan. There are therefore as many selection plans as there are breeds.

Several 'tools' are used for selection.
- Artificial insemination, which is essentially undertaken by collection centres and placement centres (9 for sheep), is regulated and involves only approved sires.
- Laboratories that perform pedigree testing and some genotyping, such as *PRNP* genotyping.

- Flock identification, which is mandatory for all of France's sheep and goats, is governed by European and French regulatory texts. It is managed by EDEs (departmental livestock breeding organisations).
- In-farm pedigree certification and performance testing, undertaken by 7 approved bodies with temporary geographic exclusivities. There are 300,000 dairy ewes (out of 1.6 million) and 300,000 meat ewes (out of 4.3 million) in this programme. Several protocols are proposed (full or simplified dairy production, reproduction, breeding, growth).
- Breeding centres for young males for the collective management of rams in breeding flocks.
- Stations for the testing of individual or progeny performance, which ensure the homogenisation of measurement conditions, complement the in-farm scheme for meat traits.
- National Genetic Information Systems (SNIGs) collect, store, process, update and distribute information required by parties involved in the selection process (particularly selection bodies, animal insemination centres, analysis laboratories). They rely on the relationship between performance testing bodies, regional information management centres and the Genetic Data Processing Centre (CTIG, located at INRA), which works in close collaboration with the Institut de l'Elevage.

The basic principles behind the selection schemes are:
- the identification, in accordance with breed standards, of fertile, top-breeding future sires from the young generation;
- their testing on a farm or in testing stations against characteristics of zootechnical interest decided upon by the selection body;
- the assessment of their genetic value, under INRA's supervision, using statistical models that are scientifically recognised by the international scientific community and software applications executed at the CTIG;
- the classification of candidates on the basis of this objective information;
- the start of reproduction, with specified mating plans, for the chosen candidates.

b. Objectives

The PNAGRT was created with four objectives: (i) eliminate the susceptibility allele (VRQ), (ii) introduce resistant animals or the semen of resistant animals to flocks affected by scrapie, (iii) increase the frequency of the resistance allele (ARR) while maintaining genetic variability and level, (iv) introduce resistant rams or the semen of resistant rams (ARR/ARR) into commercial flocks.

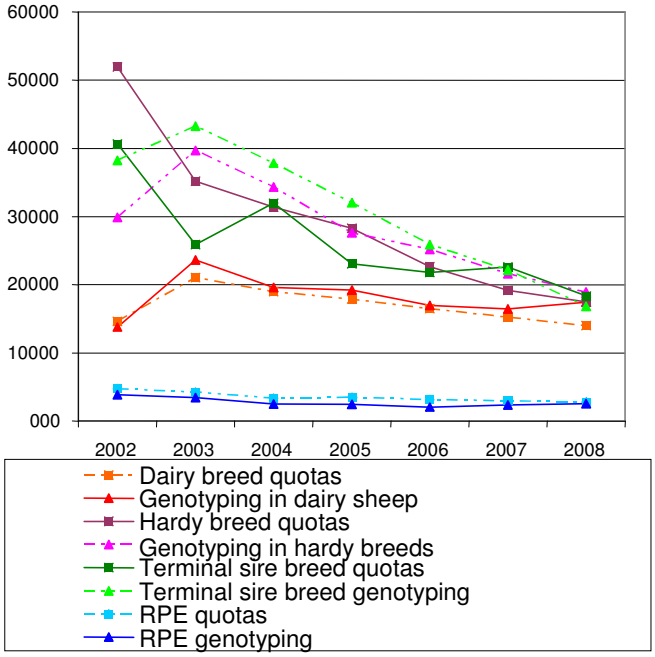
c Implementation methods.

Implementation methods, and particularly the amount of genotyping to be undertaken each year and the choice of animals to be targeted for this genotyping, were adapted to each breed, to take into account allele frequencies at a given time.
All the male adults in the selection bases were genotyped at the start of the programme, to obtain an overview and eliminate VRQ carriers. Then, every year, young rams were genotyped before entering the rallying centre (breeding centre or individual testing station depending on the case) in order to select ARR homozygotes. Additional quotas for the genotyping of breeding rams were organised in some breeds to meet the demand of commercial flocks.
The use of genotyping quotas for females mostly targeted replacement ewe lambs for meat breeds and elite ewes for dairy breeds.
In total, more than 670,000 sheep were genotyped.

Genotyping figures by major sheep type are given in figure 1.

Figure 1

Annual genotyping stipulated by the PNAGRT steering committee (quotas) and performed by selection enterprises according to breed type (RPE: Rare Breed)



B- PNGART assessment

The following data were compiled recently by head engineers at the PNAGRT from databases centralised at the CTIG. These compiled data were the subject of a comprehensive report that was published in 2009 (Sidani, 2009) in which information was given in detail for each breed.

a. In breeder flocks

i. Elimination of the VRQ allele

Figures 2a, 2b and 2c give the change in VRQ allele frequencies in lambs from various breeds. In all cases, this frequency decreased rapidly, especially when the initial frequency was high. It can be noted that, while it makes up no more than a few percent of the total, the VRQ allele is never completely eliminated.

Figure 2a

Frequency of the VRQ allele in specialized meat breeds

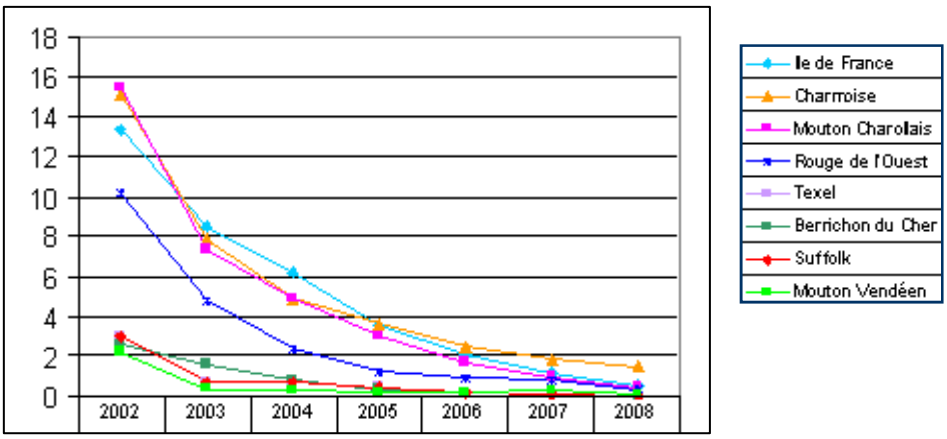


Figure 2b
Frequency of the VRQ allele in hardy breeds

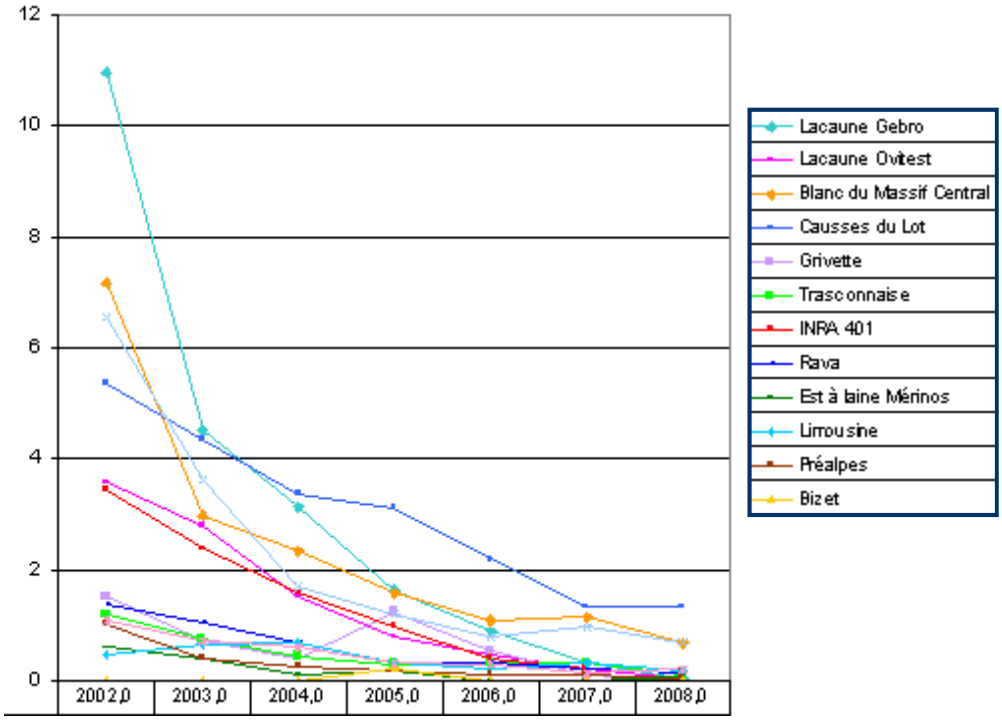
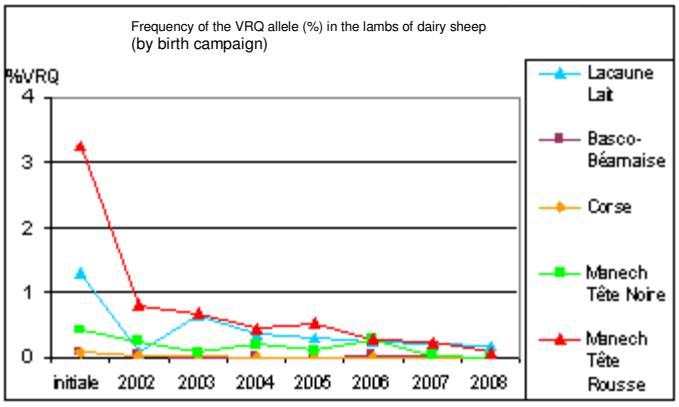


Figure 2c
Frequency of the VRQ allele in dairy breeds



ii. Selection of the ARR allele

Figures 3a, 3b and 3c give the change in ARR allele frequencies in active males from various breeds. In all cases, this frequency increases. While it is still low in the Est à Laine Mérinos breed, it almost always exceeds 50%. Terminal sire breeds, whose rams are widely used in commercial crossbreeding to produce butcher lambs, have very high allele frequencies that almost always exceed 70%.

Figure 3a
Frequency of the ARR allele in specialized meat breeds

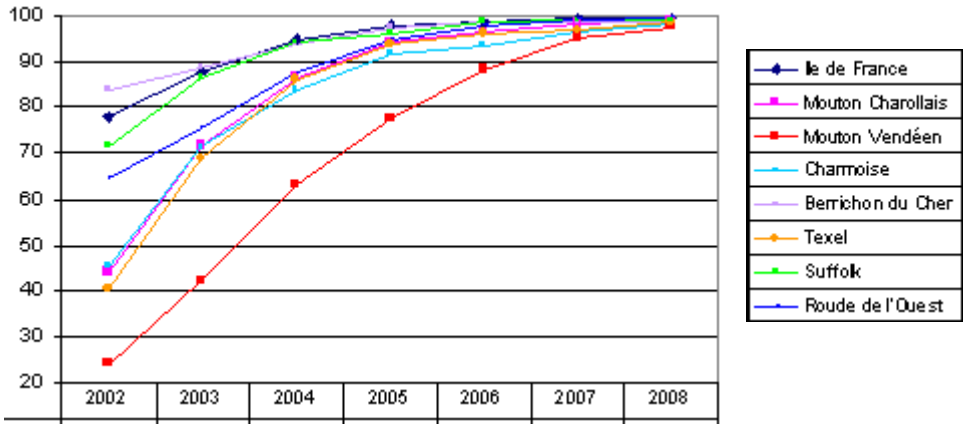


Figure 3b
Frequency of the ARR allele in other meat breeds

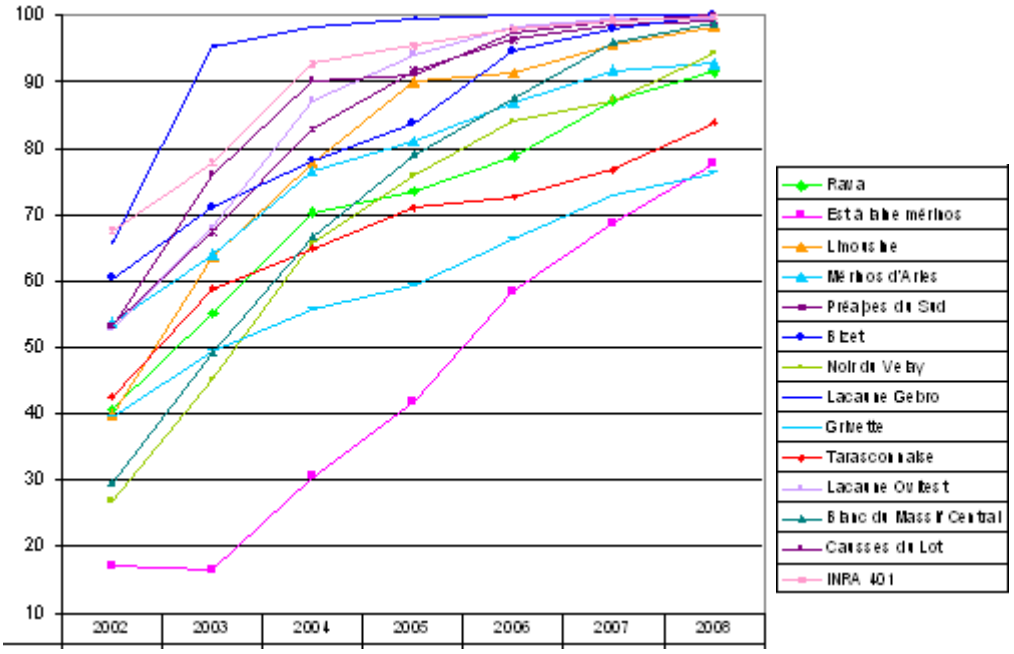
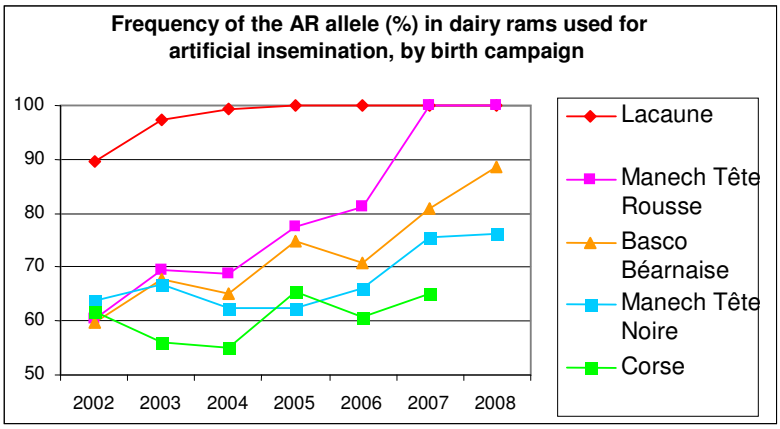


Figure 3c
Frequency of the ARR allele in dairy breeds



b. In commercial flocks

The genotypes of rams introduced from selection bases into commercial flocks of meat breeds improved considerably between 2002 (30% ARR/ARR rams) and 2008 (90% ARR/ARR) (Figure 4). However, the total number of disseminated rams dropped significantly in 2007 and 2008 (Table 1), to reach a value that was clearly below the dissemination capacity of the breeder flocks. It can be noted, however, that these figures underestimate the actual number of rams sold by selecting breeders to producers, and that a market of ewe lambs complements the programme in undoubtedly non-negligible proportions.

Figure 4
Genotypes of rams sold by selection farms to commercial farms

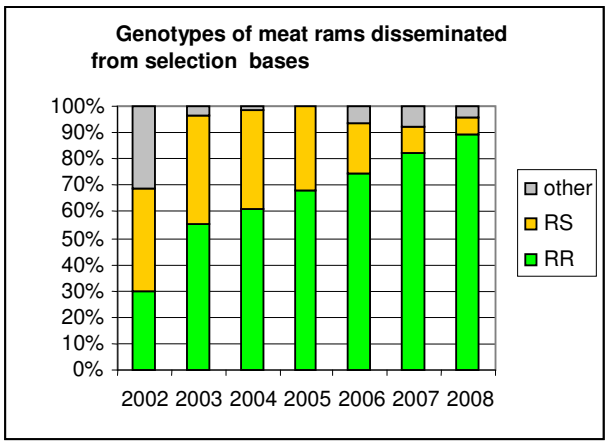


Table 1
Number of rams disseminated from selection bases of meat sheep

	2002	2003	2004	2005	2006	2007	2008
Number of disseminated males	12236	12174	11900	12045	11400	9720	7888