



## **EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards), 2014. Scientific Opinion on the scrapie situation in the EU after 10 years of monitoring and control in sheep and goats**

### **EFSA Publication**

*Link to article, DOI:*  
[10.2903/j.efsa.2014.3781](https://doi.org/10.2903/j.efsa.2014.3781)

*Publication date:*  
2014

*Document Version*  
Publisher's PDF, also known as Version of record

[Link back to DTU Orbit](#)

*Citation (APA):*  
EFSA Publication (2014). *EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards), 2014. Scientific Opinion on the scrapie situation in the EU after 10 years of monitoring and control in sheep and goats*. European Food Safety Authority. the EFSA Journal Vol. 12(7) No. 3781 <https://doi.org/10.2903/j.efsa.2014.3781>

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## SCIENTIFIC OPINION

### Scientific Opinion on the scrapie situation in the EU after 10 years of monitoring and control in sheep and goats<sup>1</sup>

EFSA Panel on Biological Hazards (BIOHAZ)<sup>2,3</sup>

European Food Safety Authority (EFSA), Parma, Italy

#### ABSTRACT

To assess the effectiveness of the strategies implemented in the European Union (EU) to control Classical scrapie (CS), epidemiological data have been compared in the context of the efforts in terms of control measures applied over time. Official EU surveillance data and results from questionnaire surveys of EU Member States (MSs) have been used along with case studies. A spatio-temporal description of the occurrence of small ruminants TSEs in MSs in the period 2002-2012 is provided, with a particular focus on CS in sheep. Based on information collected from MSs, the potential effectiveness of breeding programmes for resistance to CS (BP-CS) in the dissemination of resistance into the general sheep population has been assessed for those countries for which the CS trend analysis has been performed. CS in sheep was reported in 17 MSs (average prevalence: 8.7 cases/10 000 tests), with heterogeneous trends and geographical distribution: among the 13 countries reporting a consistent number of cases, the trend analysis shows a statistically significant decreasing trend only for six of them. Variations in the implementation of genetic and non-genetic measures for the control of CS may explain the failure to improve the disease situation in the remaining seven MSs. At a national level, a reduction in CS seems to be linked to better-achieving BP-CSs. Control options applied to CS in sheep and goats indicate that a CS eradication policy that relies solely on the detection of infected flocks by *post-mortem* testing and subsequent depopulation would be unlikely to succeed. A minimum frequency of the ARR allele in a sheep population above which CS may be expected to fade-out could be estimated for each specific national sheep population. Recommendations for additional/alternative measures to control CS in sheep and goats are formulated.

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#### KEY WORDS

Atypical scrapie, breeding programme, Classical scrapie, goat, sheep, surveillance

<sup>1</sup> On request from the European Commission, Question No EFSA-Q-2012-00646, adopted on 10 July 2014.

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<sup>3</sup> Acknowledgement: The Panel wishes to thank the members of the Working Group on Scrapie situation in the EU: Pier Luigi Acutis, Olivier Andreoletti, Antonello Carta, Christian Ducrot, Jean-Michel Elsen, Thomas Hagenaaers, Angel Ortiz-Pelaez, Giuseppe Ru and Marion Simmons for the preparatory work on this scientific opinion and EFSA staff members: Pietro Stella and Luis Vivas-Alegre for the support provided to this scientific opinion.

Suggested citation: EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards), 2014. 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. doi:10.2903/j.efsa.2014.3781

Available online: [www.efsa.europa.eu/efsajournal](http://www.efsa.europa.eu/efsajournal)

## SUMMARY

Following a request from the European Commission (EC), the EFSA Panel on Biological Hazards (BIOHAZ) was asked to deliver a scientific opinion on the scrapie situation in the European Union (EU) after 10 years of monitoring and control in sheep and goats. In particular, in order to evaluate the measures in place and assess the progress accomplished, the EC consulted EFSA to gain a better understanding of the dynamics of the epidemiological situation of Classical scrapie (CS) and Atypical scrapie (AS), and a retrospective analysis of the effectiveness of the control tools encompassed by EU legislation.

The opinion provides background information in relation to CS and AS in sheep and goats, including information on the nature of the agents, genetic susceptibility of the hosts, transmission pathways and epidemiology of the diseases.

Based on information from the EU TSE surveillance database, CS and AS in sheep and goats in the period 2002-2012 have been analysed to provide a spatio-temporal description of the occurrence of the diseases, after appropriate statistical adjustment accounting for the main known confounding factors. Due to the heterogeneity of the distribution of CS within the different EU Member States (MSs), and, to some extent, of the monitoring and control measures implemented, it was not considered meaningful to present an overall EU trend of the disease. Therefore, the temporal trends have been considered country-by-country.

CS in sheep was reported in 17 MSs, with an overall average prevalence of 8.7 cases per 10 000 rapid tests performed. Both the temporal trend and geographical distribution of CS showed great heterogeneity across the MSs. Among countries reporting a sufficient number of cases of CS in sheep over the years, six MSs showed a statistically significant decreasing trend, and seven showed a trend not statistically different from a flat one.

CS in goats was reported in eight MSs, with an overall prevalence of 9.8 cases per 10 000 rapid tests performed. This is mostly explained by the unique epidemic in one MS, while the overall prevalence in the remaining seven MSs was 2.2 cases per 10 000 rapid tests. A statistically decreasing trend was detected in one MS for the period 2002-2012, and in two more MSs for the period 2007-2012.

Although it was not possible to identify causes that can explain objectively the failure to improve the situation of CS in some MSs, the assessment of country-specific data, obtained through *ad hoc* surveys to MSs, and related to the implementation of surveillance and of genetic and non-genetic control measures, allowed the formulation of some hypotheses. In the case of sheep, these included ineffective implementation of genetic and non-genetic measures for the control of the disease, whereas in goats these included the absence of genetic measures and the variability of the non-genetic measures applied.

AS in sheep was reported in 21 MSs, with an overall prevalence of 5.8 cases per 10 000 rapid tests performed. A similar prevalence over time and space was observed, with no large epidemics, and only sporadic detection in five MSs. Only two MSs show a statistically significant trend, decreasing in one case and increasing in the other case.

AS in goats was reported by five countries, at a very low prevalence and with no statistically significant trend in any of them.

The different control options available for CS in sheep and goats are discussed. Firstly, non-genetic control measures are described, with the support of data from two case-studies in non-EU countries applying exclusively non-genetic measures for a period of time. Detection and eradication measures in affected flocks were effective in reducing the observed prevalence of CS in a population with a high prevalence of disease. However, it is concluded that, due to the pathogenesis and the epidemiological characteristics of CS, and to the high persistence of the CS agent in the environment, a CS eradication

policy that relies solely on detection of infected flocks by *post-mortem* testing and subsequent depopulation would be unlikely to succeed.

Secondly, the use of genetic measures, i.e. breeding programmes for resistance to CS (BP-CS), is discussed. During the last ten years, BP-SC have been implemented by 17 MSs as a strategy to control the disease. Based on information collected from MSs, the potential effectiveness of these BP-CS in the dissemination of resistance into the general sheep population was assessed for those countries for which the CS trend analysis was performed. There was a clear heterogeneity in the characteristics of the BP-CSs implemented by the different MSs. There was also heterogeneity within MSs according to different geographical areas, ARR allele frequency in the general population prior to BP-CS implementation, breeds and production types. Given the characteristics of each national BP-SC, a deterministic model was used to estimate the ARR/ARR frequency in the general sheep population over time. Subsequently, the outputs of the model were compared with the national CS situations. The results obtained suggest that, at national level, a favourable reduction in CS seems to be linked to better-achieving BP-CSs.

Given the very strong resistance to CS of sheep of the homozygote ARR genotype, one may expect that a minimum frequency of the ARR allele in a sheep population exists, above which CS may be expected to fade-out. Some case-studies are presented, showing that this hypothetical minimum frequency is not universal and that it is affected by parameters such as disease prevalence and the national characteristics of the sheep industry. In those cases studied, the required minimum frequency ranged between 53 % and close to 100 %, in a context where no additional control or eradication measures were applied.

Additional/alternative measures to control CS in sheep and goats are recommended. These focus on: i) the improvement of surveillance and control measures and their adaptation to the individual MSs, ii) the reinforcement and improvement of the policy of breeding for resistance in sheep, iii) the introduction of breeding policies in goats, and iv) knowledge transfer on scrapie.

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## BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

The situation of scrapie has been actively surveyed in the Member States since the implementation of a compulsory programme for the monitoring of TSEs in sheep and goats in 2002, on the basis of a random sampling of healthy slaughtered animals on one hand and fallen stock on the other. Targeted population and sample sizes have evolved over time.

Mandatory eradication measures have simultaneously been enforced by Regulation (EC) No 999/2001<sup>4</sup>, hereafter designated as the TSE Regulation, in holdings where TSE cases were confirmed, combining culling, movement restrictions and reinforced surveillance measures.

Recognising that some polymorphisms of the *PRNP* gene are associated with differences in the phenotypic expression of prion diseases in sheep (incubation period, physiopathology and clinical signs), several Member States have been implementing since the 1990s breeding programmes aimed at increasing the level of alleles associated with resistance (ARR) and decreasing the frequency of alleles associated with susceptibility (VRQ) in their sheep population. As of 2004, the EU made compulsory the introduction by the Member States of a breeding programme to be applied to the flocks of high genetic merit, until it became facultative again in 2007.

This global strategy for monitoring and controlling TSEs in sheep and goats has now been in place for approximately 10 years. Among other goals, one of its underlying objectives is the eradication of Classical scrapie in EU population of sheep and goats. Today, the situation of Classical scrapie appears to be heterogeneous among the Member States, with no clear trend perceived by the Commission with regards to the evolution of its prevalence rate at the scale of the European Union. In order to assess the progress accomplished and evaluate the measures in place, the Commission needs a better understanding of the dynamics of the epidemiologic situation of Classical scrapie, and a retrospective analysis of the efficiency of the control tools foreseen by the TSE Regulation. There is a similar need for a better understanding of the dynamics of the Atypical scrapie situation in the EU.

## TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

EFSA is requested to provide a scientific opinion on the following questions:

1. On the basis of the results of the TSE monitoring programme laid down in the TSE Regulation, what is the trend since 2002 of the situation of Classical scrapie and Atypical scrapie in sheep and in goats respectively, in the EU as a whole and in the 27 Member States individually? Where no favourable trend can be observed, what are the identifiable causes for failure to improve the situation of Classical scrapie?
2. Has the evolution of the Classical scrapie situation been statistically different in the MS which have implemented a breeding programme from 2004 to 2011 compared to the MS without a breeding programme in the same period?
3. On the basis of the above analysis, can a minimum level of frequency of the ARR allele in the sheep population in a MS be defined or estimated above which Classical scrapie can be expected to fade-out, in a context where no control and eradication measure is being applied?
4. In a context where no breeding programme is implemented, are the present mandatory measures in terms of active monitoring, eradication and control of Classical scrapie effective to achieve a decline of this disease and its eradication on the long term?
5. What additional measures can EFSA recommend in view of achieving the eradication of Classical scrapie in the MS?

<sup>4</sup> Regulation (EC) No 999/2001 of the European Parliament and of the Council of 22 May 2001 laying down rules for prevention, control and eradication of certain transmissible spongiform encephalopathies.



## ASSESSMENT

### 1. Introduction

#### 1.1. Approach to answer to the terms of reference

The different Terms of Reference (ToRs) of the mandate for the present Scientific Opinion are tightly linked, and the answers to the single ToRs often need consideration of aspects dealt with when replying to the others. For example, as explained later in detail, the observed epidemiological trends of the disease depend on a series of factors, obviously including the control measures implemented at international, national, and local level. Consideration to the latter thus needs to be given in order to discuss the observed trends and the potential reasons for the positive or not positive evolution of scrapie (ToR 1). On the other side, there is a need to first analyse the evolution of Classical scrapie (CS) to allow the comparison of the trend of the disease in countries implementing or not certain control measures (ToR 2).

This first chapter of the Opinion aims at providing some background information in relation to CS and Atypical scrapie (AS) in sheep and goats, including information on the nature of the agents, genetic susceptibility of the hosts, transmission pathways and epidemiology of the diseases.

The second chapter presents the legal background for the monitoring and surveillance of Transmissible Spongiform Encephalopathies (TSEs) in small ruminants in the European Union (EU), and the data gathered by Member States (MSs) and the European Commission. It discusses in detail the sensitivity of the EU monitoring system, and the factors that affect surveillance. This is expected to provide useful background to inform the assessment of the scrapie trends in the EU.

The analysis of the trends of both CS and AS in sheep and goats is presented in the third chapter of the Opinion. A spatio-temporal description of the occurrence of the diseases is provided, after appropriate statistical adjustment accounting for the main known confounding factors. Due to the heterogeneity of the distribution of CS within the different EU MSs, and, to some extent, of the characteristics of the monitoring and control measures implemented, it would not be meaningful to present an overall EU trend of the diseases, and therefore the trends are to be analysed and discussed country-by-country, assessing, when data allow, the evolution of the disease over the years. This chapter provides useful information to answer the first part of ToR 1. Potential reasons for the absence of a decreasing trend, which answers the second part of ToR 1, are discussed later in the opinion (i.e. in Chapter 4), since there is a need to first consider in detail the measures implemented to control the disease.

Chapter 4 focuses on the control options available for CS in sheep and goats, differentiating and discussing genetic and non-genetic measures. Firstly, non-genetic control measures are described, with the support of data from two case-studies in non-EU countries applying exclusively non-genetic measures for a period of time. Conclusions from these case-studies are used to provide an answer to ToR 4 of the mandate.

Secondly, the use of genetic breeding programmes for resistance to CS (BP-CS), as a strategy to control the disease implemented in many EU MSs in the last ten years, is discussed. ToR 2 asks for a comparison of the evolution of the CS situation between MS having or not having implemented a BP-CS. However, since there were no MSs without a BP-CS with sufficient cases of CS in order to estimate a trend, such comparison was not possible. Therefore, based on information collected from MSs, the potential effectiveness of BP-CS in the dissemination of resistance into the general sheep population was assessed for those countries for which the CS trend analysis was performed. Subsequently, the evolution of the CS situation between MSs, depending on the potential effectiveness of their BP-CS, was compared.



The answer to ToR 3 of the mandate is also provided in Chapter 4, making use of case-studies (countries or regions) to discuss the possibility to define a minimum frequency of the ARR allele in a sheep population above which fade-out of CS is expected.

Finally, as requested in ToR 5, the last part of Chapter 4 includes a series of recommendations for additional/alternative measures to control CS in sheep and goats, based on the current state of knowledge. All information and conclusions discussed in earlier parts of the document are used to substantiate the recommendations formulated.

## 1.2. Classical scrapie

### 1.2.1. Background

Scrapie in small ruminants is the archetype of the TSEs, or prion diseases, and has been recognised as a disease in sheep and goats for almost 300 years although many aspects of the disease are still poorly understood. It was reported for the first time in sheep in the United Kingdom in 1732 and a few years later, in 1759, in Germany. Since then, scrapie has become endemic in the national flocks of several countries (Detwiler, 1992). CS has also been reported as a naturally-occurring disease in goats since it was first described in France in the 1940s (Chelle, 1942).

TSEs in small ruminants are characterized by long asymptomatic incubation periods that usually range between two and seven years, during which time infected animals are a potential source of contamination (vanKeulen et al., 1996; Andreoletti et al., 2002a; Baylis et al., 2004). The disease is thought to have spread across the world through the export of asymptomatic infected animals (for review see Detwiler and Baylis (2003)).

### 1.2.2. Genetic susceptibility

CS is an infectious disease of small ruminants for which the susceptibility is strongly influenced by different polymorphisms of the *PRNP* gene that encodes for prion protein (PrP). In sheep the polymorphism at codons 136 (A or V), 154 (R or H) and 171 (R, Q or H) have been demonstrated to be of major importance (Cloucard et al., 1995; Hunter et al., 1996). Under natural exposure conditions VRQ/VRQ, ARQ/VRQ and ARQ/ARQ genotype animals are considered to be the most susceptible to CS, whereas homozygous or heterozygous AHQ and heterozygous ARR animals only show a marginal susceptibility. ARR/ARR sheep are considered to be strongly (but not absolutely) resistant to CS (Elsen et al., 1999; Groschup et al., 2007). Beside these three major polymorphisms other polymorphisms like the K176 or the T137 have more recently been recognized to be associated with resistance to CS infection (Vaccari et al., 2009b), and experimental studies have identified a resistance to Bovine Spongiform Encephalopathy (BSE) in sheep with the T112 polymorphism (Saunders et al., 2009), although the significance of this substitution for CS susceptibility has not been established.

In goats, the information related to PrP polymorphisms associated with susceptibility to CS is more limited than for sheep, and the data available for naturally-infected goats have mainly resulted from field studies (Gonzalez et al., 2009; EFSA, 2012; Corbiere et al., 2013a; Ortiz-Pelaez et al., 2014b) that yielded small numbers of positive animals. The goat *PRNP* gene is highly polymorphic, but many of the polymorphisms have been observed only in one or two countries. However, eight out of the 29 reported amino acid changes seem to have worldwide distribution (by haplotype ranking, from most common: P240, R143, S127, H154, K222, Q211, M142, S146) and at least five of these have been suggested to be associated with TSE susceptibility (Vaccari et al., 2009a). Case/control studies carried out in Italy and France have demonstrated a potentially highly protective effect of the K222 allele (Acutis et al., 2006; Vaccari et al., 2006; Barillet et al., 2009; Corbiere et al., 2013a) against CS. This view was recently reinforced by the apparent resistance of animals with the K222 allele to infection following experimental challenge with a CS isolate (Acutis et al., 2012). Similarly, the Q211 allele has been associated with an increase in resistance to CS in infected French herds (Barillet et al., 2009; Corbiere et al., 2013a). The H154 allele has also been associated with some level of resistance to CS

in studies carried out in Greece, France, Italy and Cyprus (Billinis et al., 2002; Vaccari et al., 2006; Barillet et al., 2009; Corbiere et al., 2013a). Studies conducted in Cyprus support the view that S146 and D146 polymorphisms of the *PRNP* gene are likely to be associated with substantial resistance to CS in goats naturally exposed to disease (EFSA, 2012; Ortiz-Pelaez et al., 2014b). This contention was further supported by the results of an experimental challenge of S146 allele carriers goats conducted in the United States of America (USA) (White et al., 2012).

Until relatively recently scrapie was thought to be a 'slow virus' disease, which was passed through familial lines, and disease control within a flock centred on the culling of maternal lines. Given what is now known on genetic susceptibility, it is not surprising that this method was successful in limiting the disease within a flock.

In addition to the importance of individual genetic susceptibility, it has been suggested that the risk of CS occurrence may also be associated to the flock-level PrP genotype profile (Baylis et al., 2000; McIntyre et al., 2008b; Tongue et al., 2009; Ortiz-Pelaez and Bianchini, 2011).

### 1.2.3. Agent diversity

Despite the relative uniformity of the clinical signs in the natural host, CS can be caused by TSE 'strains' with different biological features. Historically, the appreciation of the potential diversity of the scrapie agent has relied on the serial passaging of natural isolates to a panel of inbred mouse lines. The strains that preferentially replicated in the mice were isolated and their biological phenotypes compared. However, the considerable strain variations initially described in these experimental situations (up to 20 strains (Dickinson, 1976)) might finally be considered to be representative of the three main distinct strains which can be recognised in a single wild-type mouse line (Bruce et al., 2002). The relationship between these mouse-adapted TSE agents and those initially present in the TSE field isolates is a matter of debate. The propagation of natural TSE isolates into inbred wild-type mouse lines requires passage through a species/transmission barrier that can result in differing outcomes depending on the donor/recipient combinations, including changes in the biological properties of the agent. Moreover, wild-type mice have been described to be refractory to some TSE isolates, such as CH1641 (Foster and Dickinson, 1988) and 'Suffolk' scrapie (Thackray et al., 2012), and to AS (see below) (Le Dur et al., 2005; Bruce et al., 2007). In that context it is clear that the conventional wild-type mouse models do not provide a comprehensive and reliable picture of the diversity of the TSE agents in small ruminants, although transgenic mice are proving to be susceptible to a wider range of TSEs, enabling more comprehensive characterisation of strains (Thackray et al., 2012).

There are many similarities between CS in sheep and goats with regard to clinical signs, pathogenesis and pathology, but less is known about the biological diversity of the TSE agents causing disease in goats. It has been established experimentally that goat CS isolates can affect sheep (Konold et al., 2013a) but there has been no systematic assessment of how much, if any, cross-species transmission may occur in a mixed population.

### 1.2.4. Disease pathogenesis

According to studies on the pathogenesis of CS in naturally infected sheep, which focus predominantly on VRQ/VRQ sheep born and raised in two individual flocks (one in the Netherlands and one in France), infection apparently occurs via the Gut Associated Lymphoid Tissues (GALT) before a rapid spread of the agent to draining mesenteric lymph nodes and later to all lymph nodes, including those that remain on prepared carcasses (Andreoletti et al., 2000; van Keulen et al., 2000; van Keulen et al., 2008). The amount of PrP<sup>Sc</sup> in lymphoid formations increases with age before reaching a plateau level. The TSE agent spreads to the central nervous system (CNS) (brain and spinal cord) where PrP<sup>Sc</sup> can be shown to accumulate from around half way through the incubation period. This neuroinvasion apparently occurs via the enteric nervous system and its nerve fibres (Andreoletti et al., 2000; van Keulen et al., 2000), although haematogenous spread has also been proposed as a

contributory/alternative route (Siso et al., 2009). From there the agent redistributes (centrifugally) to the peripheral nervous system and skeletal muscle (Andreoletti et al., 2004). In blood, infectivity and PrP<sup>Sc</sup> can be detected as early as at three months of age, and persist throughout the incubation period (Lacroux et al., 2012). This pattern of dissemination is consistent with most of the data reported with regard to natural CS cases. However VRQ/VRQ is considered to be the most susceptible/permissive sheep PrP genotype for the majority of CS strains (Baylis et al., 2004; Hagenaars et al., 2010). In sheep bearing other PrP genotypes the kinetics of agent distribution in affected animals can vary substantially. For example, in heterozygote ARR sheep and under natural exposure conditions, the PrP<sup>Sc</sup> distribution seems to be mostly confined to the CNS (vanKeulen et al., 1996; Andreoletti et al., 2002b). Additionally, in several CS cases in ARQ/VRQ and ARQ/ARQ sheep (Jeffrey et al., 2002; Ligios et al., 2006) PrP<sup>Sc</sup> accumulation in the CNS has been reported in the absence of detectable PrP<sup>Sc</sup> in the lymphoid tissues. Experimental studies confirm the observations from natural disease that amplification and accumulation of PrP<sup>Sc</sup> occurs in LRS tissues, and is host genotype dependent (Gonzalez et al., 2014a), but does not necessarily have a marked effect on the outcome of the infection (Gonzalez et al., 2014b). Meta-analysis of data from ARR/ARR sheep confirms that these animals are extremely resistant to challenge with CS (Jeffrey et al., 2014).

In goat the kinetics of agent distribution in the organs of affected animals can vary substantially depending on *PRNP* genotype, including cases in which PrP<sup>Sc</sup> accumulation in the central nervous system was reported in the absence of detectable PrP<sup>Sc</sup> in the lymphoid tissues (Gonzalez et al., 2009).

### 1.2.5. Exposure and transmission

It is still unclear precisely which sources of infectivity and routes of transmission are possible, and which have the greatest effect on the spread and maintenance of infection in a population.

It is uncertain if true vertical transmission can occur. Although there are no data providing direct demonstration of germ cell involvement, data recently reviewed in the context of the EFSA Opinion on the risk of transmission of CS via *in vivo* derived embryo transfer in sheep (EFSA BIOHAZ Panel, 2013) reinforce the view that CS could be vertically transmitted. Recent improvements in PrP detection methods have led to the demonstration of disease-associated PrP in foetuses from CS-infected ewes, indicating the potential for *in utero* infection (Carmen Garza et al., 2011). Infectivity has also been detected by bioassay in the mesenteric lymph node of a foetus from a CS-affected ewe (Spiropoulos et al., 2014).

It is commonly hypothesised that under conditions of natural exposure, infection with CS mainly occurs around birth (maternal transmission) and that placenta, which can accumulate large amounts of prions in incubating animals, plays a major role in this process (Race et al., 1998; Tuo et al., 2002; Lacroux et al., 2007; O'Rourke et al., 2011). For specific flock-level outbreaks this hypothesis has been supported by epidemiological evidence for exposure and/or susceptibility declining with age (Matthews et al., 2001; Nodelijk et al., 2011) and for increased transmission during lambing (Touzeau et al., 2006). It has been shown that disposing of the placenta in the compost and spreading sheep compost on the land were associated with increased probability of scrapie in a sheep flock (Healy et al., 2004). Moreover a seasonality in the occurrence of the disease has also been associated with lambing time (McIntyre et al., 2008b). The genotype of the foetus is pivotal in determining the extent to which PrP can accumulate in the placenta so a breeding for resistance programme can lead to a distinct improvement on this aspect of disease control very quickly, by reducing the potential amount of infectivity produced by this route by infected animals (Andreoletti et al., 2002a).

Milk has also been demonstrated to be a route of transmission from dam to lamb. Both colostrum and milk were shown to contain infectivity and their capacity to transmit disease to suckling lambs was demonstrated (Konold et al., 2008; Lacroux et al., 2008; Konold et al., 2013b). The results obtained in these studies clearly demonstrated that even a limited amount of colostrum/milk from CS infected ewes is able to transmit CS to genetically susceptible lambs. Lympho-proliferative mastitis seems to enhance the efficacy of the transmission; nevertheless colostrum/milk collected from ewes displaying

an apparently healthy mammary gland was also efficient in transmitting the disease. The presence of infectivity in goat milk has also been recently confirmed (Konold et al., 2013b).

It has been shown in scrapie and other TSEs (i.e. Chronic Wasting Disease) (Gough and Maddison, 2010) that faeces, urine and saliva can all carry infectivity, but the extent to which these contribute to the spread of scrapie in commercial situations is not clear. It is thought that faecal shedding is likely to be linked to the accumulation of PrP<sup>Sc</sup> in the GALT of susceptible animals, which then acts as a source of infectivity which could be shed in the faeces. It is well-established that peripheral accumulation of PrP<sup>Sc</sup> is very strongly associated with genotype, with widespread dissemination of PrP<sup>Sc</sup> in the most susceptible genotypes. The presence of a resistant allele in a heterozygous animal, while not effective at preventing disease, will greatly reduce the peripheral accumulation of PrP and, by assumption/extrapolation the extent to which that animal represents a source of infection for its flock mates.

CS infectivity is very robust, and cannot readily be inactivated by standard microbiological disinfection procedures. Once shed into the environment TSE agents have been shown to resist to degradation over long periods in soil (Genovesi et al., 2007; Wiggins, 2009; Smith et al., 2011). There is also evidence of environmental persistence on farm equipment such as pens and troughs, in addition to pasture (Maddison et al., 2010).

#### 1.2.6. Epidemiology

Disease can be introduced into populations by the movement of animals (Palsson, 1979; Healy et al., 2004; McIntyre et al., 2008b) and it has also been established that healthy adult animals introduced into a flock and/or a contaminated environment can develop disease, without exposure to lambing environments or close familial contact (Detwiler and Baylis, 2003; Ryder et al., 2004; Dexter et al., 2009). It has also been demonstrated under controlled conditions that close contact between infected and naive animals (even at an early preclinical stage) results in effective transmission between animals of susceptible genotype (Konold et al., 2008; Ryder et al., 2009). The efficacy of such lateral transmission appeared to be lower in older animals than in younger animals. The mechanism underlying such transmission remains unclear and both inter-individual horizontal transmission and/or environmental sources could be at their origin. However in general the occurrence of the disease has been associated with large flock size, which may increase the probability of interindividual contacts (Healy et al., 2004; McIntyre et al., 2008b; Stevens et al., 2009).

A key management strategy for individual on-farm disease reduction has been to maximise lambing hygiene, and reduce as much as possible the contact between animals and placental debris post-partum (Dexter et al., 2009).

Iatrogenic TSE transmissions in sheep have also been reported on several occasions. In the UK, brain, spinal cord and spleen tissues from young sheep were used to produce an inactivated vaccine against ovine encephalomyelitis or 'loupings-ill' (Flavivirus). The administration of this vaccine was identified as the cause of scrapie outbreaks in a number of flocks (Gordon, 1946). More recently, in Italy, several hundred CS cases were observed in sheep and goats that had been vaccinated against *Mycoplasma agalactiae* with a vaccine produced using homogenised, filtered ovine brains, mammary glands and lymph nodes (Capucchio et al., 1998): recently a cohort study confirmed the impact of this iatrogenic transmission on the spread of CS in Italy (Bertolini et al., 2012).

The within-flock prevalence of CS is affected by all of the parameters discussed above. Very high prevalence can be obtained in experimental flocks where a high proportion of susceptible genotypes are actively maintained, and husbandry practices favour the spread of infection (Elsen et al., 1999; Dexter et al., 2009). The estimation of individual within-flock prevalence depends on the selected flocks and the follow-up period during which positive cases were confirmed. In some cases it is not a real prevalence of infection (proportion of positive animals at one point on time). The results obtained by monitoring infected flocks may be different from the estimates derived from surveillance data. In

one longitudinal study of 15 individual commercial infected sheep flocks, the flock-level prevalence estimates varied from 0 to 15.4 per cent when culled animals were screened by immunohistochemistry (IHC) for evidence of infection (Tongue et al., 2005). McIntyre et al. (2008b) identified 415 cases of CS in 30 infected flocks, with numbers of cases per flock varying from 1 to 131, with seven flocks having only a single case of CS. Using the snapshot of 213 infected flocks at the time of cull and testing, as part of compulsory eradication measures, an average of 0.65 % of tested sheep presented detectable infection by the available approved diagnostic methods at the time of testing; 68.5 % of the flocks did not have any other case (prevalence 0 %) and 22 % of the flocks had a within-flock prevalence higher than 1 % (maximum of 8.5 %) (Ortiz-Pelaez and Del Rio Vilas, 2009). In all cases the variability of within-flock prevalence was very high. A similar wide range of within-herd prevalence has also been reported in goats in several countries (Gonzalez et al., 2009; Corbiere et al., 2013a; Ortiz-Pelaez et al., 2014b), as discussed in more detail in Section 2.4.5.

Reducing the spread of CS based on primary prevention would require the elimination of the main risk factors. Unfortunately it is very difficult to reduce or eliminate the exchange of animals between farms, to decrease the size of flocks or even act on the age distribution of the animals. Moreover within flocks effective disease control is very complex, and needs to take into account the sources of contamination, effectiveness of cleaning/decontamination procedures, and the restriction of exposure of susceptible animals to such sources. The most powerful tools presently available for disease control in any flock which is not disease-free and closed are the effective identification and removal of infected animals, and, in the case of sheep, the control of the genetic composition of the flock, i.e. the methods covered by the current regulations. For a field study showing the success of selective breeding to control CS at the flock level see Nodelijk et al. (2011). Mathematical modelling studies have been applied to data of within-flock outbreaks and of data at national-flock level, yielding a range of epidemiological insights (for a review see Gubbins et al. (2010)). Concerning within-flock outbreaks, some of the insights might be outbreak specific. More generic insights include the following:

- The duration of within-flock CS outbreaks is of the order of 5 years or longer.
- Most cases arise through horizontal transmission (as opposed to vertical transmission).
- The hypothesis of increased transmission during lambing has been supported by an analysis of CS incidence in French study flock.

Concerning the national-flock level, modelling has been used to estimate infection levels from detected case prevalence, and to assess the efficacy of control strategies including the impact of selective breeding. General insights include:

- Trading restrictions have a limited impact on controlling CS transmission risks as compared to selective breeding and culling.

Many infected sheep do not survive to show clinical signs, and a large proportion of cases remains undetected/unreported to passive surveillance.

### **1.3. Atypical scrapie**

#### **1.3.1. Background**

In 1998, the definition of ovine TSEs was extended by the discovery, in Norway, of an experimentally transmissible, PrP-related, neurological disease of sheep that was clearly distinguishable from the CS cases that had been reported so far. It was therefore considered to be an 'atypical' form of scrapie, also named 'Nor98' scrapie (Benestad et al., 2008). Following its recognition in sheep, AS was also detected in goats in France (Le Dur et al., 2005; Arsac et al., 2007), Spain (Vaccari et al., 2009a), Switzerland (Seuberlich et al., 2007) and Italy (Colussi et al., 2008).



### 1.3.2. Genetic susceptibility

Importantly, the susceptible genotype range is also different from CS, with the genotypes most resistant to CS occurring frequently in the atypical population and vice versa. While a clearly increased risk for developing AS is associated with AF<sub>141</sub>RQ and AHQ alleles, the VRQ allele seems to be at lower risk. Strikingly ARR allele carriers (both homozygous and heterozygous) can develop the disease (Moum et al., 2005; Arsac et al., 2007; Moreno et al., 2007). The differences in observed susceptibility of small ruminants to classical and AS illustrate that successful infection with TSE is strongly influenced by the nature of the agent involved, and the genetics of the host. The relative effectiveness of CS control measures means that AS is now more common in some populations than CS.

Affected goats from France and Switzerland carried the H154 mutation, and an Italian case control study demonstrated that the H<sub>154</sub>Q<sub>222</sub>S<sub>240</sub> allele is a risk factor for AS (Colussi et al., 2008). This allele is homologous to the ovine AHQ allele, which is associated with high susceptibility to AS in sheep, suggesting that the agent–host interaction is similar in the two species. Mutation L<sub>141</sub>F, associated with very high risk of AS in sheep, or any other mutation at codon 141, has not been reported in the caprine *PRNP* gene.

### 1.3.3. Agent diversity

In AS cases the abnormal PrP<sup>Sc</sup> that accumulates in the brain of positive animals is only partially proteinase K (PK) resistant (Buschmann et al., 2004) and displays a multi-band pattern as shown by Western blot (WB) that contrasts with those normally observed in small ruminant TSE cases (Benestad et al., 2003). The pathology of AS is also unique (Moore et al., 2008). However, unlike CS, the biological properties of these atypical TSE isolates (regardless of host genotype) are very consistent, even in experimental rodent assays (Le Dur et al., 2005; Griffiths et al., 2013).

### 1.3.4. Disease pathogenesis

The pathogenesis of AS remains poorly documented. Abnormal PrP has never been demonstrated in peripheral tissues collected from field cases or experimental cases (Andreoletti et al., 2011; Benestad et al., 2008; Simmons et al., 2011). However, recent information obtained in both natural and experimental atypical cases demonstrated that low levels of infectivity can be present in skeletal muscle, peripheral nerves and lymphoid tissues of affected animals in the absence of detectable PrP<sup>Sc</sup> (Andreoletti et al., 2011; Simmons et al., 2011).

### 1.3.5. Exposure and transmission

The apparent restriction of the infectious agent to the CNS has been widely interpreted to be supportive of the hypothesis that AS could be a spontaneous disorder of PrP folding and metabolism, occurring in aged animals without external cause (Benestad et al., 2008). In addition, no statistical difference in the detectable AS prevalence was observed between the general population and the flocks where a positive case had been identified (Fediaevsky et al., 2010), indicating that it does not present, epidemiologically, like an infectious disease. This has been interpreted as evidence that AS may not be contagious at all.

While the contagiousness of AS under natural conditions is still debated, the transmissibility of the AS agent by the intracerebral route is clearly established in both rodent models (transgenic animals over-expressing the ovine PrP gene (Le Dur et al., 2005)) and in sheep (Simmons et al., 2007; Simmons et al., 2010). Data from an oral challenge study in sheep also indicate that very early exposure (within 24 hours of birth) can lead to transmission of the disease, resulting in clinically affected animals displaying a similar clinic-pathological pattern to those observed in natural cases (Simmons et al., 2010). It is probably too early, therefore, to conclude if this is a spontaneous, non-contagious disorder or if it can behave like other TSE agents circulating in small ruminants.

### 1.3.6. Epidemiology

The clinical features observed in AS differ significantly from those observed in CS affected sheep (Konold et al., 2007) which may account for the low suspect referral rate, as might the average age at onset, with atypical cases usually presenting at a substantially older age (more than 6 years old) than CS cases (Benestad et al., 2008).

Following the introduction of active surveillance of fallen stock and healthy slaughter populations, AS has been identified as occurring at a low but very consistent prevalence in small ruminant populations in which there has been screening. It has been found in populations in which CS has not been reported, such as the USA and Canada (Benestad et al., 2008), and more recently also in Australia and New Zealand (Kittelberger et al., 2010), two countries that had so far been considered by the OIE as TSE free. A retrospective study carried out in tissues banks allowed the identification of atypical cases in sheep samples collected in UK as far back as 1987 (Webb et al., 2009). The analysis of data collected through the epidemio-surveillance system in the UK between 2002 and 2006 suggests that AS prevalence in this population could have remained stable over this period (McIntyre et al., 2008a; Ortiz-Pelaez and Arnold, 2013). Together these elements suggest that this form of disease might have been present but undetected in the small ruminant population at least for several decades.

AS now represents a substantial proportion of the TSE cases identified in the EU small ruminant population. Several studies described the apparent prevalence of AS in sheep slaughtered for human consumption (healthy slaughtered animals) or collected as fallen stock. In a study between 2002 and 2007 that included 11 European countries, the mean prevalence of this disease was estimated to reach 5.5 cases per ten thousand in abattoir surveillance, and 8.1 cases per ten thousand in fallen stock (Fediaevsky et al., 2010). The apparent prevalence of AS in sheep did not present with any important variations between countries (Fediaevsky et al., 2008) or over time (Fediaevsky et al., 2008; McIntyre et al., 2008a). However, these values are likely to be underestimates of the real situation. Indeed a recent study provided evidence that samples from the central nervous system containing high infectious titre (as assessed by bioassay) could remain negative in the tests currently used in active surveillance programs for the detection of PrP<sup>Sc</sup> in field cases (Andreoletti et al., 2011). This suggests that a significant number of animals which may be incubating AS would remain undetected even when tested.

### 1.4. Concluding remarks

- TSEs in small ruminants have a long asymptomatic phase, during which animals can circulate in the population.
- With regards to CS:
  - Susceptibility is strongly influenced by different polymorphisms of the *PRNP* gene. In sheep, the A<sub>136</sub>R<sub>154</sub>R<sub>171</sub> allele provides substantial resistance to the infection in both heterozygote and homozygote animals. In goats, the K<sub>222</sub>, the S<sub>146</sub> and the D<sub>146</sub> alleles also provide substantial resistance to infection.
  - CS can be caused by TSE ‘strains’ with different biological features. There are many similarities in the clinical signs, pathogenesis and pathology of CS in sheep and goats, but less is known about the biological diversity of the TSE agents causing disease in goats.
  - Detection of infected animals and the resultant sensitivity of active surveillance are dependent on the PrP<sup>Sc</sup> distribution within each animal:
    - In the most susceptible genotypes, initial infection occurs via the gut-associated lymphoid tissues, then other lymph nodes. The agent spreads to the brain and



spinal cord approximately half way through the incubation period, and from there redistributes centrifugally to the peripheral nervous system and skeletal muscle.

- In other *PRNP* genotypes the kinetics of agent distribution in affected animals can vary substantially. PrP<sup>Sc</sup> accumulation in the nervous system has been reported in the absence of detectable PrP<sup>Sc</sup> in the lymphoid tissues.
- Horizontal transmission is considered to be the main route of transmission, through exposure to oral infection (e.g. mainly via placentae or milk from infected animals) or to contaminated environments. It is still unclear precisely which sources of infectivity and routes of transmission are possible, and which have the greatest effect on the spread and maintenance of infection in a population.
- The genotype of the foetus is pivotal in determining the extent to which PrP can accumulate in the placenta so a breeding for resistance programme can lead to a distinct improvement on this aspect of disease control very quickly.
- Infected asymptomatic individuals can also disseminate the infectious agents to susceptible offspring and other in-contact animals, and the environment, where the agent can persist for long periods.
- Animal movements, flock size and age are important risk factors, but are difficult to address by preventive measures.
- Disease prevalence in affected flocks is extremely variable.
- The most powerful tools presently available for disease control in any flock which is not closed, and disease-free, are the effective identification and removal of infected animals and the control of the genetic composition of the flock, i.e. the methods covered (for sheep) by the current EU legislation.
- With regard to AS:
  - The genotypes most resistant to CS occur frequently in the AS-infected population. The H154 mutation is considered to be a risk factor for AS in goats. The H<sub>154</sub>Q<sub>222</sub>S<sub>240</sub> allele is homologous to the ovine AHQ allele, which is associated with high susceptibility to AS in sheep, suggesting that the agent-host interaction is similar in the two species.
  - The biological properties of atypical TSE isolates (regardless of host genotype) are very consistent.
  - The late age at onset and the relative protease sensitivity of the atypical PrP limits the sensitivity of AS case detection through current surveillance programmes.
  - AS pathogenesis is poorly documented. PrP<sup>Sc</sup> has never been demonstrated in peripheral tissues but low levels of infectivity can still be present in skeletal muscle, peripheral nerves and lymphoid tissues of affected animals.
  - AS 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. However, disease can be transmitted experimentally by the oral route.
  - AS has been identified as occurring at a low but very consistent prevalence in every small ruminant population in which there has been screening. Retrospective surveys suggest that this

form of disease might have been present but undetected in the small ruminant population for several decades at least.

- AS has been found in populations in which CS has not been reported.

## 2. Monitoring of TSEs in small ruminants

### 2.1. EU legal background

Legislation related to TSEs in the EU was enforced as a consequence of the emergence of BSE in cattle in the UK in the late 80s. The first legislative act linked to TSE dates back to 1989 and established trade restrictions of certain live cattle from the UK to the other MSs<sup>5</sup>. Other measures were progressively implemented in the EU, especially linked to cattle BSE, in order to prevent and limit the spread of the disease to ruminants (e.g. feedban, measures applied to suspect and confirmed TSE cases, trade restrictions) and to humans (e.g. removal of specified risk material (SRM) from animals slaughtered for human consumption).

Whereas BSE was included in the the Community list of animal diseases subject to notification as early as 1990, scrapie became a compulsorily notifiable disease in all MSs in 1993 (Council Directive 91/68/EEC). Requirements for the detection of both BSE in cattle and scrapie in ovine and caprine animals were first established in 1998 (Decision 98/272/EC), focusing on animals showing clinical signs compatible with TSE and on certain categories of high risk animals (i.e. animals originating from countries with indigenous TSE, animals consuming potentially contaminated feedstuffs, and animals with TSE-infected parents). This Decision also introduced into the EU legislation the obligation to notify to the competent authorities, the suspected presence of any TSE in any animal species.

The key piece of legislation integrating all measures for the prevention, control and eradication of TSE Regulation (EC) No 999/2001 ('TSE Regulation'), dates back to 2001, which, after many subsequent amendments, is still in force today. The TSE Regulation sets out the framework for all the measures related to the prevention and control of scrapie in the EU, including notification and monitoring of animal TSE, the feed ban, SRM removal, movement restrictions and other measures for suspect and confirmed cases, and the placing on the market of animal products.

The monitoring systems for scrapie in sheep and goats have evolved over the years. Initially the only testing was of animals showing scrapie-like symptoms, but from 2002 sample-based surveys (active surveillance) were implemented in two (Regulation (EC) No 1248/2001) additional categories of small ruminants: healthy animals slaughtered for human consumption (SHC) and animals not slaughtered for human consumption (fallen dead on farm) (NSHC) over 18 months of age, to be tested using a rapid screening test and a confirmatory test.

The introduction into the legislation of the use of discriminatory tests to differentiate BSE from scrapie in positive TSE cases in small ruminants dates back to 2005 (Regulation (EC) No 36/2005). During the same year a goat was confirmed positive for BSE in France (Eloit et al., 2005). As a result, TSE testing requirements for goats were increased in the EU. A similar increase in TSE tests to be carried out in sheep was implemented in 2006, due to the identification of two possible BSE-like cases in sheep in France and one in Cyprus, which were subsequently confirmed to be scrapie rather than BSE<sup>6</sup>. In the absence of further BSE positive findings in sheep or goats, the target for TSE testing was decreased again in both species in 2007. In the same year, the requirement to differentiate between CS and AS cases was introduced into the EU legislation. Currently, the number of tests to be carried out in both sheep and goats over 18 months of age is dependent on the adult population size, with a requirement to test up to 10 000 representative samples for each species and risk category in MSs with

<sup>5</sup> Commission Decision 89/469/EEC of 28 July 1989 concerning certain protection measures relating to bovine spongiform encephalopathy in the United Kingdom, OJ L 225, 3.8.1989, p.51.

<sup>6</sup> See: [http://europa.eu/rapid/press-release\\_IP-06-288\\_en.htm](http://europa.eu/rapid/press-release_IP-06-288_en.htm)

populations larger than 750 000. Additional samples are required from animals belonging to infected flocks culled following compulsory eradication measures, and, voluntarily, in other categories of animals.

## **2.2. Information to be reported in the EU TSE Database**

The TSE Regulation requires all EU MSs to submit a yearly report to the Commission including the results of the monitoring of TSE in ruminants. The Commission produces an annual report summarising the information provided by all MSs<sup>7</sup>. In relation to small ruminants, MSs report to the Commission information on the general results of the monitoring programme carried out, on TSE suspected cases and TSE positive cases, which are gathered into the EU TSE Database.

In particular, MSs report the number of ovine and caprine animals tested within each subpopulation in the framework of the monitoring programme, together with the method for sample selection and the results of the rapid and confirmatory tests. In relation to suspected TSE cases, MSs report the number of suspected cases placed under official movement restrictions, the ones subjected to laboratory examination per animal species, including the results of the rapid and confirmatory tests, and the number of flocks where suspected cases in ovine and caprine animals have been reported and investigated. Finally, with respect to positive cases of CS, AS and BSE, MSs report the geographical distribution, including the country of origin, the year and, if possible, the month of birth of the animal, the results of the primary molecular testing for discriminating scrapie and BSE cases and, for sheep, the genotype and, if possible, the breed.

Throughout the years this information was collected by the European Commission from MSs in different ways, until a harmonised database and submission system were implemented in 2006, which also enabled the collection of additional information. MSs directly provide national data to the EU TSE database by means of two different types of report: 'monthly reports' and 'case reports', for both sheep and goats.

'Monthly reports' are submitted monthly by MSs and include aggregated information in relation to all TSE tests performed in sheep and goats and include data on the animals tested (i.e. age, target group), on the flock (i.e. whether the flock is under official control following to TSE cases or not, and geographical location), the first test (i.e. which rapid test was used as the initial screening test) and the total number of animals tested, including positive, negative and inconclusive results.

'Case reports' are submitted yearly by MSs and include detailed individual information related to positive TSE cases diagnosed in sheep and goats. Data should be submitted on the positive animal (i.e. scrapie type, unique national case number, month and year of birth, country of origin, breed and genotype), on the flock of origin of the animal (national flock identification number, geographical location), and on the tests performed (i.e. type and result of rapid test(s) and confirmation method(s) used to identify the case, discriminatory method(s) used to differentiate CS from BSE).

## **2.3. A questionnaire survey on the monitoring sampling strategy in EU Member States**

As further discussed below, the design and practical implementation of surveillance activities, and the control and eradication measures in place, can substantially influence the collected data and their interpretation. In order to collect information on the sampling strategy and control measures implemented in EU MSs, a questionnaire was developed and circulated to all MSs. The questionnaire included, for both active surveillance target groups (i.e. healthy slaughtered animals (abattoir survey) and animals not slaughtered for human consumption (fallen stock)), a set of questions on the sampling design, representativeness of the sheep and goats tested, potential bias, evolution of the strategy and screening testing protocol over the years, specific monitoring measures implemented in the country, etc. In addition, it included questions on the measures implemented for the control and eradication of classical and AS in sheep and goats, any derogations implemented, the epidemiological investigations

<sup>7</sup> See: [http://ec.europa.eu/food/food/biosafety/tse\\_bse/monitoring\\_en.htm](http://ec.europa.eu/food/food/biosafety/tse_bse/monitoring_en.htm)

carried out in outbreaks, etc. A copy of the questionnaire circulated to MSs and an overview of the replies collected is included in Appendix A.1.

The information gathered from MSs was taken into consideration when interpreting the results of the analysis of the trends of classical and AS in the EU MSs, also in association with the potential impact of the control measures implemented, in order to inform the response to Term of Reference 1 of the mandate for this Opinion (see Sections 3.2 and 4.5.5).

## **2.4. Sensitivity of the current EU monitoring system**

### **2.4.1. Passive surveillance**

Initially, identification of CS relied on clinical suspicion of disease, and voluntary reporting of this suspicion by the farmer to the relevant veterinary authorities (passive surveillance). Such disease monitoring relies on farmer knowledge and motivation, and is easily affected by the presence of specific economic incentives or disincentives for disease detection. For example, compensation for affected animals will be likely to increase reporting, whereas commercial penalties such as animal movement restrictions or compulsory culling may be a disincentive to reporting suspicion in a previously unaffected flock. Passive surveillance is more likely to record cases from flocks with a previous history/knowledge of scrapie, rather than identifying new affected flocks and in general is not able to provide additional and complementary information in comparison with the active system (Bertolini et al., 2011). An anonymous postal survey of farmers in the UK at the height of TSE awareness suggested that only 13 % of farmers who thought they might have had scrapie had actually reported this (Hoinville et al., 2000). In the USA, it has been reported that over 80 % of the variability in the incidence of scrapie was the result of reporting (Kuchler and Hamm, 2000). Farmers may seek to benefit from the favourable regulatory context and compensation schemes linked to statutory control and eradication measures (Ortiz-Pelaez and Del Rio Vilas, 2009). This might increase farmers' efforts to report disease. Overall, therefore, passive surveillance has a very variable efficiency. Given the extremely limited information regarding the clinical presentation of AS, and the fact that it is so dissimilar from CS, passive surveillance is particularly poor at detecting this form of disease.

### **2.4.2. Active surveillance**

In the EU, the active surveillance for TSE in small ruminants, as currently understood, has been implemented since 2002. It relies on the testing of a sample of the apparently healthy animals slaughtered for human consumption (abattoir survey) and found-dead animals (fallen stock). The data collected through surveillance have clearly demonstrated that earlier evaluations of the prevalence of TSE in small ruminants and their geographical distribution (on the basis of passive surveillance) were largely underestimated, and the within-flock prevalence was on average 20 times higher than the apparent prevalence in the general population identified by active surveillance (Fediaevsky et al., 2008).

### **2.4.3. Factors affecting active surveillance – test sensitivity**

Various PrP detection methods are applied in the context of statutory surveillance (ELISA, WB, IHC) but all of these are immunodetection methods. None of the antibodies currently used in these tests can distinguish between the normal host cellular PrP and the disease related abnormal isoform PrP<sup>Sc</sup>. However, the majority of tests exploit the relative protease resistance of PrP<sup>Sc</sup> by introducing a preparatory step of PK digestion in the sample preparation, which leaves only the disease-related protein for detection. This system has proved robust for BSE and CS, but the discovery of AS, which is more PK sensitive (Groschup et al., 2007) has led to concerns about test sensitivity for this form of disease.

The methods currently approved for use within the EU for statutory screening purposes must meet certain minimum standards relative to each other, but, historically, some of the tests used for scrapie surveillance have had very poor sensitivity for AS.

The European Commission (EC) originally commissioned an evaluation exercise of rapid *post-mortem* TSE tests in 1999 (Decision 2000/374/EC). Several tests were assessed using brain tissue from clinical cases of BSE in cattle. Three tests were approved under the TSE Regulation. A subsequent laboratory evaluation commissioned by the EC examined the performance of further proposed *post-mortem* rapid tests using brain tissue from clinical cases of BSE in cattle which resulted in two further tests being approved for use (Regulation (EC) No 1053/2003). In 2003, an EFSA Working Group on TSE testing designed a refined laboratory evaluation of selected rapid *post-mortem* BSE tests leading to seven new tests recommended for approval by the European Commission in the framework of the TSE Regulation.

No evaluation of rapid TSE tests on material from small ruminants by the European Commission was possible before February 2004. In the absence of such data, tests performing satisfactorily on bovine tissues were provisionally approved for small ruminants and used for surveillance of TSE in sheep and goats during 2002-2004. In 2005 an EU evaluation exercise of rapid *post-mortem* TSE tests intended for small ruminants was undertaken. This involved an evaluation of diagnostic and analytical sensitivity, and diagnostic specificity and repeatability of six rapid *post-mortem* tests on samples from natural scrapie cases. Additionally the capability of these tests and their diagnostic sensitivity for the detection of the newly identified 'atypical' scrapie strain (Nor98) in sheep tissue were evaluated. Based on these data, tests were specifically approved by the EC for the *post-mortem* testing of slaughtered small ruminants in accordance with the TSE Regulation (as amended by Regulation (EC) No 253/2006).

Further modifications were made to Annex X of the TSE Regulation in April 2008 (Regulation (EC) No 315/2008), defining twelve approved tests for use in the rapid testing of BSE in cattle and nine tests for use in the rapid testing of TSE in sheep and goats. At this point, some tests were not able to meet all the requirements for AS and therefore were not recommended for use for TSE monitoring in small ruminants, and were subsequently delisted from Annex X of the TSE Regulation. This test approval list has remained stable since 2008, although market forces have resulted in the loss of some tests from use.

It should be noted that all of these evaluations were based on sheep samples, and approval extrapolated to use in 'small ruminants'. At no point positive goat samples have been included in any manufacturer test development or independent evaluation sample panel.

All the currently approved tests are required to fall within an analytical sensitivity of a maximal  $2\log_{10}$  inferiority of the most sensitive test. Despite the potential for apparent differences in analytical sensitivity it was concluded by EFSA (EFSA BIOHAZ Panel, 2009) that "*no potential differences in field detection performance can be inferred on the sole basis of the difference in analytical sensitivity reported in this study*".

While testing laboratories are kept 'under control' by the regulatory requirement to apply tests within recognised quality systems (ISO 17025 or equivalent (Regulation (EC) No 882/2004)), the initial selection of animals and sampling of material falls largely outside this procedural control.

#### **2.4.4. Factors affecting active surveillance – individual animal sampling**

The basis for the current active surveillance screening activities across the EU is the detection of PrP<sup>Sc</sup> accumulation in the brainstem. Regardless of the analytical sensitivity of the test used, sample location is key to good diagnostic sensitivity. PrP<sup>Sc</sup> is detectable in the brainstem in pre-clinical disease (see Section 1.2.4) and this is the reason why at an individual animal level, the testing of clinically suspect animals for confirmation of disease (i.e. passive surveillance) is very effective. The accumulation of



PrP<sup>Sc</sup> within the lymphoreticular system (LRS) can also be used to detect preclinical infection, if necessary *in vivo* for high value animals, but successful detection is only possible for disease in animals with genotypes that predispose to peripheral PrP accumulation (see Section 1.2.4), so a negative result has to be carefully qualified by context. The combination of tissue choice, age at testing and the accuracy of sampling will all have an effect on the overall diagnostic sensitivity of screening. In brainstem, the anatomical distribution of PrP<sup>Sc</sup> is very localised to the dorsal nucleus of the vagus nerve (DNV) in early CS, and the nucleus of the trigeminal tract in AS (even at clinical endpoint). Robust and accurate sampling of these target areas is essential to give confidence in a negative biochemical result. IHC has the advantage in this regard because anatomical context is retained, but this is not practical as a rapid screening test for large populations.

#### **2.4.5. Factors affecting active surveillance – population sampling and surveillance strategies**

As previously described, EU TSE active surveillance requires MSs to carry out a targeted sample-based monitoring on adult small ruminants.

In general, the purposes of surveillance may be different: substantiating freedom from disease, monitoring the epidemiological evolution of disease in time and space, or detecting cases of disease to facilitate its control (Hoinville et al., 2013).

##### **2.4.5.1. Surveillance to monitor the epidemiological evolution in time and space.**

In the context of this Opinion, the results of the EU sample-based surveillance strategy are used to monitor the evolution of the occurrence of scrapie and to assess the effectiveness of the control measures implemented by MSs. However, a sample-based active surveillance programme may enable the monitoring of the general evolution of the scrapie epidemic in a country without being able to detect a significant proportion of the incident outbreaks.

The representativeness and comparativeness of the sampling strategy and data collected (e.g. from one year to another) are essential to avoid biases in the estimation of the prevalence. When interpreting the prevalence estimates obtained, factors that have an impact on the probability of an animal to be positive also have to be taken into account.

A number of factors potentially affect the validity of the prevalence estimates obtained:

- The surveillance streams targeted by the surveillance (SHC vs. NSHC) and their relative proportion may bias the overall estimates, since the NSHC population is more likely to contain positive animals (Hopp et al., 2003; Del Rio Vilas et al., 2007). Indeed, clinical suspects that are not detected by passive surveillance (e.g. the farmer is not willing to report) may be included in the NSHC stream. However, exceptions are possible for various reasons:
  - some animals die in remote areas and the carcass are never collected and tested, or are in a condition not suitable for testing and are disposed of on farm;
  - some animals not falling within the scope of the fallen stock group might none-the-less be reported as fallen stock, leading to a reduction of the higher risk nature of the NSHC stream (Del Rio Vilas et al., 2007);
  - the same incentives/disincentives as described for passive surveillance may also affect the submission of carcasses from farms with no history of disease.

In this case the confounding effect due to the stream can be handled, if data are available, by conventional statistical techniques of confounding adjustment, allowing the estimation of statistically-unbiased prevalence.

- The age of the population selected for testing, which is generally variable over time and space (e.g. years and countries), may be too low to ensure the detection of pre-clinical cases. In France, the performance of active PrP<sup>Sc</sup> detection in goats was assessed in eight scrapie infected goat herds (Corbiere et al., 2013b). The sensitivity of detection using posterior brainstem appeared to be strongly dependent on the age of tested individuals, which is consistent with the known pathogenesis of TSE. Similar data were obtained from studies of a large UK goat herd (Gonzalez et al., 2009) and four scrapie affected goat herds in Cyprus (Ortiz-Pelaez et al., 2014b). These data confirm that the age of the tested individuals (incubation stage) strongly influences the probability of being detected by surveillance. Even in this case a confounding adjustment would be effective but the availability of accurate age data of tested animals is generally more problematic.
- EU TSE surveillance may not take into sufficient account the heterogeneity of the sheep population and of the scrapie distribution within each MS. Within the EU legislation it is stated that sampling shall be representative of each region and season and avoid the over-representation of any group (e.g. by origin, age, breed, production type); however, no detailed instruction is explicitly provided.
- In a flock or herd with low disease prevalence, the sample size may not be large enough to detect the disease.

In EU surveillance the sampling size only partially takes into account the size of the national sheep population. However, this factor only influences the precision of the prevalence estimate and not its validity.

How large the discrepancy is between the observed prevalence from surveillance and the true prevalence of scrapie infection in the population is not an easy question to answer. To do so, Gubbins and McIntyre (2009) used a back-calculation model, integrating data from multiple surveillance streams. The analysis carried out suggests that, in the period (1993-2007) and country (Great Britain) studied, the true prevalence was about three to about six times higher than the case prevalence detected in healthy slaughter, depending on a scenario parameter for the proportion of the incubation period that need to be elapsed for detecting CS preclinically by the rapid test. In other situations the discrepancy may be less, for example if the age range of animals in the healthy slaughter stream is coinciding better with age range in which the majority of detectable preclinically infected animals are expected to be.

#### 2.4.5.2. Surveillance to detect cases of disease and facilitate its control

As mentioned above, surveillance may also aim at maximizing the detection of cases of disease to facilitate its control. For instance, the National Scrapie Eradication Program (NSEP) in the USA aims, through the *post-mortem* testing of small ruminants, at eradicating CS from the national small ruminant population by 2020 (USDA, 2010).

If the aim is detection for disease control, the relative size of the sample tested compared to the size of the sheep population becomes a very important parameter. The strategy should be to target high risk subpopulations and perform as many tests as possible in order to detect outbreaks. In such a situation, bias is no longer a major concern because the goal is not to obtain a prevalence estimate. Overall, when having the same sample size, the testing programme would be much more effective in controlling scrapie in a country with small sheep population compared to a country with a larger sheep population, because the percentage of animals tested will be much higher.

However studies based on current active surveillance programmes in goats demonstrated that whatever the number of screening tests performed, *post-mortem* surveillance has limited success in identifying infected animals (Gonzalez et al., 2009; Corbiere et al., 2013b; Ortiz-Pelaez et al., 2014b). Similar findings were reported by Hopp et al. (2003) when assessing the performance of TSE active surveillance in the Norwegian sheep population.



In particular Corbiere et al. (2013b) provided quantitative parameters to estimate the performance of scrapie active surveillance at population level, through simulation studies of the capacity of TSE surveillance programs to detect CS infected herds under different testing scenarios. In a first scenario, it was hypothesized that every goat aged over two years that was slaughtered for human consumption or eliminated at a rendering plant would be tested (178 000 animals in total). In this scenario, the estimated proportion of infected herds that would be identified after one year of surveillance using *post-mortem* PrP<sup>Sc</sup> detection tests on posterior brainstem would be 51.0 % (95 % CI: 49.7-52.4). In a second scenario, it was considered that 20 000 tests would be randomly performed each year, 50 % in fallen stock and 50 % in healthy slaughtered animals. In that scenario, after one testing year, only 11.8 % (95 % CI: 10.7 - 12.7) of the CS infected herds would be identified. The comparison between the scenarios shows the relevant impact of sample size on the capacity of outbreak detection.

Moreover in both scenarios the capacity of the surveillance programme to detect infected herds was found to be strongly influenced by the herd size; the smaller the herd, the lower the detection probability. For instance, while scenario 1 was estimated to detect around 75 % of infected herds that contained more than 200 adult goats, only approximately 30 % of the infected herds containing less than 50 adult goats could be detected.

## 2.5. Concluding remarks

- Passive surveillance is very ineffective at identifying new affected flocks or herds, especially if the prevalence is low and if suspicion reporting may lead to socio-economical consequences such as animal movement restrictions or compulsory culling.
- Active surveillance, enforced since 2002 as a targeted sample-based monitoring of adult small ruminants, represents a major improvement of TSE surveillance in the EU.
- The EU TSE surveillance in small ruminants allows the monitoring of the evolution of the disease and the assessment of the effectiveness of control measures implemented by the MSs. It may not take into due account the heterogeneity of the sheep population and scrapie distribution in each MS: deviation from the representativeness of the sampled population may result in reduced validity of the prevalence estimate.
- The observed prevalence obtained through the EU TSE surveillance programme in small ruminants is affected by two main factors: i) the sensitivity of the detection method given the age of the tested animal, the stage of the incubation period and the sampling/testing procedures, and ii) the distribution of testing by surveillance stream. The availability of the relevant data allows the statistical control of their confounding effects.
- Surveillance programmes could be designed to ensure detection of as many scrapie cases and outbreaks as possible as a tool to facilitate the control or the eradication of the disease in a country.

## 3. Trends of Classical and Atypical scrapie in the EU (2002-2012)

### 3.1. Objectives, data and analytical methods

The objective of this analysis was to provide a spatio-temporal description of scrapie occurrence by species, stream, scrapie type and MS, comparing the occurrence between years within MSs.

Two main sources of information were available: the EU TSE database, which collects standardised surveillance data on all testing activities in all MS, and a questionnaire designed specifically to capture additional data specific to aid the interpretation of the data (see Section 2.3). Due to the biased and variable nature of passive surveillance, data from clinical cases were excluded from the analysis, and only the more unbiased active surveillance data, namely the animals slaughtered for human

consumption (SHC) and the animals not slaughtered for human consumption (NSHC) were used for describing national trends in scrapie prevalence. Additionally, flock/herd identifiers made it possible to further filter the data to avoid the inclusion of multiple cases from single premises. Standardised data were only available for individual MSs from the point at which they joined the EU.

A number of limitations/drawbacks were identified in the EU TSE database, such as duplication of records (as a consequence of multiple testing), data being unavailable for statistical analysis at flock level, poor and incomplete data with regards to breed, age or geographical origin of tested animals.

The precision and validity of the crude prevalence rates obtained through the analysis of active surveillance data may have been affected by the targeted and sample-based design of both the SHC and NSHC surveys. Country-specific temporal trends are in general heterogeneous, precluding any meaningful interpretation of the overall temporal trend at the EU27-level. Therefore the analysis and interpretation of the temporal trends has been conducted only at MS level.

Data analysis was conducted separately by species (sheep vs. goats) and disease (CS vs. AS). In each individual subset, descriptive frequency tables were produced showing the breakdown of animals tested, and cases by country, year, surveillance stream (SHC and NSHC) and rapid test. The potential for a confounding effect of stream in the case of CS in both sheep and goats became evident after comparing the stream-specific prevalence and the different distribution of the number of tests carried out in each stream by country or by year. Non-significant differences in the prevalence of AS by stream were observed.

A spatial description of the presence of the diseases (scrapie types) in the two populations of small ruminants was carried out by producing two sets of maps:

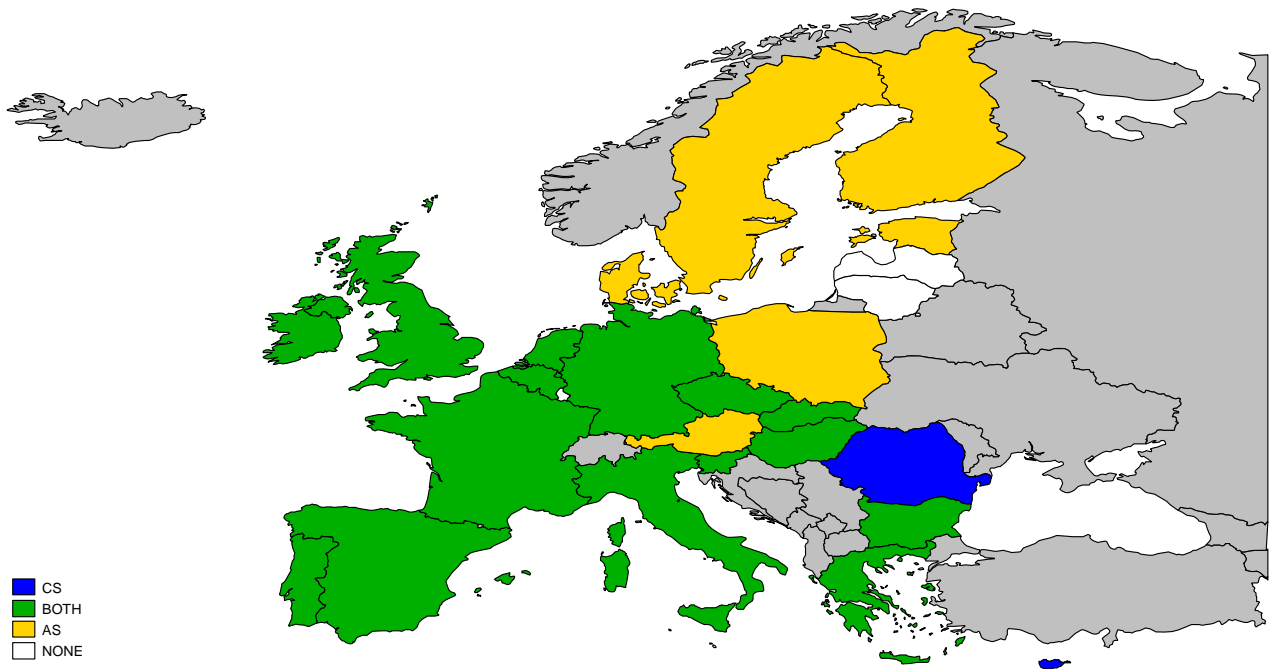
- the occurrence of CS and AS for the period 2002-2012 by species and MS;
- surveillance stream-adjusted (for CS) and crude (for AS) prevalence rates for the period 2002-2012 by species and MS, produced through proportional symbol mapping. The point markers were drawn using absolute minimum and maximum reference values: that allowed each map to be compared with the other maps (e.g. the prevalence of CS in sheep with that in goats). The adjustment on surveillance stream was carried out by means of a direct standardization using the proportion of tests carried out in the MSs in the NSHC vs. SHC in sheep and goats respectively.

Negative binomial models were used to fit 'count of cases detected' and 'year' to estimate the country-specific and stream-adjusted annual prevalence ratios (PRs). Significance levels of the slope of the linear function for individual MS and years were used to determine statistically significant temporal trends. In a preliminary analysis, the type of rapid test, if changed over the period, was included as a covariate in country-level models. However, since no confounding effect was evident, the type of rapid test was not included as a covariate in the final analyses. For more details see Appendix B.

## **3.2. Results and considerations**

### **3.2.1. Classical scrapie in sheep**

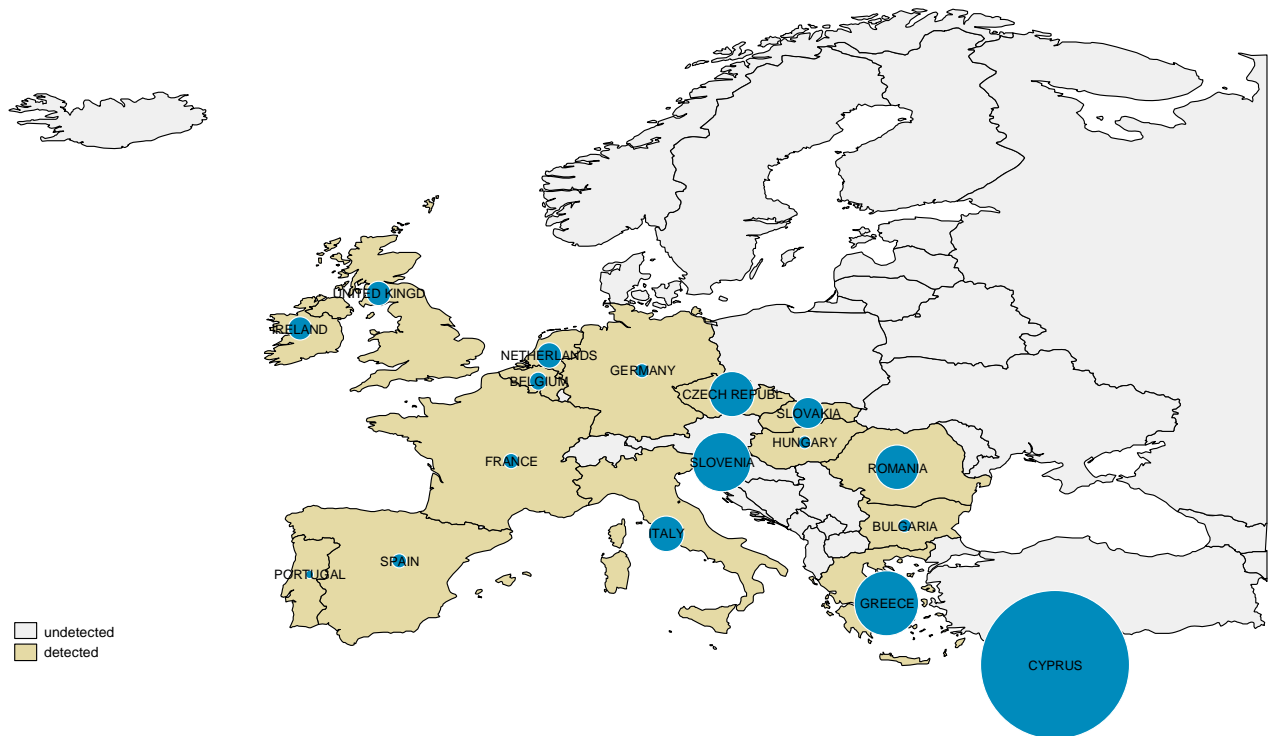
Based on EU-wide surveillance data, CS in sheep was detected in 17 out of 27 MSs between 2002 and 2012 (Figure 1). During the period covered by the present Opinion, the following countries did not report cases of CS: Austria, Denmark, Estonia, Finland, Latvia, Lithuania, Luxemburg, Malta, Poland and Sweden.



**Figure 1:** Geographical distribution of ovine CS and AS within EU27. Countries in green reported both CS and AS; countries in blue reported only CS; countries in yellow reported only AS; white is used for countries where scrapie has been never reported

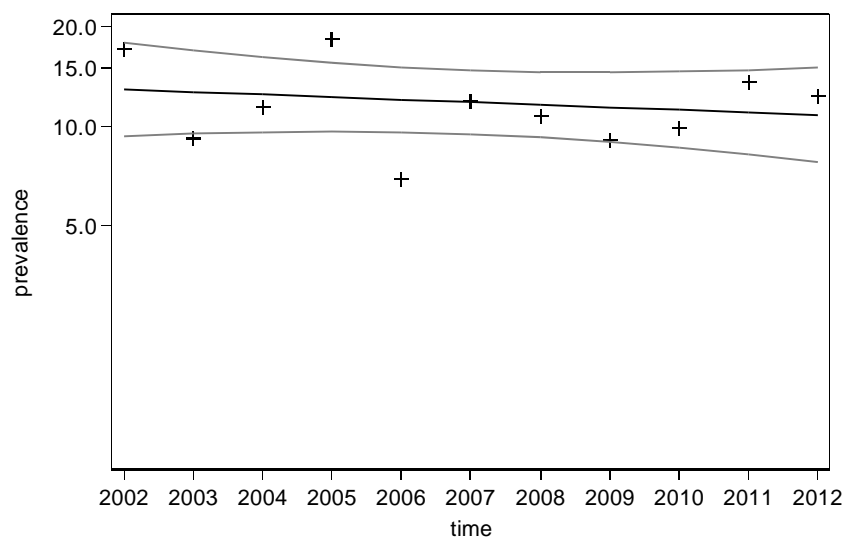
In these 17 countries some 4.7 million sheep were tested leading to the detection of 4 132 CS cases, equal to an overall prevalence of 8.7 cases per 10 000 rapid tests. About one third of the cases (1 464) were detected in the SHC stream whereas the remaining cases (2 668) were detected in the NSHC stream. Considering the same group of countries, the stream-specific prevalence was 5.5 and 12.8 cases per 10 000 rapid tests in the SHC and the NSHC, respectively.

The stream-adjusted prevalence of CS in sheep by country is shown in Figure 2 (in the case of Belgium only the prevalence within NSHC was considered: the standardisation by stream was not carried out because there was only one case detected within the SHC stream out of a small number of animals tested).



**Figure 2:** Prevalence of CS in sheep within EU27. Number of cases/10 000 rapid tests standardized by stream, i.e. SHC vs. NSHC over the period 2002-2012. The proportion of tests carried out in all the 27 MSs in the NSHC vs. SHC in sheep has been used to define the baseline population for the direct standardisation. The blue markers are proportional to the prevalence and comparable with those presented in the other maps of scrapie prevalence.

At the EU27 level over the period 2002-2012, CS in sheep showed annual stream-adjusted prevalence rates ranging between 5 and 20 cases per 10 000 rapid tests. The overall temporal pattern is not consistent with any statistically significant trend (PR 0.98, 95 % CI: 0.94-1.03) (Figure 3).



**Figure 3:** Temporal trend of CS in sheep at the EU27 level. Crosses (+) indicate the annual stream-adjusted prevalence (cases per 10 000 rapid tests) whereas the lines show respectively the linear trend (black line) with its 95 % confidence limits (grey lines). The adjustment on stream was obtained by fitting a negative binomial model (internal reference).

However, the interpretation of the overall trend at the EU-level is not meaningful: rather than indicating a general stable situation in the EU, it is the result of the aggregated heterogeneous epidemiological situation at country level, with large differences in the temporal trend between countries.

The within country differences in the prevalence over the years help us to understand better the evolution of CS during the period covered by this Opinion. Bulgaria, Germany, Hungary and Portugal reported CS only sporadically, preventing any analysis of the temporal trend, with few cases detected every year and a very low prevalence of disease. In these four countries the annual prevalence rates were never higher than 10 cases per 10 000 rapid tests. Given the limitations of the data for these countries, it was decided to restrict any further investigation to the remaining 13 countries.

As mentioned above, the analytical strategy focussed on country-level trends. Negative binomial models were applied to fit ‘count of cases detected’ to estimate the country-specific and stream-adjusted annual prevalence ratios (PRs) by year. A PR larger than one indicates an increasing trend whereas a PR less than one is associated with a decreasing trend; the PR is statistically significant when its 95 % confidence interval does not include one (that would indicate a flat trend). After excluding Bulgaria, Germany, Hungary and Portugal from any further temporal analysis, based on modelling outputs, the countries were divided in two main categories as follows:

- Countries with an observed statistically significantly decreasing trend over the period 2002-2012: Cyprus, France, Ireland, the Netherlands, Slovenia and the UK. Table 1 reports the annual PRs by country obtained in this group of countries, with France showing the most substantial decrease.
- Countries with an observed trend not statistically different from a flat one over the period 2002-2012: Belgium, Czech Republic, Greece, Italy, Romania, Slovakia and Spain. All the countries showed the absence of any statistically significant trend. However, in Belgium and Czech Republic scrapie cases have no longer been detected since respectively 2008 and 2009: in both the countries, since 2008 the national active surveillance programmes have been restricted to the NSHC only.

**Table 1:** The six EU countries showing a statistically significant decreasing trend over the period 2002-2012. Countries are ranked on the basis of the prevalence ratios (PR)

Country	Prevalence ratios (PR)	95 % CI
France	0.61	0.56 - 0.67
Slovenia	0.68	0.54 - 0.85
Cyprus	0.73	0.61 - 0.87
United Kingdom	0.74	0.68 - 0.82
The Netherlands	0.75	0.69 - 0.81
Ireland	0.84	0.78 - 0.90

The country-specific trends over the period 2002-2012 are shown in Figures 4 and 5.

Within the six countries with a statistically significant decreasing trend, there are two subgroups clearly identified. This is supported by the information gathered from MSs through a survey on the monitoring sampling strategy in EU MSs (see Section 2.3), to which the paragraphs below refer.

Cyprus, Ireland, The Netherlands and Slovenia are small countries with a small ovine population with homogeneous characteristics in terms of number of breeds, husbandry and management. Ireland is an upper bound of this group with a considerable number of breeds (54) and a sheep population of approximately 3.5 million. In general these four countries present a robust implementation of their surveillance systems in terms of representativeness and coverage. In some instances they exceed the statutory requirements. For example, Ireland has in place a standard operating procedure (SOP) for the

SHC to ensure that the correct ratio of eligible animals are selected randomly, and identified, with a record generated of all flocks sampled on a particular day that can be used to demonstrate that a random sample is achieved. Equally the NSHC monitoring system requires quarterly analysis of the scrapie monitoring database to ascertain how representative of season and region the sampling is. In the case of Cyprus, collection and sampling of found dead sheep are conducted by a private contractor continuously monitored by the Veterinary Services by pre-printing each driver's report and issuing instructions as to whether samples should or should not be collected from a specific farm. The Veterinary Services issue lists of non-infected flocks/herds, from which drivers should collect at least one sample if possible. The driver's report also includes the total number of samples collected per farm in the year to date, to indicate to the driver whether a sample should be collected. The Netherlands systematically test 10 % of all sheep over 18 months slaughtered for human consumption, with a minimum of 1 per slaughterhouse. In Slovenia, between 2003 and 2010, all fallen stock over 18 months were tested. Since 2011, and despite logistical and resource constraints, a small sample is selected randomly as per an annual plan: every first dead animal from a flock of more than 100 sheep or more than 50 goats is selected for testing, ensuring sampling is evenly distributed through regions, breeds, production categories, age groups, and avoiding multiple samples from the same flocks.

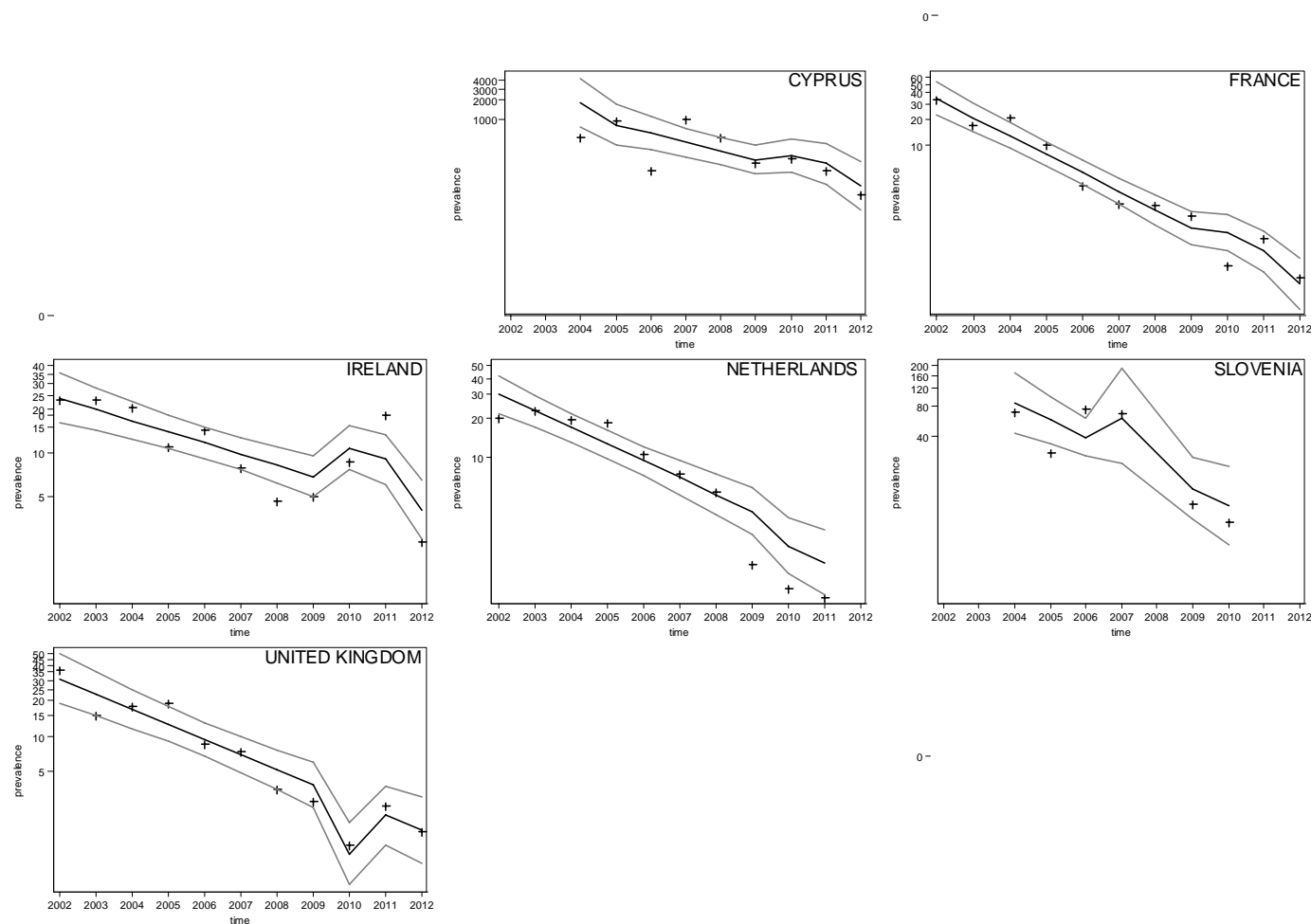
In the case of France and the United Kingdom, with the third and largest sheep populations respectively in the EU, they are at the other extreme of the spectrum with multiple breeds, varied husbandry systems and geographical dispersion. However, these two countries have in place robust surveillance systems with strong veterinary governance and a long history of monitoring and controlling TSEs, as in the case of the United Kingdom and the BSE epidemic. Both countries have systems in place to ensure the representativeness of the surveys. For instance, in NSHC, the *“number of animals to be tested are annually defined by sampling center and month. Instructions are given to choose randomly eligible animals in order to reach this number”* (France), *“approved Category 1 incineration, rendering and intermediate plants with a throughput of at least 1,000 adult sheep and goat carcasses may apply to become sampling sites for the TSE Fallen Sheep and Goat surveys”* (United Kingdom). To ensure that sampling covers all parts of the country and that samples are taken at all times throughout the year, the delivery agency *“allocates each participating plant monthly quotas of the number of samples to be taken”* (United Kingdom).

All these aspects would have allowed this group of countries to obtain a valid prevalence estimation.

With regards to follow up of the outbreaks detected through sampling, four countries declared that all rapid test positive (rapid test) cases were traced back, which resulted in the confirmation of infected flocks and the implementation of control measures (Cyprus, Ireland, France and Slovenia); 90 % (United Kingdom) and 29 % (The Netherlands).

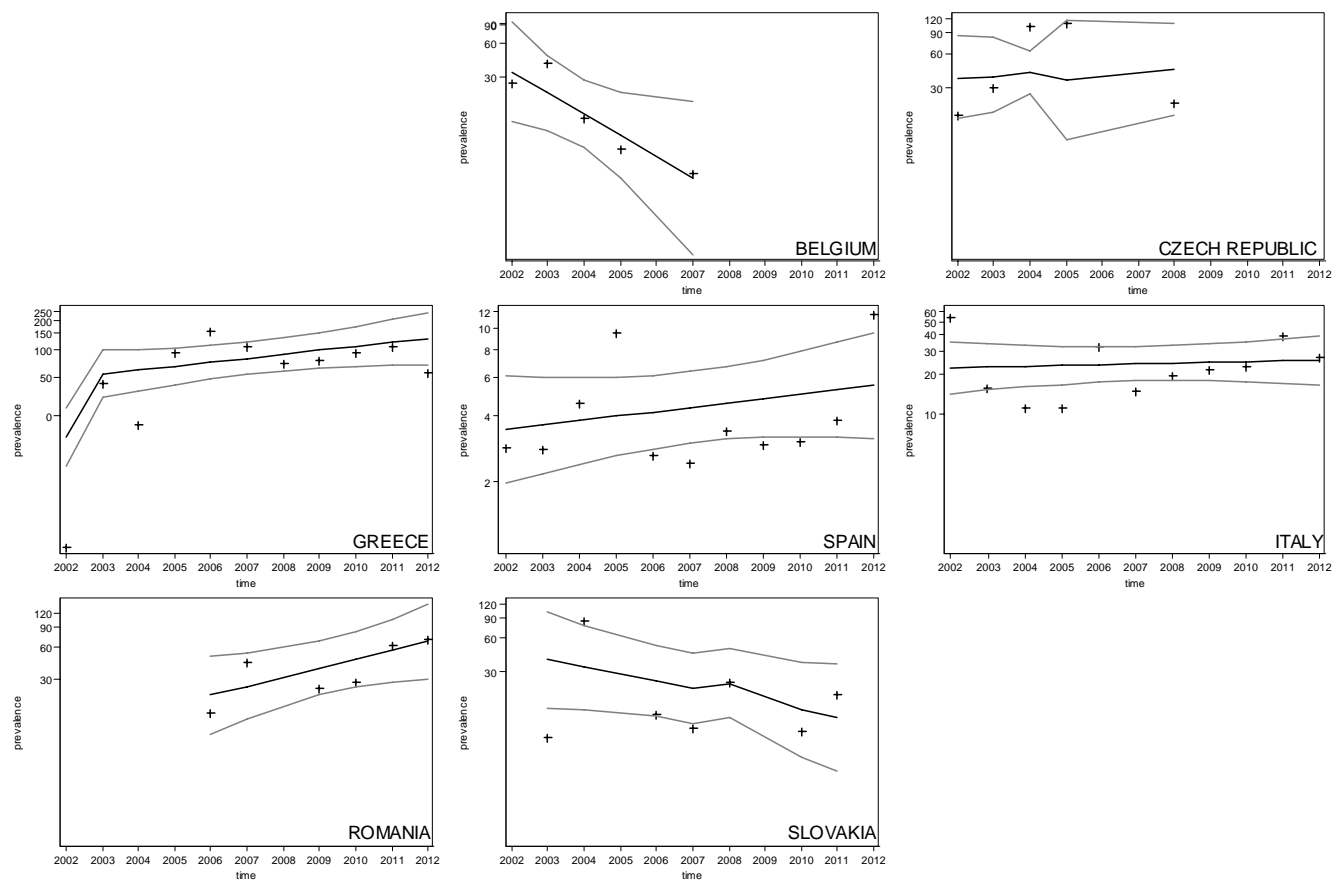
Unlike the other countries, France systematically exceeded the required annual sample size. In the period 2006-2007, a major monitoring effort is evident (about 488 and 339 thousand sheep tested respectively); since then the sample size in the NSHC has exceeded the EU requirements by four to seven times. In this case the application of the statistical control for stream confounding was particularly helpful in preserving the possibility of sensible comparisons between the prevalence estimates. However, an additional consequence of this massive targeted testing may have been a dramatic increase in the capacity of outbreak detection and therefore of effective application of control measures.

The potential reasons for the trend not being statistically different from a flat one for the other seven countries are discussed later in the Opinion (see Section 4.5.5), since detailed consideration needs to be given to the combined impact of the various control measures implemented in those countries (dealt with in Chapter 4).



**Figure 4:** Temporal trend of CS in sheep in countries where a statistically significant decreasing trend was confirmed. Crosses (+) indicate the annual stream-adjusted prevalence (cases per 10 000 rapid tests) whereas the lines show respectively the linear trend (black line) with its 95 % confidence limits (grey lines). The adjustment on stream was obtained by fitting a negative binomial model (internal reference).





**Figure 5:** Temporal trend of CS in sheep in countries where the trend was not statistically different from a flat one. Crosses (+) indicate the annual stream-adjusted prevalence (cases per 10 000 rapid tests) whereas the lines show respectively the linear trend (black line) with its 95 % confidence limits (grey lines). The adjustment on stream was obtained by fitting a negative binomial model (internal reference).

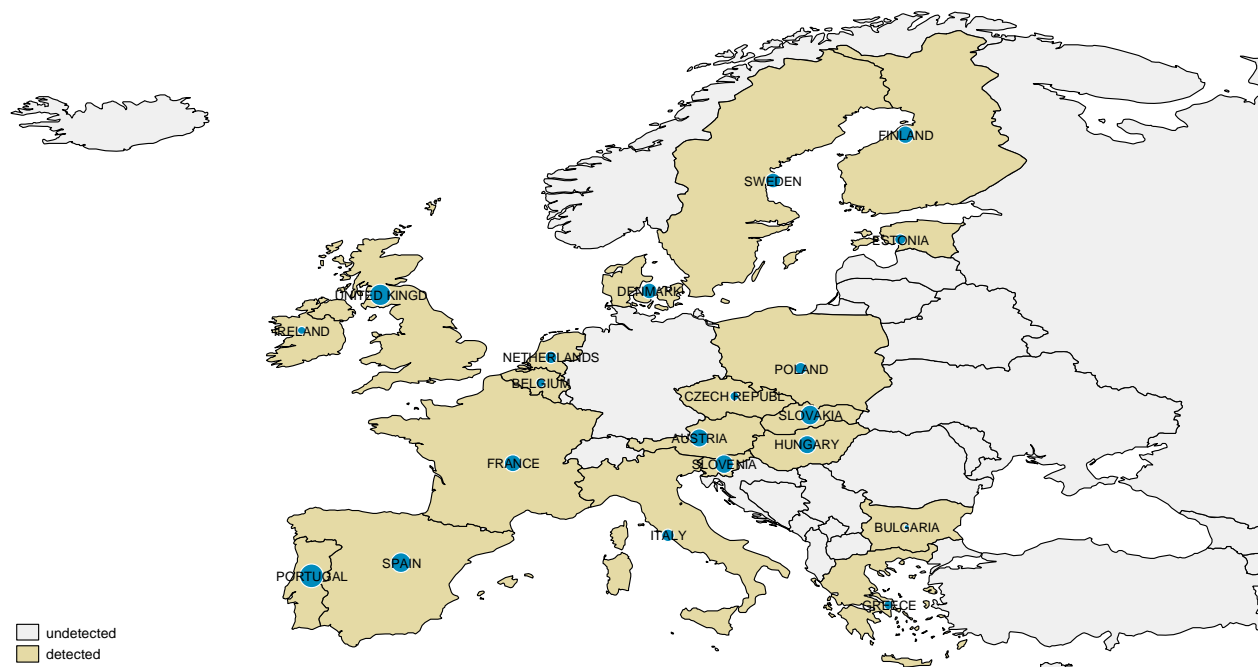
### 3.2.2. Atypical scrapie in sheep

Over the period 2002-2012, AS was reported in 21 countries (see Figure 1) and it was the only ovine TSE detected in six of them (Austria, Denmark, Estonia, Finland, Poland and Sweden). Within the EU27, the disease has never been reported in Cyprus, Latvia, Lithuania, Luxembourg, Malta or Romania. In some countries cases were identified sporadically, as in Austria (2011 and 2012), Bulgaria (2008 and 2012), Czech Republic (2007), Estonia (2010 and 2011, when a case was detected out of only 10 SHC sheep tested) and Slovenia (2010 and 2011).

As a consequence of the limited ability, in the past, of some rapid tests to detect cases of AS, the analysis was restricted to few commercial rapid tests, used mainly from 2006 onwards (see Appendix B). Thus all the calculations were restricted to 2.51 million (known) tests and the mapping and trend analysis were restricted to the period 2006-2012. Prior to 2006, the data from the EU TSE database does not include data on the rapid test used, and therefore animals tested with rapid tests unable to detect the disease during those years could not be excluded from the denominators. Conversely, some of the cases detected since 2006 were excluded from any statistical analysis since data on the rapid test used were not available (9 cases) or related to a rapid test with limited ability to detect cases of AS (14 cases). Moreover, Germany was excluded from the analysis, since data regarding the type of rapid test used over time is not available even after 2006.

The active surveillance carried out since 2006 led to the detection of 1 466 cases equal to an overall prevalence of 5.8 cases per 10 000 rapid tests. The stream-specific prevalence rates are very similar (5.6 and 6.1 per 10 000 rapid tests in SHC and NSHC, respectively) confirming the absence of a confounding effect from the surveillance stream for AS.

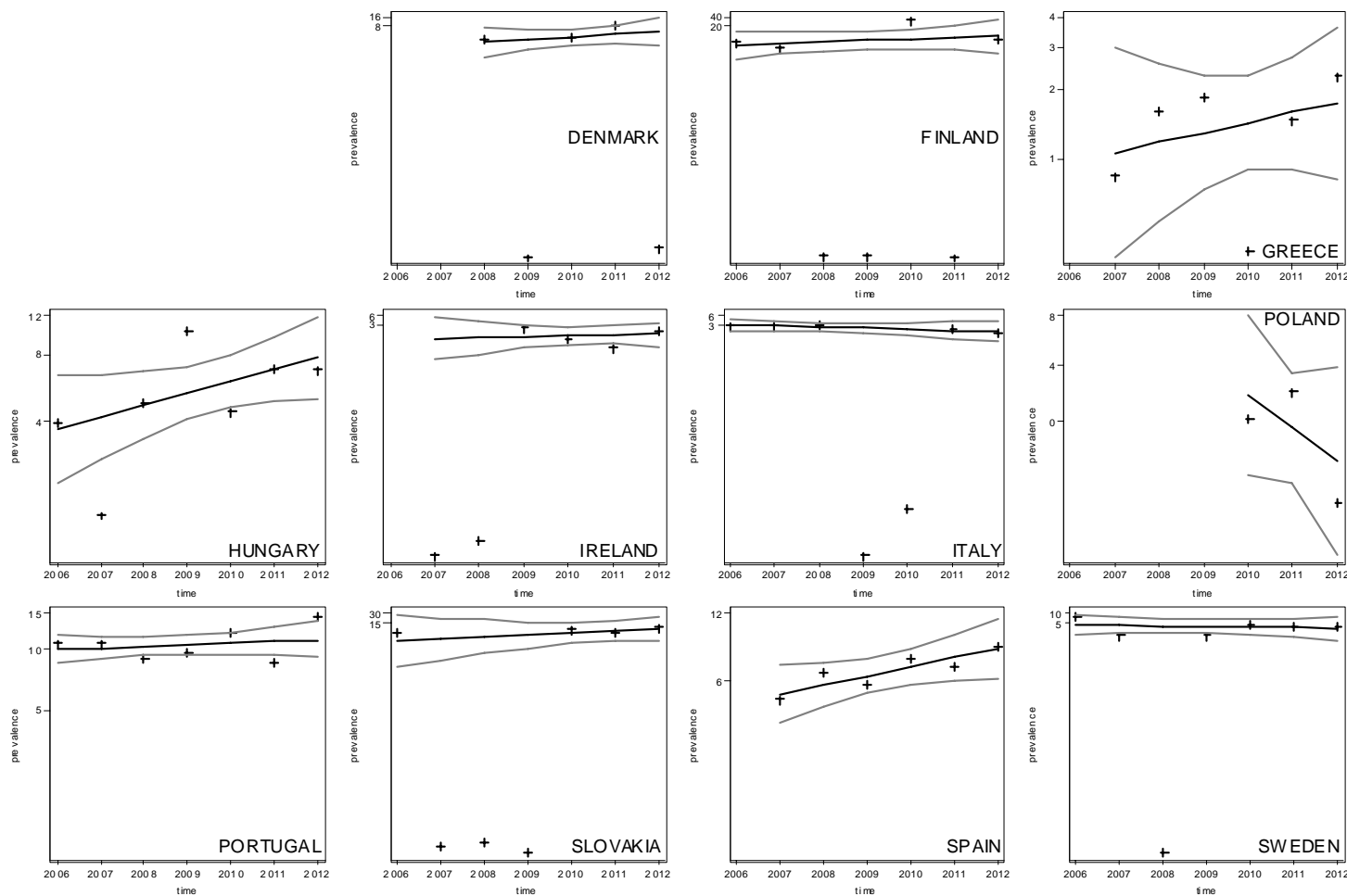
The crude prevalence between 2006 and 2012 (Figure 6) was similar throughout the EU27 without the heterogeneity evident for CS.



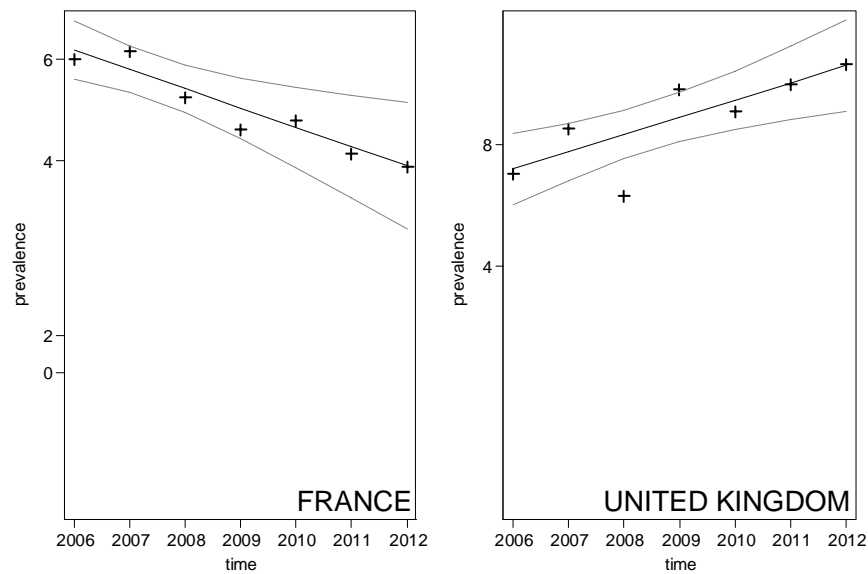
**Figure 6:** Crude prevalence of AS in sheep within EU27 (Estonia prevalence data refer only to the NSHC stream and Germany is not shown). Cases/10 000 rapid tests are calculated over the 2006-2012 period. The blue markers are proportional to the observed crude prevalence and comparable with those presented in the other maps of scrapie prevalence.

Figure 7 shows crude annual prevalence rates (cases per 10 000 rapid tests) by country restricted to animals tested with rapid tests able to detect AS, excluding countries where AS cases were not reported in at least three different years (e.g. Belgium). The Netherlands was also excluded as only cases detected over the last two-year period, 2011-2012, met the test type requirement. A statistical assessment of the slope of the individual regression models has been carried out (fitting country-specific Poisson Regression models). France and the United Kingdom (Figure 8) showed a statistically significant trend: in the case of France the trend was decreasing (annual PR=0.92, 95 % CI: 0.88-0.97) whereas in the UK a statistically increasing trend has been detected (annual PR=1.10, 95 % CI: 1.04-1.17): in all the remaining countries the PRs were not statistically significant. The decline observed in France was less than the one observed for CS in the same country.

Overall it is not possible to draw any conclusions from these findings, since there has not been any apparent difference in the implementation of control measures during this time window.



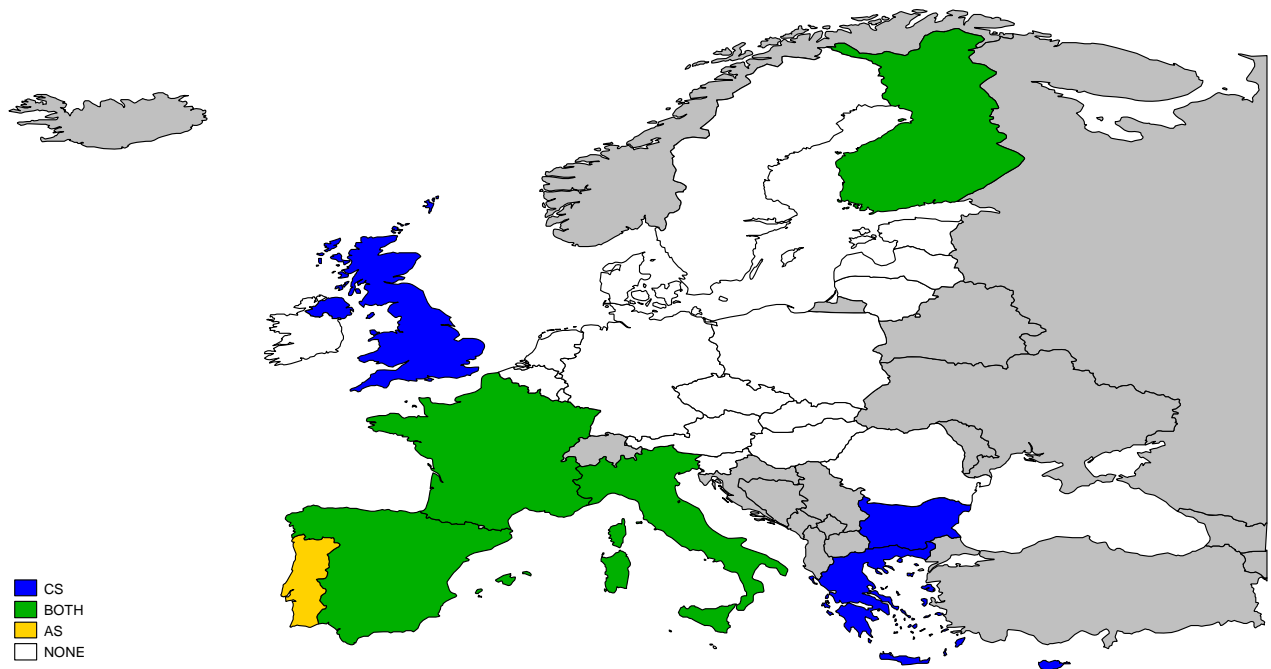
**Figure 7:** Temporal trend of AS in sheep in countries where the trend was not statistically different from a flat one. Crosses (+) indicate the annual prevalence (cases per 10 000 rapid tests) whereas the lines show respectively the linear trend (black line) with its 95 % confidence limits (grey lines).



**Figure 8:** Temporal trend of AS in sheep in France and the UK. In the case of France the trend was significantly decreasing (annual PR=0.92, 95 % CI: 0.88-0.97), whereas in the UK a statistically increasing trend was detected (annual PR=1.10, 95 % CI: 1.04-1.17). Crosses (+) indicate the annual prevalence (cases per 10 000 rapid tests) whereas the lines show respectively the linear trend (black line) with its 95 % confidence limits (grey lines).

### 3.2.3. Classical scrapie in goats.

Based on EU-wide active surveillance data, CS in goats was detected in 8 out of 27 MSs between 2002 and 2012 (Figure 9). Bulgaria, Cyprus, Greece and the UK reported only cases of CS whereas both CS and AS were detected in Spain, Italy, Finland and France.

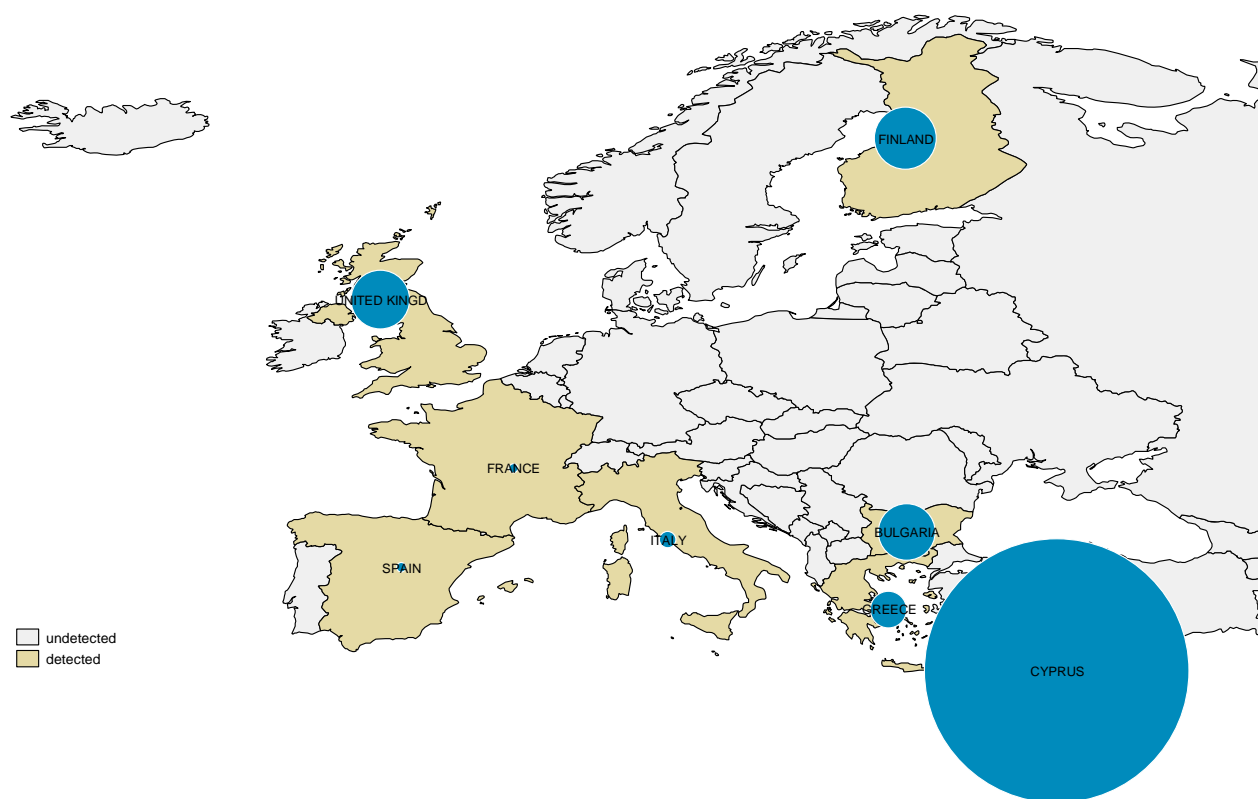


**Figure 9:** Geographical distribution of caprine CS and AS within EU27. Countries in green reported both CS and AS; countries in blue reported only CS; countries in yellow reported only AS; white was used for countries where caprine scrapie was not reported.

In the eight countries where cases of scrapie have been confirmed in goats, about 1.4 million goats were tested and 1 405 cases of CS were detected, equal to an overall prevalence of 9.8 cases per 10 000 rapid tests. The annual crude prevalence in Cyprus was between 10 and 864 times higher than those observed in the remaining countries where the disease was reported. If the calculation of the prevalence is carried out after the exclusion of Cyprus, the overall prevalence in the remaining seven countries is 2.2 cases per 10 000 rapid tests.

About 47 % of the total cases (659) were from the SHC, whereas the remaining cases (746) were detected in the NSHC. The overall stream-specific prevalence in the eight countries was 8.3 and 11.6 cases per 10 000 rapid tests in SHC and NSHC, respectively. The exclusion of Cyprus from the calculation of the stream-specific prevalence led to the following figures: 1.0 and 3.7 cases per 10 000 rapid tests in SHC and NSHC, respectively.

The heterogeneous prevalence of CS in goats by country over the period 2002-2012 is shown in Figure 10 through the stream-adjusted prevalence by country.



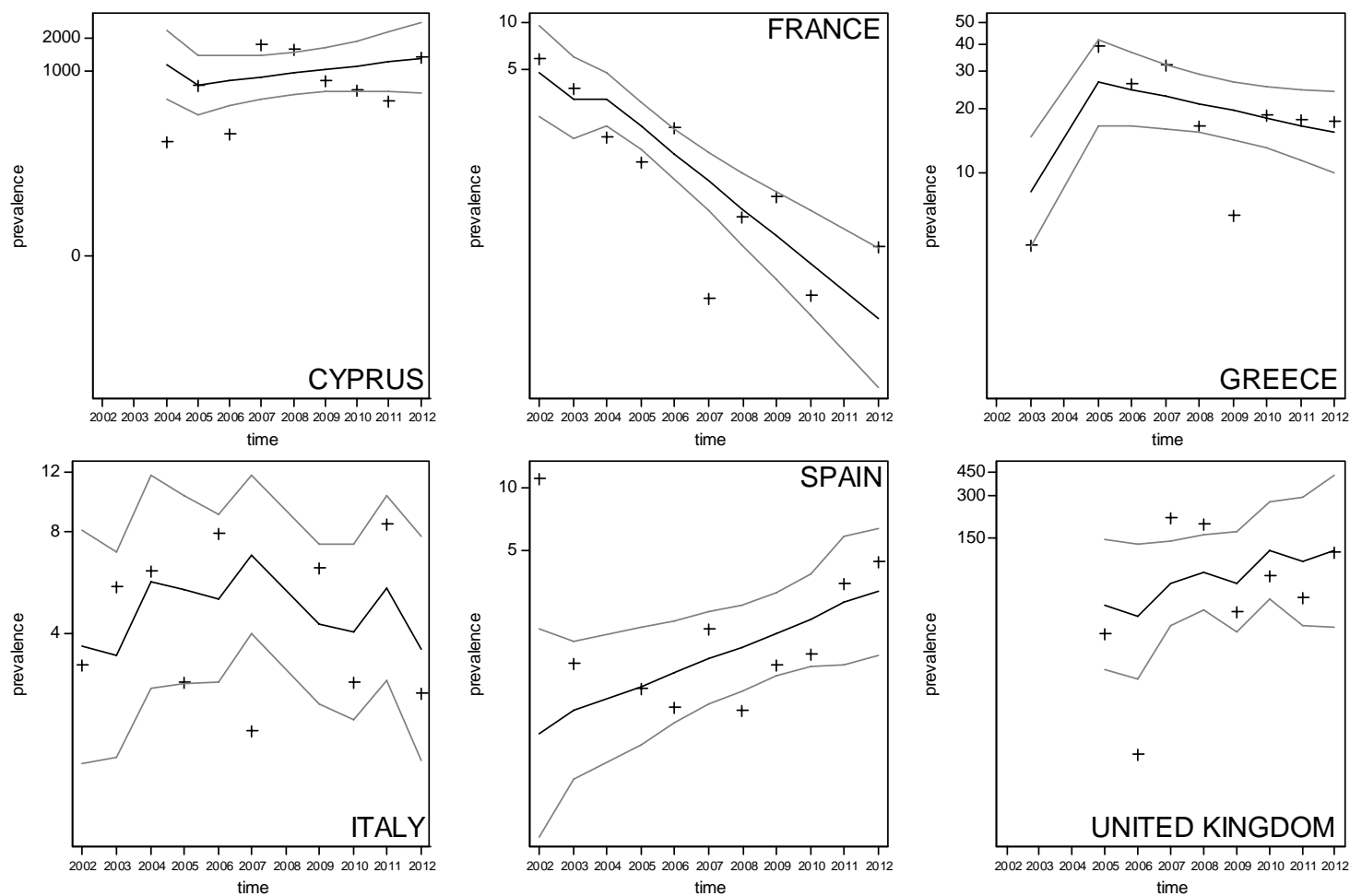
**Figure 10:** Prevalence of CS in goats within EU27. Number of cases/10 000 rapid tests standardized by stream, i.e. SHC vs. NSHC over the 2002-2012 period. The proportion of tests carried out in all the 27 MSs in the NSHC vs. SHC in goats has been used to define the baseline population for the direct standardisation. The blue markers are proportional to the prevalence and comparable with those presented in the other maps of scrapie prevalence.

The assessment of the statistical significance of the national trends related to the period 2002-2012 has been carried out by fitting negative binomial models (Figure 11). Two countries were excluded from the analyses as they reported the disease only in two different years (i.e. Bulgaria in 2009 and 2010 and Finland in 2002 and 2005). Based on the models, France showed a statistically decreasing trend with an average reduction of nearly 40 % year by year (PR 0.66, 95 % CI: 0.57-0.77) whereas Cyprus showed a statistically increasing trend (PR 1.10, 95 % CI: 1.07-1.13). The French case may reflect in part also the impact of a strong surveillance pressure able to detect and manage a large proportion of outbreaks. As observed for sheep, the surveillance effort applied on the French goat

population largely exceeded the EU requirements: between 2005 and 2007 about 495 000 goats were submitted for rapid testing and since then the goats tested in the NSHC stream annually has been about 54 000 compared with the expected 10 000 rapid tests.

In the case of Cyprus and the UK, if the analyses were restricted to the period 2007-2012, both countries would show a statistically decreasing trend (PR 0.91 95 % CI: 0.88-0.95 and PR 0.79, 95 % CI: 0.69-0.91 respectively). The particular management and husbandry practices of goat herds in Cyprus can explain the increase in prevalence in the early years, which only benefitted from the reduction of scrapie in sheep from 2007 onwards. In the case of UK, with very few mixed holdings, this effect is more unlikely.





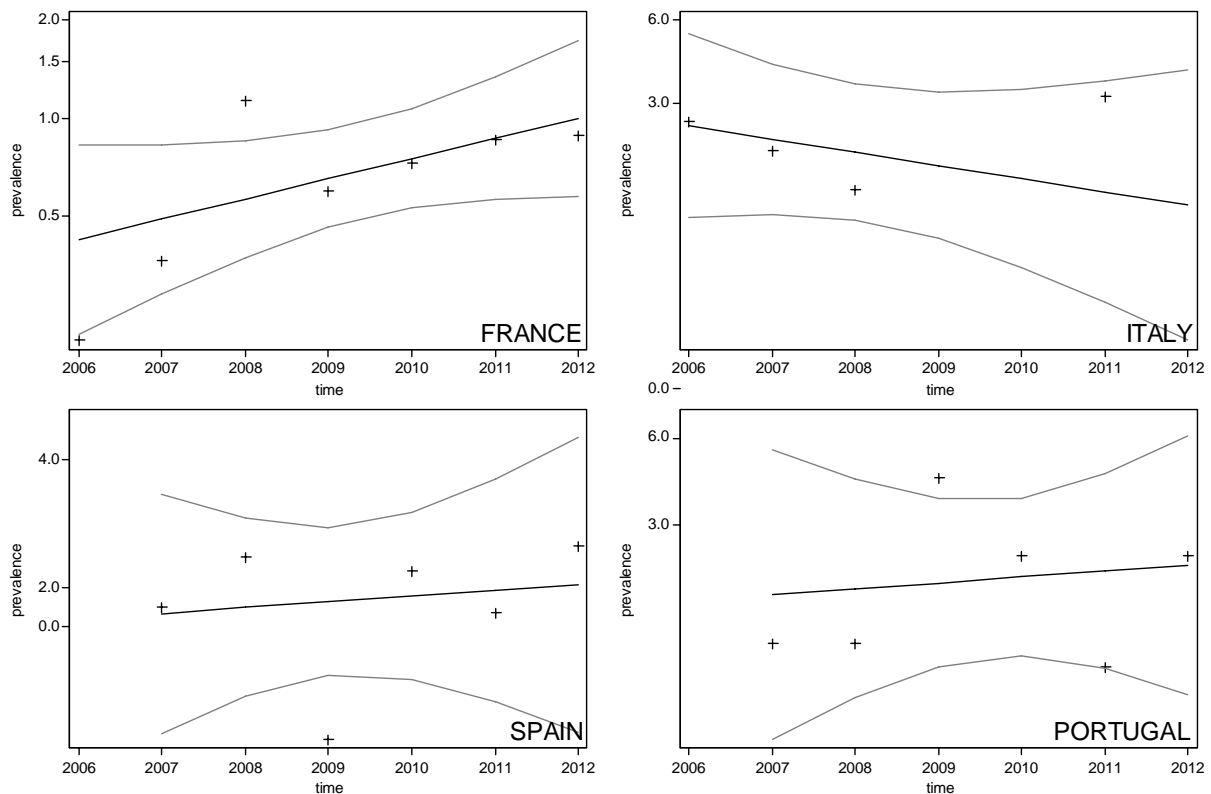
**Figure 11:** Temporal trend of CS in goats in EU countries where the disease was reported over at least three years. Crosses (+) indicate the annual stream-adjusted prevalence (cases per 10 000 rapid tests) whereas the lines show respectively the linear trend (black line) with its 95 % confidence limits (grey lines). The adjustment on stream was obtained by fitting a negative binomial model (internal reference). France shows a statistically significant decreasing trend.

### 3.2.4. Atypical scrapie in goats

Over the period 2002-2012, AS in goats has been reported in five countries (Figure 9), i.e. Finland (just one case in 2009), France, Italy, Portugal (where it was the only caprine TSE detected) and Spain.

As for sheep, data analysis was restricted to the period 2006-2012. Based on the application of rapid tests able to detect AS, about 764 000 animals were tested in the five countries where AS was initially reported. The total number of cases detected was 84, equal to an overall prevalence of 1.1 cases per 10 000 rapid tests. As in sheep, the stream-specific prevalence rates in goats are very similar (0.9 and 1.2 per 10 000 rapid tests in the SHC and NSHC, respectively). Finland was excluded from the analysis, since it reported only one case of AS in one year.

Figure 12 shows temporal trends of AS in goats in the four countries for which the analysis was carried out. None of those countries showed any statistically significant trend.



**Figure 12:** Temporal trends of AS in goats. No country shows a trend statistically different from a flat one. Crosses (+) indicate the annual prevalence (cases per 10 000 rapid tests) whereas the lines show respectively the linear trend (black line) with its 95 % confidence limits (grey lines).

### 3.3. Concluding remarks in relation to Term of Reference 1

- The overall EU27 surveillance effort between 2002 and 2012 led to the testing of five million sheep and 1.7 million goats. The idiosyncrasies of the EU database, the quality and completeness of the data and the heterogeneous implementation of surveillance at country level have determined the analytical options to measure the temporal variation of scrapie occurrence over the reporting period.

- The precision and validity of the crude prevalence rates obtained through active surveillance may have been affected by the targeted and sample-based design of both the SHC and NSHC surveys. The crude prevalence rates should not be compared between countries.
- In order to analyse prevalence trends:
  - The statistical analysis was restricted to the MSs reporting scrapie cases in the framework of EU active surveillance.
  - Cases identified through passive surveillance, or as a result of culling animals from known infected flocks, were removed from the data sets prior to analysis.
  - In the case of CS, and in order to prevent the validity of the estimates of the prevalence rates being compromised by the confounding effect of surveillance stream, stream-adjusted estimates were systematically obtained through the application of ad hoc statistical techniques.
- Over the period 2002-2012, the overall average prevalence of CS in the sheep population was 8.7 cases per 10 000 rapid tests (considering the 4.7 million sheep tested in the 17 MSs where CS has been reported). The geographical distribution of CS cases in sheep shows great heterogeneity in the level of occurrence in MSs: in some cases only a few, or no, cases were detected, whereas Cyprus experienced a large epidemic.
- Country-specific temporal trends of CS in sheep are heterogeneous, preventing any meaningful interpretation of the overall temporal trend at the EU27-level. Therefore the analysis and interpretation of the temporal trends must be conducted at MS level. With regard to CS in sheep, the results of the analysis allow the classification of the EU27 MSs into four groups:
  - countries where CS has been detected, with a statistically significant decreasing trend (Cyprus, France, Ireland, The Netherlands, Slovenia and the United Kingdom): all countries showed effective surveillance programmes, in particular France, where the surveillance pressure was stronger than in any other country and was likely to facilitate the detection and management of a large proportion of outbreaks;
  - countries where CS has been detected with an observed trend not statistically different from a flat one (Belgium, Czech Republic, Greece, Italy, Romania, Slovakia and Spain): however, Belgium and the Czech Republic have not reported scrapie cases since 2008 and 2009 respectively;
  - countries where CS has been reported only sporadically (Bulgaria, Germany, Hungary and Portugal);
  - countries with no cases of CS during the period 2002-2012 (Austria, Denmark, Estonia, Finland, Latvia, Lithuania, Luxemburg, Malta, Poland and Sweden).
- Over the period 2002-2012, active surveillance enabled the detection of AS in sheep in 21 MSs. Since the ability to detect cases of AS is essentially restricted to few rapid tests, all the calculations were done with the 2.5 million (known) tests carried out with these rapid tests in the 21 MSs. This restricted the temporal analysis to the period 2006-2012. The overall prevalence of AS for this period was 5.8 cases per 10 000 rapid tests.
- Where detected, AS in sheep showed a similar prevalence over time and space: no large epidemics were reported and five countries detected AS in sheep only sporadically. Only two countries showed a statistically significant trend, with a reduction in the annual prevalence rates in France and an increase in the United Kingdom.

- CS in goats has been detected in eight MSs, where 1.4 million goats have been tested. The overall prevalence of CS in goats (9.8 cases per 10 000 rapid tests) is mostly explained by the unique epidemic in Cyprus which paralleled the epidemic in sheep. The prevalence in the remaining seven countries where CS in goats was detected was 2.2 cases per 10 000 rapid tests. Statistically decreasing trends were evident respectively for France over the entire period (2002-2012) and for Cyprus and the United Kingdom after 2007. The favourable French trend may reflect in part the impact of a strong surveillance pressure able to detect and manage a large proportion of outbreaks in small ruminants.
- AS in goats was reported by five countries, at a very low prevalence and with no statistically significant trend in any of them.

#### **4. Measures for the control and eradication of Classical scrapie**

##### **4.1. Factors interacting in the control of Classical scrapie**

The epidemiological trend of CS in a given country, estimated through the trend of the prevalence rate of CS over the years, is the direct result of the balance between the number of new cases that result from the spreading of the disease in the country, the removal of CS-infected animals and the prevention of new infections, i.e. the result of the ability of the surveillance system to detect cases and of the implementation of control measures.

The spreading of CS, i.e. the occurrence of newly infected animals within farms, depends on the percentage of susceptible animals (that diminishes strongly when the dissemination of the ARR allele is high) and the farming activities that can favour disease transmission within farms (mainly lambing practices that may increase the risk of infection of newborn lambs, and other sheep, by infected ewes at lambing). Between farms, the risk of spread is affected by the trade level of live (infected) animals and the physical contacts or sharing of pastures/holdings with sheep from other flocks.

The removal of infected animals or the prevention of new cases of infection are the direct consequences of the combination of the ability of the surveillance system to detect infected animals (the surveillance pressure and effectiveness) and the control strategy implemented in infected farms. The latter itself is made of two complementary components: culling strategy in detected infected farms in order to remove animals of susceptible genotypes (selective culling), and breeding for resistance in these infected farms so that only animals carrying the ARR allele are maintained or introduced into the farm, which reduces the infection pressure and the capacity of the disease to spread.

The effectiveness of a given control strategy can therefore differ between countries if the farming practices (allowing spreading) and the prevalence of the ARR allele are different. For example, the observed effectiveness of the control programme will be higher in countries with low spreading rate of the disease (for example when there is a high percentage of closed farms).

Surveillance pressure is another strong driver of the effectiveness of any control strategy, although this is not its principal purpose. Initially surveillance systems aim mainly at providing data to monitor the epidemiological situation and trend of CS in a country. However, each detection of a case also allows the implementation of appropriate within-flock control measures, which have an impact on the situation of CS in the case flock, and other flocks, for a long period. For example, if control measures following case detection imply that only genetically resistant animals can remain in the flock, this flock would not be a source of CS or a cause of further disease dissemination.

##### **4.2. Questionnaire surveys on the control measures implemented in EU Member States**

As already described, a number of measures may be implemented with the aim of controlling and eradicating CS. These can be roughly classified into two main types of measures:

- i. non-genetic sanitary policies, aimed at detecting infected animals, depopulating, cleaning and disinfecting infected flocks, and limiting animal movements to contain the spread of the disease to other animals/flocks;
- ii. genetic breeding for resistance, aimed at decreasing the susceptibility to CS in the sheep population by increasing the frequency of resistant alleles at specific codons of the *PRNP* gene.

As mentioned in Section 2.3, a first questionnaire was circulated to EU MS to collect information on the control and eradication measures implemented in the different countries over the years. A copy of the questionnaire circulated to MSs and an overview of the replies collected are included in Appendix A.1.

Other requests for information were sent to MSs in order to obtain detailed information in relation to the implementation of BP-CS in sheep. A questionnaire had already been circulated by the European Commission to all MSs in 2010, and the replies collected were considered by EFSA in the framework of the mandate for this Opinion (see Appendix A.2). With the aim of gathering more detailed information, in 2013 the European Commission requested all MSs having a BP-CS in place to provide details on the requirements of BP-CSs and annual reports, in accordance with the TSE Regulation, and this information was also considered by EFSA (see Appendix A.2). Finally, an additional questionnaire was developed and circulated by EFSA to MSs in 2013, which allowed the collection of additional data on the sheep population structure, on the genotyping activities carried out, on the rules for the selection of rams in high genetic merit flocks (HGMF) and on their dissemination to commercial flocks (CF). A copy of this questionnaire is included in Appendix A.3, with an overall summary of the replies gathered. The information collected from MSs was used to assess the type of BP-CSs implemented and allow its comparison with the trends of CS (see Chapter 3), in order to inform the response to ToR 2 of the mandate for this Opinion.

### 4.3. EU legal background

The control of CS in small ruminants is complex, mainly due to the difficulties of breaking the transmission cycle, the inability to identify infected animals before the onset of disease and the difficulty in decontaminating an infected environment, which increases the risk of indirect transmission (Windl and Dawson, 2012). In addition, movement of animals between flocks/herds is also likely to be an important factor in facilitating spread. The knowledge acquired on the genetic susceptibility of sheep to CS has offered new possibilities to control this disease in sheep by means of genetic selection based on certain polymorphisms of the prion protein gene.

Regulation (EC) No 270/2002 introduced the requirement that the prion protein genotype of a random subsample of the ovine animals tested from the population slaughtered for human consumption (or live). This subsample had to represent at least one per cent of the total sample for each MS, and should not be less than 100 animals.

At EU level a first survey of the prion protein genotype in sheep breeds in all MSs was launched in 2003. Commission Decision 2002/1003/EC required each MS to complete a survey, by July 2003, of the prion protein genotype of sheep from flocks of high genetic merit. The survey protocol required that at least 50 samples should be collected from each breed, chosen to be representative of the entire breed in the MS. Importantly, it was required to include rams used for artificial insemination, but it was not required to include rams kept solely for the purpose of breeding with commercial ewes.

At the same time it became a requirement that the prion protein genotype of positive cases of scrapie in sheep had to be determined.

In 2003, the Regulation was again amended (Regulation (EC) No 2245/2003) to state that the genotype should be determined in a minimum of 600 animals in MS with an adult sheep population of more than 750 000 animals. In the case of other MS the minimum sample should consist of at least

100 animals. It is important to note that at this point the Regulation explicitly stated that the sampling should be representative of the entire ovine population, i.e. it was no longer linked to the HGMF.

With Commission Decision 2003/100/EC, each MS was required to introduce a BP-CS in each of its sheep breeds which were native, or which formed a significant population in its territory aimed at increasing the frequency of the ARR allele within the sheep flocks, while reducing the prevalence of those alleles which have been shown to contribute to susceptibility to TSE. It was mandated that the BP-CS should concentrate on flocks of high genetic merit. Participation in these programmes was voluntary prior to 2005. Thereafter it was mandatory, with some derogations based on implementation of national scrapie control programmes or recognition of scrapie free status (e.g. derogations granted to Denmark, Sweden, Finland, Austria). The implementation of BP-CSs became voluntary again for MSs from July 2007 onwards, and have now stopped in a number of MSs (see Section 4.5.3).

Data on specific polymorphisms in goats that may be relevant to CS susceptibility have also been published (see also Section 1.2), showing a high genetic heterogeneity by breed of such polymorphisms.

Apart from genetic selection, a number of further control measures have been laid down in the EU Regulation. In particular, since 2001 specific measures have been in place when a TSE is suspected and/or confirmed in sheep and goats, i.e. a series of movement restrictions of animals in the holding until clinical, epidemiological and diagnostic examinations are carried out, the need to carry out an epidemiological investigation to identify the sources of infection and other animals at risk, and the killing and rendering of positive animals and/or other animals at risk.

Over the years measures have been reviewed and adapted regularly, with a number of derogations also incorporated into the legislation. A differentiation of the measures to be taken has been introduced on the basis of the polymorphisms of the prion protein gene in sheep. For example, derogations to the destruction of sheep from infected flocks can be granted when these carry resistant genotypes, and different movement restrictions and rules on the introduction of new animals in holdings are based on genotypes. Control measures can be also partially different depending on the type of TSE identified: measures are stricter in the case of BSE, while specific derogations may be granted when CS or AS cases are identified. In addition, some conditions (e.g. a low frequency of resistant genotypes available in the sheep breed or holding) are defined for which derogations, such as a delay in the killing and destruction of the animals, may be possible. The replacement of killing and destruction of healthy animals from infected flocks with slaughter for human consumption is also a possibility, provided rapid tests are carried out with negative results. Protective measures have also been incorporated into the legislation in relation to milk and milk products coming from CS infected flock for the supplementary feeding of lambs.

Similar to the measures taken in cattle, EU legislation also sets measures aimed at preventing the entry into the food chain of certain tissues that may contain high levels of PrP<sup>Sc</sup> in small ruminants. A list of SRM that should be removed from sheep and goats slaughtered for human consumption is therefore defined and includes the skull (including the brain and eyes), the tonsils and the spinal cord of animals over 12 months of age, and the spleen and ileum of animals of all ages.

#### **4.4. Non-genetic strategies to control Classical scrapie**

Often, at least during the last decade in the EU, CS control programmes have included a combination of genetic and non-genetic control measures.

France may represent an interesting case study as it has applied enhanced surveillance measures, and shows a decreasing trend for CS in sheep and goats and for AS in sheep. The decreasing trend in CS in goats and in AS in sheep and goats suggests that an intensive surveillance activity by itself (shifting from a sample-based to an exhaustive strategy) may represent not only a monitoring tool but also a disease control measure. That would be consistent with what has been observed in the case of caprine CS in Cyprus, however, other control measures may have played a role in the case of this country.



However, in some cases only non-genetic sanitary measures have been applied, to different extents. This is the case, for example, for scrapie eradication plans that have been implemented in Iceland (1947 to the present day) and in the USA (1952-2000). The study of these specific control programmes can enable some assessment of the possibility of controlling/eradicating CS using only non-genetic sanitary policies in areas where the disease is endemic.

#### **4.4.1. Scrapie control and eradication programmes in Iceland**

Scrapie was first reported in Iceland in 1878, and was initially limited to the northern region of the country.

In 1933 Jaagsiekte, Maedi-visna and Johne's disease were also introduced to Iceland, and in the framework of the control of these diseases the country was divided into 36 quarantine areas, divided by natural boundaries or man-made fences (see Appendix C). Scrapie was present in three of the quarantine areas. All the sheep in the areas where scrapie was observed were destroyed during the years 1946 to 1949. Most of the farms which were depopulated were restocked within the same year, but some were left without sheep for up to three years. Scrapie recurred within two to four years following repopulation in certain farms that had displayed a high incidence of the disease (Detwiler, 1992), and by 1953, when the first scrapie survey was carried out, the disease has spread from the Northern part of the country to other quarantine areas (see Appendix C).

Considering the possibility that scrapie might spread throughout Iceland leaving no scrapie free areas, it was decided, in 1978, to implement a specific control and eradication policy for scrapie. The approach was based on the culling of scrapie affected sheep in areas already infected and the culling of whole affected flocks in newly infected areas, with restocking not permitted for at least two years, and restrictions placed on the movement of sheep and hay (see Appendix C). Before restocking, thorough cleaning and disinfection of the farm environment and machinery used, including replacement of surface soil in some cases, was carried out, and the first harvest of hay from potentially infected fields was not permitted to be used as forage for the new stock. Government inspection was required to ensure that procedures were completed to the standards set by the scheme. In addition, the use, for animal feeding, of any offal from abattoirs in scrapie-infected areas was prohibited (Detwiler, 1992).

This plan has been continuously applied since then. In 1986, when the epidemic reached its peak and the disease had spread to 25 of the restriction zones, and again in 1993, further enhancements of the programme were made. These involved various practical aspects of handling scrapie cases, and since 2012 different measures have been applied to flocks depending on whether they are infected with CS or with AS only. Measures have been accompanied by financial assistance and compensation to farmers.

Throughout this period, extensive surveillance has been maintained on farms and at slaughter to identify newly-infected flocks. The surveillance methodologies evolved along with the knowledge and techniques in the TSE field. Initially surveillance relied solely upon clinical examination, but since 1978 it was reinforced by brainstem testing by histological examination and, later, detection of PrP<sup>Sc</sup> by IHC. Georgsson et al. (2006) carried out a retrospective epidemiological study based on data from the period 1978-2004. This showed that, despite the eradication measures put in place, recurrences of the disease had occurred on 33 farms. Recurrences were observed most frequently 4-7 years after restocking of the farms, but cases were also observed as late as 12-19 years following restocking, the high persistence of the scrapie agent in the environment (see Section 1.2.5) playing an important role in this.

Rapid testing was introduced in 2004, and since then an average of about 3 500 sheep per year have been tested, which corresponds to about 10 % of the slaughtered population and about 0.7 % of the adult sheep population. Testing has been focusing on healthy slaughtered animals, with very few fallen stock sampled. Following this strategy, 19 scrapie index cases have been detected in the period 2004-2012 (15 CS and four AS cases) (see Appendix C).

Appendix C provides more detailed information with regard to the demography of the small ruminant population in Iceland, the testing policy applied and the epidemiology of classical and AS in Iceland, together with a historical background on the measures applied in the country to control and eradicate the disease, as provided by the Institute for Experimental Pathology (IEP) and the Icelandic Food and Veterinary Authority (MAST).

Overall, the scrapie eradication policy in Iceland has resulted in a substantial decrease in disease prevalence. However, despite these 35 years of continuous effort, and the drastic sanitary measures that had been applied, the disease has not yet been eradicated. This may, at least in part, be attributable to the reduced sensitivity of the surveillance methods used historically (clinical ascertainment, histopathology, limited active surveillance of fallen stock).

#### **4.4.2. Scrapie control and eradication programmes in the USA**

The first case of scrapie in the USA was diagnosed in 1947 in a Michigan flock with sheep of British origin imported from Canada. Efforts to eradicate scrapie in the USA started in 1952. The initial eradication measures relied on the depopulation of identified infected flocks: once the disease was confirmed, the flock was quarantined and depopulated, and exposed sheep sold from the flock were traced and slaughtered. In 1957 depopulation was extended to source flocks and to all those animals sold from source flocks.

Modifications of this approach were made throughout the years; however, the main focus remained on total flock depopulation. This changed as of 1983, when the policy was amended with the adoption of the bloodline/surveillance programme. This required removal of the maternal bloodlines of a scrapie-infected sheep or goat from the flock/herd, followed by a reinforced surveillance of the remaining animals for 42 months for evidence of scrapie. The change was made both because of a lack of resources to continue funding of total depopulation, and to limit underreporting of the disease by farmers, and because of the failure of the depopulation policy in eradicating scrapie. However, in 1985 it was concluded that the bloodline/surveillance programme should be abandoned because it had not been effective.

From 1991 the scrapie eradication programme for scrapie was again modified: the new programme included depopulation with indemnification for all known infected and source flocks, measures on identification for movements of sheep from infected or source flocks, and a voluntary flock certification programme, aimed at monitoring flocks over a period of five years or more and identify flocks free from scrapie. The programme, focused on risk reduction and sound husbandry practices, consisted of four scrapie risk levels (Detwiler, 1992; Wineland et al., 1998).

Wineland et al. (1998) analysed the situation of scrapie in the USA over the 1947 to 1992 period, based on reported scrapie cases. During those years, a total of 1 117 clinically affected sheep in 657 flocks were reported. During the period 1965-1992 the authors identified a slight, significantly upward trend of scrapie-positive flocks, which was in part linked to the various changes in the control measures put in place. The limited data available did not allow the estimation of the true prevalence of the disease, but overall there seemed not to be a clear impact of the control and eradication policies on the scrapie prevalence in the USA during this period.

A study performed in the early 2000s in around 12 500 adult sheep presented for slaughter over the different US regions (USDA, 2003) estimated an overall US national prevalence of 0.2 %, based on IHC testing of obex, tonsil and retropharyngeal lymph node.

In the early 2000s the policy was once again modified to integrate the use of PrP genetic criteria into the depopulation/repopulation of affected flocks (Detwiler and Baylis, 2003), and a new National Scrapie Eradication Program (NSEP) was designed, including a surveillance programme (Regulatory Scrapie Slaughter Surveillance (RSSS)) that requires sampling to be performed in different target groups (slaughtered animals, fallen stock, suspect animals) (USDA, 2010).

#### 4.4.3. Concluding remarks related to Term of Reference 4

- As shown by the Iceland case-study, detection and eradication measures in affected flocks are effective in reducing the observed prevalence of CS in a population with a high prevalence of disease.
- The overall effectiveness of such a policy relies heavily on the detection rate of outbreaks in the population.
- However, because of the persistence of the agent in environment, repopulating scrapie infected farms with non-resistant genotype animals can lead to reoccurrence of the disease.
- Due to the pathogenesis and the epidemiological characteristics of CS, and to the high persistence of the CS agent in the environment, a CS eradication policy that relied solely on detection of infected flocks by *post-mortem* testing and subsequent depopulation would be unlikely to succeed.

#### 4.5. Breeding for resistance

Based on the accumulating evidence of the role of the genetic susceptibility in the occurrence of CS, breeding for resistance was suggested in the mid 1990s as a new, potentially successful strategy to control CS (Schreuder et al., 1997): favouring the diffusion of ARR carriers would have the effect of reducing the susceptibility to CS of the ovine population. A few years later, in an ad hoc EFSA Opinion (EFSA, 2006) the advantages with regards to ovine CS of a controlled approach based on breeding for resistance were clearly highlighted: “*the selection process will lead to:*

- *a major reduction of human exposure risk, because there is no agent (or extremely low agent levels) in tissue from ARR carriers exposed animals; because inter-individual transmission is theoretically impossible or reduced, considering that ARR animals are not sufficiently susceptible and, if infected, the involvement of the lymphoreticular system or tissues other than central nervous system is highly reduced or absent;*
- *a major control of the animal health problem considering that inter-individual transmission is reduced.”*

##### 4.5.1. Definition of a breeding programme

A breeding programme (BP) aims at modifying the distribution of one or more traits in a group of animals in a direction considered as positive. This is achieved in practice through the identification, phenotyping, selection and reproduction of breeding animals. In the case of the *PRNP* gene, after identification, animals have to be genotyped at the *PRNP* locus and those carrying favourable alleles used as breeding animals in order to increase the resistant allele frequency in the next generations.

In this sense, the strategy of simply culling susceptible animals and replacing them with resistant animals is not strictly a BP, although this approach, which is generally used in outbreaks and in some cases tends to be replicated even in healthy flocks, has a dramatic effect on allele frequency.

All BPs require three main components:

- (1) Phenotyping step, including an identification and recording system, with information on pedigree, phenotype and genotype. In the framework of BP-CS, this step in practice consists in the genotyping of animals, and therefore this Opinion will refer to this step as the ‘genotyping step’ from here onwards.
- (2) Selection step, including the definition of rules for the qualification of reproducers based on the records, and rules for a differential use of animals as reproducers depending on their qualification.

- (3) Dissemination step (i.e. to disseminate resistant reproducers to otherwise non-participating flocks), if the previous steps are applied just to part of the population involved in the programme.

If these requirements are universal, the practical organisation of a BP is extremely versatile. The simplest situation is that, within a flock, selection is organised by the flock owner. In the most sophisticated plans, males are assembled in breeding centres, individually phenotyped for traits such as feed conversion rates and functional aptitudes, and the best animals progeny tested on sex limited traits or carcass composition, with an extensive use of artificial insemination to disseminate the very best in the whole population.

Generally, BPs are applied to groups of animals belonging to the same breed with a pyramidal management of the population: at the top a subset of flocks (hereafter described as nucleus population or high genetic merit flocks (HGMF)) owned by breeders sharing the same objectives and rules for the improvement of their animals where the three steps are applied and, at the bottom, flocks that commercialise the majority of animal products (commercial flocks (CF)), and to which the dissemination step is applied. The relative size of HGMF with respect to CF has to be taken into account when a BP-CS is conceived.

A selection scheme focusing only on the improvement of resistance to CS is a very simple case since the trait is unique, only one gene (*PRNP*) is involved and the relationship between its alleles and the trait (i.e. resistance to CS) is very strong. This scheme would simply require single locus genotyping (the genotyping step), rules for the elimination of carriers of unfavourable alleles and for the use of the ARR carriers (the selection step) on a large scale, including their dissemination in commercial population (the dissemination step).

Even if breeding for CS resistance appears simple, effective implementation of such designs in the field is not straightforward. The genotyping step requires long term identification of animals on a large scale, organization of DNA sampling campaigns in dispersed flocks, the setting up of a network of laboratories able to perform correct genotyping with high quality control, and centralised data banks with all the human and computing resources for fast and exact merging of data. The selection and dissemination steps need the definition of unambiguous rules, and ways to require breeders to apply them correctly.

Partly to face this complexity, in many countries it appeared more efficient to put the implementation of BP-CS in the hands of a first tier (HGMF) of farmers able to manage selection tools. This generally accepted solution has two consequences: 1) CS resistance is only one of the traits considered in the breeding objectives and BP-CS design, and 2) the final success of BP-CSs, to be measured at the whole population level, depends upon an efficient dissemination step from HGMF to CF.

When selection for CS resistance exploits a pre-existing breeding structure built to genetically improving production traits, it must be accepted that selection of the *PRNP* gene can affect other traits of interest by different mechanisms (Elsen et al., 2006; Dawson et al., 2008). The *PRNP* gene may be either directly involved or closely linked to a gene affecting the genetic determinism of a trait. In the latter case, the potential effects depend on the strength of the linkage and the phase that may be positive (favourable alleles inherited with resistant *PRNP* alleles) or negative (unfavourable alleles inherited with resistant *PRNP* alleles). The linkage phase is expected to differ between populations or between families within a given population, according to the distance between the 2 loci, so that associations that are beneficial in one population (family) may be deleterious in another. Several studies showed that no association exists between the *PRNP* gene and the most relevant economic traits in the sheep industry (Vitezica et al., 2005; Vitezica et al., 2006; Vitezica et al., 2007; Sweeney and Hanrahan, 2008; Gubbins et al., 2009). Regardless of this, the introduction of CS resistance as a selection objective leads to a lower selection intensity on the other traits. Moreover, a loss of genetic gain on production traits is expected because of the different selection pressures applied on resistant and susceptible genotype classes. Indeed, resistant rams are selected even if they show low genetic

merit for the other selection traits, whereas susceptible rams are selected only if they show high genetic merit for the other selection traits. A BP-CS which exploits a pre-existing breeding structure may benefit, in terms of effectiveness, when relationships with selection for production traits are considered (see for example the BP-CS implemented in Sardinia (Italy), described in Appendix E).

Thus, inclusion of selection for CS resistance in already existing BPs cannot be summarised as the simple implementation of a genotyping scheme. Genotyping for *PRNP* is one of the steps of BP-CS, to be followed by an appropriate qualification of selected candidates and their consistent use as reproducers. This implies that the emphasis put on selecting ARR carriers and/or eliminating susceptible allele carriers (selection pressure) is generally limited. Practically, this means that some ARR carriers may be excluded from reproduction due to their low estimated breeding values (EBV) for the other selection traits or vice versa some homozygous susceptible animals may be used as breeding animals due to their high genetic merit for the other selection traits, unless CS resistance is an overwhelmingly desirable trait (e.g. in Cyprus). The way of managing the selection for more traits can strongly affect the result of the BP-CS. Different choices are probably driven by decisions related to CS prevalence and the starting allele frequencies.

In some other countries BP-CSs were not restricted to the first tier, but rather implemented in a uniform way in all flocks, including commercial ones (e.g. The Netherlands, 2005–2007). In other cases (e.g. Sardinia (Italy), see Appendix E), CF were involved in the genotyping and selection steps only after HGMF reached high resistant allele frequencies with the aim of accelerating the substitution rate of susceptible animals or as HGMF was not large enough to fulfill the needs of ram replacement in the overall sheep population.

#### 4.5.2. Principles for assessing the effectiveness of breeding programmes

The effectiveness of a BP-CS in a given population would ideally be measured by the observed evolution of *PRNP* genotype distribution in the whole population (HGMF and CF). Unfortunately, this information is accurate only, very generally, in the HGMF. Thus, the potential evolution of *PRNP* genotype distribution in CF has to be inferred from information about the BP-CS organisation using appropriate mathematical models describing the selection in HGMF and dissemination to the CF. However, such models are always an oversimplified representation of the real implementation of the selection tools, and the actual selection based on *PRNP* genotypes has to be estimated from the observed genotype evolution in HGMF. Thus, three components are needed:

- a) The observed evolution of *PRNP* genotype distribution.

This is the most comprehensive indicator of effective selection pressure. This evolution has to be evaluated in a reference class of animals along generations, to avoid bias. For example, it may be a random sample of animals before any selection step, all rams entering the breeding centre, or a sample of females kept as new reproducers. Finally the simplest approach would be a comparison of *PRNP* allele/genotype distribution in similar animal class at the start of the selection scheme and later on during its implementation. It provides information on the consistency of the decision rules established by the breeding organization.

- b) The potential evolution of *PRNP* genotype distribution.

The effectiveness of efforts made to improve CS resistance, by a breeder organisation or a MS, may be estimated using ad hoc mathematical models to forecast the evolution of the *PRNP* distribution given the situation and programme implemented. Based on population and quantitative genetics concepts, mathematical models help in optimising the design and evaluating the efficiency and profitability of BP-CSs. Simple models estimate long-term annual genetic progress, i.e. the linear evolution of the trait mean as an effect of selecting a population in the same way over a long period of time (Rendel and Robertson, 1950; Smith and Cimasoni, 1967; Bulmer, 1971; Bichard et al., 1973; Elsen and Mocquot, 1976). More classical population genetics models are also available, which describe the evolution of allele frequencies at a locus submitted



to a selection pressure (Crow and Kimura, 1970; Bulmer, 1971; Hill, 1974; Fournet-Hanocq and Elsen, 1998). Again, such models may be used for management or evaluation purposes (Hinks, 1970; Brascamp et al., 1993; Schaeffer, 2006). The consequences of BPs mixing the 'improvement of production' traits (polygenic) and resistance to CS (monogenic inheritance) may be predicted by merging these mathematical models (Lande and Thompson, 1990; Dekkers and van Arendonk, 1998; Manfredi et al., 1998; Pong-Wong and Woolliams, 1998).

Major inputs of the models are: (i) the distribution of the *PRNP* genotype before selection, and (ii) the expected selection pressures put on these genotypes, generation after generation.

Such modelling gives the expected evolution of *PRNP* genotype distribution, which depends on the starting frequencies of genotypes, the number of animals genotyped, and the contribution of each reproducer's class to the next generation. Such classes are defined by the sex, age and reproductive roles of individuals. For instance, rams used in the flocks may be a mix of animals classified as representing the medium of the range after the observation of their pedigree and their phenotypic value, and animals classified as elites after a progeny test. The potential effectiveness indicates the expected time for the BP-CS to reach its objective, and its relative costs in terms of genotyping.

c) The observed selection of *PRNP* genotypes.

This approach aims at estimating, from the observed evolution of the *PRNP* genotypes, what effort was put on the selection for this trait in practice, and can be obtained from field data. It should be estimated for those selection steps which include, at least partially, a choice of animals based on their genotype, and quantifies the distance between the intention of the plan and what has been achieved in practice. Indeed, modelling the potential evolution of the distribution of selected traits is always an oversimplified representation of the real implementation of the selection tools. For various reasons (e.g. the individual farmer's preferences for certain morphological types, calendar constraints linked to seasonality and market prices, limited acceptance of the shared selection rules, selection for production traits, variability of allele frequency across other areas, breeds, or flocks within a MS) only a small part of the planned selection pressure is generally applied in practice. The concept of effective (observed) 'selection differential' defined by Falconer in 1960 is a response to this difficulty (Falconer, 1981; Thompson, 1986). The observed selection differential, rather than estimating the evolution from the announced selection effort, measures the selection effort from the observed difference between candidates and selected animals. Considering both the potential evolution of *PRNP* genotype evolution and the selection effort, helps to evaluate how an announced BP was applied in the field, and its management.

The three approaches described above are complementary, and need to be applied together. The *observed evolution of PRNP genotype distribution* is a simple and global measure of the achieved effectiveness of a BP-CS. The potential evolution of *PRNP* genotype distribution is more difficult to estimate, both because it needs the development of an *ad hoc* model, and because this model has to be fed by input data that are not easily available. However, the comparison between them gives an evaluation of the quality of the application of a BP-CS in the field. Moreover, comparing the potential and observed evolution of *PRNP* allele frequencies allows discrimination between negative results (poor evolution of the *PRNP* genotype frequency) due to incorrect design of the selection scheme, and the unsatisfactory management of promising plans. Finally, the *observed selection of PRNP genotypes*, which needs to record the details of *PRNP* frequency evolution, identifies the limiting factors of the application of BP-CSs (e.g. at which selection steps the genotypes were incorrectly considered when choosing future reproducers).

If these indicators are needed to understand the evolution of the *PRNP* genotype frequencies in HGMF, they must be complemented by an assessment of the dissemination of the expected progress in CF, the second tier of the whole population.



Most often, this dissemination is operated simultaneously in different ways: it concerns males (possibly semen only) and females; males may be sold by individual breeders to CF owners (a very general situation) or under the control of the breeding scheme organization (e.g. the flock book) after a qualification step (as young rams, after a performance test period or at the end of a progeny test). The dynamics of genotype frequencies in a hierarchical population with a nucleus creating genetic progress and a second tier making profit of this progress via the diffusion of reproducers from the nucleus, has been deeply studied in the past (Bichard et al., 1973; Elsen, 1980; Shumbusho et al., 2013). When the trait of interest is polygenic it has been demonstrated that, as soon as a non-zero proportion of the reproducers of the second tier originated from the first, and whatever this proportion, the two sub populations progress at the same speed (equal annual genetic progress), with a constant lag between nucleus and commercial which depends both on the proportion (higher proportion, lower lag) and on the selection differential applied on the animals sent to the second tier. The dynamic for monogenic traits (the *PRNP* case) is more complex since a constant selection differential does not give a constant response due to the change in gene frequency (Dekkers and van Arendonk, 1998; Costard et al., 2009).

For various reasons, only a limited proportion of the rams used in the commercial flocks may come from the nucleus, even if the ratio between the size of the HGMF and the total number of replacement reproducers in the CF is large enough to supply all of them. Here again, this evolution should be estimated in a certain class of animals at the start of the selection scheme and later on during its implementation.

The potential evolution of *PRNP* genotype distribution in the global population may also be estimated using ad hoc mathematical models. The most important elements of the models are the proportion of new reproducers which were born in HGMF (males and females) and the way they were selected (based on the *PRNP* genotype). The maximum number of reproducers sent to CF is proportional to the size of HGMF ('Nn'), while the total number of replacement reproducers in the CF is proportional to the size of this tier ('Ng'). Thus the ratio 'Nn/Ng' is a good indicator of the capacity of the nucleus to fulfil its role, and provides information about the potential evolution of *PRNP* genotype distribution in the global population. However, field data to monitor the actual flow of reproducers from HGMF to CF is needed. In practice, many factors may affect the predicted flow. For instance, the absence of a centralised management of reproducers may have the consequence that a relevant portion of ARR animals are excluded from reproduction since they exceed the ability of a single breeder to raise them.

#### 4.5.3. Breeding programmes for resistance to Classical scrapie in EU Member States

Information on the implementation of BP-CS in the EU has been gathered by different means, as anticipated in Section 4.2. In the EU, a total of 17 MSs have implemented or are currently implementing a BP-CS, the earliest being implemented in 1998 in the Netherlands, while the most recent started in 2012 in Romania. Austria, Bulgaria, Denmark, Finland, Latvia, Lithuania, Malta, Poland, Portugal and Sweden have not implemented a BP-CS. Details on the start and end years of BP-CS implementation in the EU MSs are reported in Table 2.

As already mentioned, a questionnaire (see Appendix A.2) was developed and distributed to the different MSs. It was constructed on four pillars, that were considered the four principal elements constituting a BP-CS:

- The population structure: numbers related to total populations, to their subdivision in flocks and to the percentage of HGMF vs total population. These were required to know the frame in which each MS has to operate.
- Genotyping effort (BP-CS's genotyping step): it is clear that this action is fundamental in order to obtain a high number of resistant animals to use in a BP-CS. Thus information was required about the number of animals genotyped in HGMF and in CF, the period over which the genotyping has been undertaken and the proportion of males that are genotyped relative to females.

- Rules for selection of rams (BP-CS's selection step): a genotyping effort can be valid only if genotyped rams are properly selected for use afterwards. Information on the presence of rules for ram selection and for follow up of the selection made were requested from MSs. In this section the starting and final frequencies of resistant animals in the genotyped populations were also requested.
- Rules for dissemination of rams (BP-CS's dissemination step): given that most of the MSs concentrated the genotyping mainly or exclusively on HGMF and in the absence, in most cases, of an accurate value related to ARR allele frequency in the general population, rules for the dissemination of resistant rams and the presence of controls on their compliance were requested, in order to indirectly evaluate the effectiveness of the flow from HGMF to CF.

Replies to the questionnaire were obtained from: Belgium, Cyprus, Czech Republic, Estonia, France, Germany, Hungary, Ireland, Italy, The Netherlands, Slovakia, Slovenia, Spain, UK.

#### 4.5.4. Assessment of breeding programmes for resistance to Classical scrapie in EU Member States

##### 4.5.4.1. Methods

The only accurate way to assess the effectiveness of the MSs' BP-CSs would be to know the precise frequency of the ARR allele, and the animals with resistant genotypes, in the general ovine population. In the absence of this information, the effectiveness of BP-CSs has been initially evaluated through a descriptive analysis of the replies to the questionnaire.

The four components of the questionnaire were evaluated separately for each MS. The evaluation was carried out based on the assumption that the diffusion of ARR allele to CF is mainly determined by:

- the relative size of HGMF vs. CF tier: the higher the relative size the higher the proportion of replacement of CF breeding rams potentially coming from HGMF;
- the proportion of new rams genotyped in HGMF: the higher this proportion, the higher the number of genotyped rams available for CF;
- the proportion of ARR carrier rams in HGMF: the higher this proportion, the higher the number of ARR carriers available for dissemination to CF;
- the presence of, and compliance with, rules modulating the diffusion of rams from HGMF to CF which permit the dissemination of ARR carriers only.

A deterministic model was then developed to: i) compare the potential and observed evolution of *PRNP* genotype distribution in HGMF, and ii) evaluate the dissemination towards CF.

The model was based on the approaches defined in Section 4.5.2. The observed evolution of the *PRNP* genotype distribution in HGMF (component 1) was compared to the potential evolution (component 2) obtained through a model describing the selection design. When a large discrepancy was observed between observed and predicted evolution of *PRNP* genotypes, the observed selection of *PRNP* genotypes in HGMF (component 3) was used to adjust the model parameters, through an adjustment factor ('rate'), which describes the selection pressure put on traits other than *PRNP* genotype. Potential evolution in the CF was estimated with this adjusted model.

The core of the model is a diffusion process as described, for instance, by Dekkers and Chakraborty (2001) and Costard et al. (2009). The population is divided into classes of animals sharing the same sex, age and tier. Each class is described by its *PRNP* genotype frequencies at a given year. The evolution of these parameters follows a Markov process which involves ageing, replacement and

selection steps. All elements of the diffusion equations are estimated from the input parameters. Details on the model used, including an explanatory diagram, are reported in Appendix F.

The quantitative assessment has been carried out only for MSs included in the analysis of the epidemiological trend (Chapter 3) and that provided replies to the survey (i.e. Belgium, Cyprus, Czech Republic, France, Ireland, Italy, The Netherlands, Slovakia, Slovenia, Spain, United Kingdom), since the aim was to compare the type of BP-CS with the observed trend of CS, as explained in Section 1.1. For the United Kingdom, because of a lack of data on some input parameters for the model, the analysis has been carried out using data relating to Great Britain only (referred to as United Kingdom (GB) from now onwards).

Table 2 contains an extraction of some data from the replies to the above mentioned questionnaire for MSs by which the BP-CS was assessed, and some other parameters, calculated from data collected through the questionnaire, which were used as inputs of the deterministic model, or that are crucial for their calculation. Where needed, values reported in Table 2 were obtained through approximations and assumptions from available data, as further explained in the table. Detailed information on the parameters used in the model is reported in Tables 10 and 11 (Appendix F).

#### 4.5.4.2. Limitations and uncertainties of the model

The deterministic model is based on a number of assumptions and approximations. Amongst these, the most important are that the progress in favourable allele frequency is only due to the selection of rams, and that genotypic frequencies in females equalled the values reported for the males, which is an optimistic assumption.

Limitations in the data available also influence the outcome of the model. For example, some parameters on the sheep population structure were not available from some MSs, and had to be estimated on the basis of specific assumptions, as indicated in Table 2. Variations in the population structure over the years were also not available. In other cases, the collected data showed some potential inconsistencies. For example, this was the case for the ARR allelic and genotypic frequency in the population in some MSs. These values were estimated assuming that the correct value was provided for the ARR allelic frequency (see Table 2 and Appendix F), which, ultimately, might not be accurate. Some data collected from MSs through a questionnaire survey (see Section 4.2) represented average values over the years, as in the case of the number of animals genotyped in HGMF and CF, while the precision of the model outputs would benefit from using actual year-by-year data.

The model assumes that sheep populations in MSs have uniform characteristics, but the actual heterogeneity of the population and of the implementation of BP-SCs also influence the evolution of the genetics over time. Based on the information provided, different types of BP-SCs and of types of dissemination of resistant rams from HGMF to CF were assumed, and used as input of the model (see Appendix F). However, these classifications are based on limited qualitative information provided by the MSs with variable degrees of detail.

An additional source of uncertainty originated from the unknown effort that each BP-CS put on the concurrent selection for non-scrapie traits: this led to the need to apply an empirical country-specific adjustment parameter (see Appendix F).

Consequently, the results obtained from the use of the deterministic model should not be considered as trustworthy point estimates of the ARR/ARR frequency in the sheep population of each MS by tier and over time. However, the model has been helpful in describing the national trends of the potential evolution of the ARR/ARR frequency, based on the characteristics of the BP-SC, and is a valuable tool to inform the comparison the potential evolution of the resistance to CS in the sheep population with the trend of CS in the different MSs.

**Table 2:** Extraction of some data on the characteristics of BP-CSs from replies obtained by MSs for which the BP-CS was assessed, and additional parameters, calculated from data collected through the questionnaire ‘Breeding programmes’, which were used as input of the deterministic model, or that are crucial for their calculation. Where needed, values reported in the table were obtained through approximations and assumptions from data available, as further explained in the footnotes. Detailed information on the parameters used in the model is reported in Tables 10 and 11 (Appendix F).

Member State	Total population	Total population in HGMF (NfH)	First-last genotyping year for which data are available	Average number genotypings / year (NG)	Proportion of genotypings in HGMF (propH)	Proportion of genotypings in males (propM)	Annual HGMF replacement rate for males (rnmG) <sup>a</sup>	Observed ARR allele frequency (HGMF)		Observed ARR/ARR frequency (HGMF)	
								first year	last year	first year	last year
Cyprus	304 894	304 894 <sup>b</sup>	2004-2012 <sup>c</sup>	86 750	1.00 <sup>b</sup>	0.60 <sup>d</sup>	20.48	0.40 <sup>e</sup>	0.99	0.16 <sup>c</sup>	0.76 <sup>b</sup>
France	5 541 149	536 466	2002-2012	70 300	1.00	0.50	7.86	0.49	0.85	0.26	0.72
Ireland	3 430 300	127 933 <sup>f</sup>	2004-2012	9 418	0.97	0.53	4.54	0.86	0.998	0.62	0.84
The Netherlands	1 334 252	66 713 <sup>g</sup>	2005-2013	26 900	1.00 <sup>h</sup>	0.42 <sup>h</sup>	20.32	0.38 <sup>i</sup>	0.70 <sup>i</sup>	0.17 <sup>i</sup>	0.52 <sup>i</sup>
Slovenia	86 535	13 111	2006-2011	3 649	1.00	0.15	5.02	0.38	0.55	0.14 <sup>j</sup>	0.13
United Kingdom (GB) <sup>k</sup>	15 200 000	7 690 000	2002-2008	700 000	1.00	0.20	2.18	0.50	0.69	0.29	0.54
Belgium	185 624	32 573	2005-2012	733	1.00	0.58	1.57	0.87	0.98	0.76 <sup>j</sup>	0.79
Czech Republic	159 324	23 217	2003-2012	4 297	1.00	0.34	7.57	0.53	0.85	0.22	0.51
Italy	7 310 739	529 741	2005-2013	34 734	0.42	0.56	1.85	0.47	0.70	0.23	0.50
Slovakia	416 952	27 184	2004-2011	3 590	1.00	0.66	10.44	0.395	0.402	0.19	0.51
Spain	16 609 069	2 157 070	2003-2012	355 214	0.91	0.09	1.56	0.28	0.48	0.11	0.25

- a Ratio of new HGMF rams genotyped to males needed for replacement (see Appendix F for details on its calculation).
- b Cyprus makes no distinction between HGMF and CF, and all flocks are considered as a single tier with respect to the BP-CS.
- c Despite the BP-CS in CY starting in 1999, data in relation to 1999 were insufficient to be used as an input in the deterministic model and therefore for this purpose the starting year was assumed to be 2004, based on answers from 2010 Commission questionnaire (see Appendix F for details).
- d In the absence of information on the division of genotyping effort between males/females, it was assumed that all male newborns were genotyped, which leads to the estimation propM=0.60.
- e Value estimated from ARR/ARR frequencies, assuming HWE is respected.
- f Number of sheep in HGMF was not reported, and has been estimated based on the number of flocks in HGMF vs. CF, and assuming a similar average size of HGMF and CF.
- g Approximate number, estimated on the basis of information provided in the questionnaire.
- h In the absence of information on the division of genotyping effort between HGMF/CF and males/females, it was assumed that all male newborns were genotyped, which leads to the estimation propM=0.42.
- i These values refer to the whole population (HGMF+CF), as reported in the answer to the questionnaire.
- j Since values collected with the questionnaire were not consistent with allele frequency (HWE not respected), these values were estimated from ARR allele frequencies, assuming HWE is respected.
- k Because of a lack of data on some input parameters for the model, the analysis has been carried out using data relating to Great Britain only.

#### 4.5.4.3. Descriptive analysis of the replies to the questionnaires

- Population structure

Ireland, France, United Kingdom (GB), Italy, Spain and The Netherlands have large populations of more than 1 000 000 heads. The HGMF sheep population in the different MSs ranges from a percentage of 3.7 % (Ireland) to a percentage of 50.6 % (United Kingdom (GB)). Most of the countries are below 15 %. Cyprus has no distinction between HGMF and CF, and all flocks are considered as a single tier with respect to the BP-CS.

- Genotyping effort

Nearly all the MSs apply genotyping to the HGMF population only. Spain and Ireland are doing some genotyping in the CFs too, while more than half of the genotyping performed in Italy targets the CF. In Cyprus all flocks are genotyped. With regard to The Netherlands, in the absence of information on the division of genotyping effort between HGMF/CF and males/females, it was assumed that all male newborns were genotyped. Genotyping involves a high percentage of flocks of the HGMF tier in almost all countries (the figure was not available for Belgium). Generally, some females are genotyped, at different percentages and with different aims.

- Rules for selection of rams

All the MSs have rules for ram selection and an audit system to check compliance with these rules, except Ireland. In all the MSs an increase of the frequency of resistant animals in the target population has been achieved. When considering the results from the last year of genotyping, the resistant animals in HGMF are on average around 54 % whereas in Spain and Slovenia they are still at relatively low frequency (25 % and 13.4 % respectively).

- Rules for the dissemination of rams

Ireland and United Kingdom (GB) have no specific rules for dissemination. Belgium, Cyprus and The Netherlands allow the dissemination of ARR/ARR rams only. The percentage of compliance with the rules is above 80 % of the flocks in nearly all MSs. Italy, Slovenia and Spain have a percentage of compliance of 61-80 % (as defined by the questionnaire). All the MSs with rules have an audit system in place. The percentage of CFs that have introduced resistant animals from the HGMF is declared to be high in Cyprus, Czech Republic, The Netherlands, Slovakia (81-100 %), 61-80 % in UK, 41-60 % in France and in Italy, 21-40 % in Slovenia and Spain. No such estimation of the value was provided with regard to the situation in Belgium and Ireland.

The overall review showed a generally satisfactory organization of the BP-CSs in the different MSs. However, some weaknesses were found, such as the low percentage of HGMF in the majority of the MSs, the lack of rules for selection and dissemination in Ireland, the still relatively low frequency of resistant animals in the target populations in Slovenia and Spain, the lack of effective rules for dissemination in Slovenia, Spain and Italy, and a relatively low percentage of CFs that have actually introduced resistant rams from HGMF in France.

#### 4.5.4.4. Quantitative assessment through the deterministic model

When analysing the input parameters of the model, a large discrepancy with the Hardy-Weinberg Equilibrium (HWE) in the initial ARR allele and ARR/ARR frequencies in three cases (Slovenia, Belgium and Slovakia) suggested that the information concerning the initial situation was not fully reliable for these values. Moreover, the adjustment factor (rate) was highly variable between countries (from 0.14 to 1.0, see Tables 10 and 11 in Appendix F).

To assess the effectiveness of the national BP-CSs, to try to infer their potential impact in the control of CS, and to compare this with national trends of CS, some parameters obtained through the

deterministic model may be particularly helpful. Figures 13 and 14 summarise the main outputs of the model, and allow some meaningful comparison by country and by CS trend group (statistically significant decreasing trend vs. trend not statistically different from a flat one, see Chapter 3) of the main achievements obtained by the national BP-CSs in HGMF, CF and in the whole population (HGMF+CF).

However, when analysing the outputs of the model, it should be borne in mind that a BP-CS, when effective, creates a progressive change of allele frequencies. Therefore, summarizing a BP-CS's effectiveness by the final situation only (e.g. the adjusted potential evolution of the ARR/ARR frequency in the whole population) is a very incomplete description of the dynamics of the BP-CS and is not sufficient, on its own, to evaluate the effectiveness of a BP-CS. Other factors have to be considered, such as:

- The initial ARR allele frequency: in a case where the initial ARR allele frequency is very low (e.g. Slovakia), even BP-CSs which rapidly increase the ARR allele frequency to intermediate levels may not be able to impact on the CS prevalence. On the other hand, when the initial ARR allele frequency is high (e.g. Ireland) the trend of the disease may improve even if the effectiveness of the BP-CS is low.
- The initial CS prevalence: in a case where the initial prevalence is low, an effective BP-CS is able to show its effect only on in the longer term.
- The accuracy of surveillance, which affects the ability to describe the evolution of CS in the population.
- The heterogeneity of CS prevalence and ARR allele frequency within a country (e.g. in terms of geographical distribution, breeds and flocks).

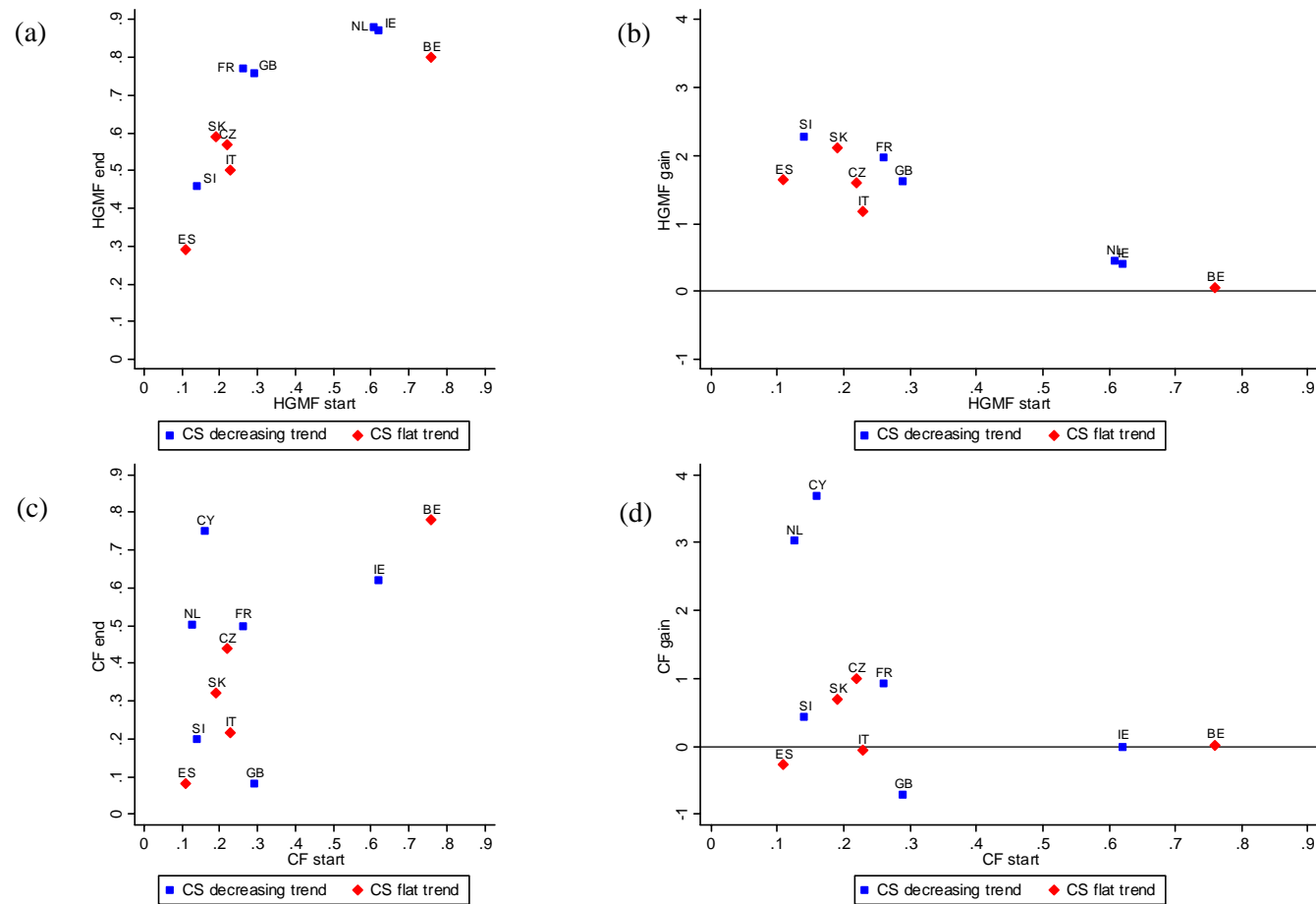
Moreover, the evaluation of the effectiveness of a BP-CS simply based on the raw increase of resistant allele frequency may underestimate the effort of MSs where the initial ARR frequency was high and, on the other hand, emphasise the achievements of MSs where the initial ARR frequency was low. This is due to the fact that the raw increase in resistant allele frequency is higher with intermediate starting allelic frequencies than with extreme allelic frequencies even when the same selection effort is applied.

Finally, from an epidemiological point of view, the prevalence of CS in a country at a given point in time depends on the genotypes frequencies present years earlier. Therefore, the potential effect of the current *PRNP* genotype distribution may result in a change of the situation only in the future, and its interpretation may not be straightforward. The current genotype frequencies are at most an indirect indicator of the resistance of the population to CS.

Figure 13 shows scatterplots of MSs, showing:

- on the X axes: the initial national observed ARR/ARR frequencies at the start of the implementation of the BP-CS (in HGMF in Figure 13(a-b), and in CF in Figure 13(c-d));
- on the Y axes: the national adjusted potential evolution of the ARR/ARR frequencies (in HGMF in Figure 13(a), and in CF in Figure 13(c)), or their relative increase in 2013 compared to the beginning of the implementation of BP-CS (in HGMF in Figure 13(b), and in CF in Figure 13(d)).





**Figure 13:** On the left, evolution of the ARR/ARR frequency in HGMF (a) and in CF (c) populations at the start of the implementation of the BP-CS and as estimated in 2013 ('HGMF end' and 'CF end') for the different countries. On the right, relative increase of the ARR/ARR frequency in HGMF (b) and CF (d) population from the start of the implementation of the BP-CS to 2013 (estimated value: 'HGMF gain' and 'CF gain') for the different countries

With regard to HGMF (Figure 13(a-b)):

- Three MSs, namely The Netherlands, Belgium and Ireland start from a high ARR/ARR frequency in HGMF and show a weak (NL, IE) or null (BE) effect of the BP-CS.
- Two MSs, namely France and the United Kingdom (GB) show an evolution from an initial unfavourable state (ARR/ARR frequency close to 30 %) to a very favourable one (high ARR/ARR frequency between 70 and 80 %); in the case of the United Kingdom (GB) this is particularly important, since the HGMF population is about 50 % of the whole population.
- Two MSs, namely Slovenia and Slovakia, show a very high relative increase of the ARR/ARR frequency; however, due to a lower starting frequency compared to France and the United Kingdom (GB), the final ARR/ARR frequency achieved is also lower.
- The last group of countries shows a variety a situations, with low ARR/ARR frequency at the beginning of the BP-CS, and a large range, but not extreme, increase of resistance.

With regard to CF (Figure 13(c-d)):

- Two MSs, namely Belgium and Ireland, start from a high ARR/ARR frequency and do not show any effect of the BP-CS.
- Two MSs, namely Cyprus and The Netherlands, show a dramatic change of their situation, from an initial unfavourable state (low ARR/ARR frequency, respectively 16 % and 13 %) to a favourable one (ARR/ARR frequency of 75 % and 50 % respectively); in the case of Cyprus this is a remarkable effect, since this tier represents the whole population.
- Two MSs, namely France and Czech Republic, show a similar evolution, doubling their initial values; however, only in France is an ARR/ARR frequency of 50 % achieved.
- The last and most numerous group shows a variety a situations, with low ARR/ARR frequency at the beginning of the BP-CS, and low or no increase of resistance; the United Kingdom (GB) shows a clear decrease in the ARR/ARR frequency in this tier.

When looking at the mean and median Y values (Table 3) in the two groups of countries defined by their CS epidemiological trend (statistically significant decreasing trend (CY, FR, IE, NL, SI, UK(GB)) vs trend not statistically different from a flat one (BE, CZ, ES, IT, SK)), they are always higher (even though without statistical significance) in the first group compared to the second one, suggesting a more favourable genetic evolution.

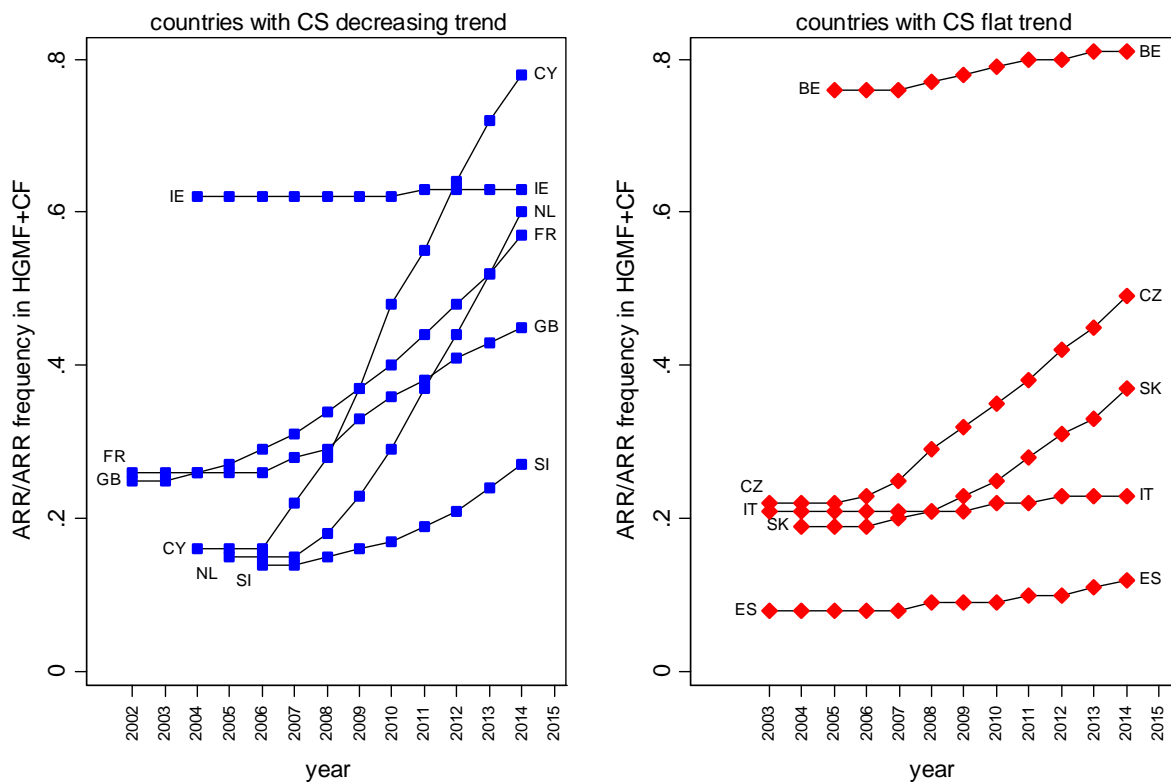
**Table 3:** Mean and median values of final (2013) ARR/ARR frequency ('end') in HGMF and CF and of their relative increase ('gain') by CS epidemiological trend.

CS trend	HGMF end	HGMF gain	CF end	CF gain
	mean	mean	mean	mean
decreasing	0.75	1.34	0.44	1.23
flat	0.55	1.31	0.37	0.27
	median	median	median	median
decreasing	0.77	1.62	0.50	0.68
flat	0.57	1.59	0.32	0.03

Figure 14 shows the prediction of the deterministic model with regard to the evolution over time of the ARR/ARR frequency in the general population (HGMF+CF) for the MSs included in the analysis.

Three tendencies can be observed:

- A relatively flat evolution of the ARR/ARR frequency in the whole population in Belgium, Italy, Ireland and Spain, even though those countries started from very different ARR/ARR frequency (from less than 10 % to more than 80 %). Three of them (Belgium, Spain and Italy) belong to the group of countries where the epidemiological trend of CS was unfavourable, i.e. not statistically different from a flat one. In Ireland, despite the fact that the ARR/ARR frequency in the whole population did not show any relevant improvement, the favourable evolution of the trend of CS may be consistent with the very high initial ARR/ARR frequency.
- A very strong evolution in most of the countries with a statistically significant decreasing trend of CS, namely Cyprus, The Netherlands, France and, to a lesser extent, the United Kingdom (GB), where the ARR/ARR frequency in the whole population increases from low levels (15-30 %) to very high levels.
- A moderate increase of the ARR/ARR frequency in the whole population for the other countries (CZ, SI, SK), with a very similar slope of the curves, but with varying starting ARR/ARR frequencies and starting years, resulting in a large variability of the final ARR/ARR frequency.



**Figure 14:** Estimated evolution over time of the ARR/ARR frequency in the whole population (HGMF+CF) in the different countries. On the left countries with blue squares are the ones with a statistically significant decreasing trend of CS in sheep; on the right countries with red diamonds are the ones with a trend not statistically different from a flat one.

Some considerations are discussed below in relation to the results of the deterministic model for the single MSs, grouped according to the observed trend of CS, according to the trend analysis described in Chapter 3. More detailed information on the parameters used for the single countries and respective results of the model are provided in Appendix F.

- Countries with a statistically significant decreasing trend in the prevalence of CS:
  - Cyprus: Cyprus is a very specific case since the whole population is involved in the BP-CS. The estimated ARR/ARR frequencies in the whole population in 2013 are high, despite the fact that the resistant allele was extremely rare in the early stages of implementation of the BP-CS. The prediction of the deterministic model is consistent with the characteristics of the BP-CS as described in Section 4.5.4.3, in particular the very large genotyping effort which allowed a strong selection of rams on their *PRNP* genotype.
  - France: The BP-CS as described in the questionnaire has a great potential to increase the frequency of the ARR allele. However, it is not running at its full speed, and selection of rams based also on additional criteria limits the selection pressure on *PRNP* genotypes. The dissemination to the CF probably did not result in more than 50 % of individuals being ARR/ARR. As many breeds are present, some not following the rules in a very strict way, this global result should be assumed to be heterogeneous within the population.
  - Ireland: The ARR/ARR frequency is very high in both the HGMF and the CF. However, this is mainly due to the quite high starting frequency, and the results of the deterministic model show a limited increasing trend in HGMF and no increasing trend in CF. Both the relative size of HGMF (less than 4 %) and the lack of rules seem to be limiting factors for good results in the CF.
  - The Netherlands: The BP-CS implemented in The Netherlands is efficient and correctly applied in HGMF. This gives a good evolution of ARR/ARR frequency in this subpopulation. If the rule of disseminating only ARR/ARR rams to the commercial flocks was respected, the deterministic model predicts that an effect of the BP-CS in the CF would have been observed for the last six years (2008-2013).
  - Slovenia: From the deterministic model, the ARR/ARR genotype frequency, starting from an unfavourable situation, improved sizeably in HGMF and at a lower level in CF, where it doubled. This is consistent with the relative size of the two tiers, and the proportion of HGMF rams genotyped. However, the potential evolution, in HGMF, before correction for the extra selection pressure on traits other than *PRNP* genotype, was much higher than the observed evolution, showing that the implementation of rules based on ram genotype would be crucial to accelerating results.
  - United Kingdom (GB): The deterministic model suggests that the applied BP-CS had great potential to increase ARR/ARR frequency in HGMF. It also shows that dissemination to the CF was inefficient, with a negative evolution of the resistance to CS. This result may be explained by: i) the almost 1:1 ratio between the two tiers, ii) the absence of dissemination rules preventing CF breeders from using susceptible rams born in HGMF, and iii) the low selection pressure applied in HGMF. A positive evolution could be predicted to be observed from 2016, but the BP-CS was stopped in 2009.
- Countries with a trend not statistically different from a flat one in the prevalence of CS:
  - Belgium: The ARR/ARR frequency is very high in HGMF and above the mean in the CF. However, the evolution in the CF is null, and the high final frequency seems to be due to a retention of the high initial frequency. One explanation may be found in the small amount of genotyping performed in HGMF (ratio of new rams genotyped in HGMF to males needed for

replacement is only 1.57), giving no room for choice of resistant rams to be sent to the CF. The low number of genotyped rams along with the rule which permits the diffusion of ARR/ARR rams only from HGMF may have reduced the potential evolution in CF mainly during the early years of implementation of the BP-CS, when the ARR/ARR ram frequency was not sufficient. However, it is likely that in the longer term the BP-CS, after increasing ARR/ARR frequency in HGMF, will produce results in CF.

- Czech Republic: The BP-CS implemented in Czech Republic could be more efficient considering the number of genotypes measured and the compulsory rules in place. The ARR/ARR frequency after 11 years of selection is much lower than expected, probably due to a strong selection of males on traits other than known *PRNP* genotype. The size of the HGMF tier is large relative to the CF, and the dissemination rate could be high.
- Italy: From the qualitative analysis it seems that poor dissemination would make the BP-CS effective only in the longer term, and no positive results would be expected to be visible at present. Similarly, the model suggests that the BP-CS may produce results in HGMF but that the impact on the general population is not clear. However, the impact of genotyping in CF was not modelled. Like France, a better interpretation of results may be achieved by using data at a breed level (see for example the BP-CS implemented in Sardinia, described in Appendix E).
- Slovakia: The deterministic model indicates a high positive trend for ARR/ARR frequency in HGMF and a positive evolution in CF. The very large amount of genotypings carried out in the HGMF, allowing a strong selection in favour of ARR carriers, probably explains these results.
- Spain: The BP-CS as described in the questionnaire has a great potential to increase resistant allele frequency. However, even a small pressure on other traits strongly limits the evolution of the ARR frequency. This is due to the relatively low proportion of the animals that are genotyped being rams, which restricts the selection rate to 0.64 on the male side. The dissemination to CF looks very limited. It was assumed in the modelling that there was a strict application of the rules (i.e. no dissemination of fully susceptible rams). The majority of the genotyping was performed on the ewes, with the declared objectives of flock qualification and mating plans. The model does not consider this part of the BP-CS. Its application by the breeders shows that *PRNP* genotype is only a marginal selection objective.

#### 4.5.5. Interpretation of the lack of a significant trend in the evolution of Classical scrapie in some countries

##### 4.5.5.1. Sheep

As described in previous chapters, the evolution of the prevalence of CS in sheep in the EU MS during the period 2002-2012 is very heterogeneous with some countries detecting a sporadic low number of cases, some countries consistently confirming cases with no significant change in the trend and other countries in which there has been a statistically significant decline during this period.

The countries where the trend in the prevalence of CS was not statistically different from a flat one over the 2002-2012 period are: Belgium, Czech Republic, Greece, Italy, Romania, Slovakia and Spain. In four additional countries (Bulgaria, Germany, Hungary and Poland), CS has been reported only sporadically over the mandate period preventing any interpretation of the epidemiological evolution.

The situation in the above countries with regards to the two main components of the control strategy of CS, namely, the cull of infected flocks supported by surveillance for the detection of new cases, and the breeding for resistance, is quite variable. It is recognized that all countries comply with the EU legislation in terms of quotas for the SHC or the NSHC, as well as with the implementation of compulsory eradication measures for infected individuals and flocks/herds.

It is not possible to identify causes that can explain objectively the failure to improve the situation of CS in specific countries where the disease has been detected consistently during the mandate. Since a significant declining trend has been observed in countries where control measures and BP-CSs are in place, it is quite plausible that the causes of the unfavourable trend might be linked to ineffective implementation of either or both strategies.

A number of potential reasons to explain the lack of a favourable trend in part of the countries can be envisaged, also in light of the information gathered from MSs through a survey on the monitoring sampling strategy in EU MSs (see Section 2.3), to which the paragraphs below refer:

- In relation to the BP-CS:
  - Short duration of implementation of the BP-CS: some countries have not implemented the BP-CSs for long enough to observe any substantial increase in the overall genetic resistance of the sheep population, for example Romania (2012) and Greece (2007).
  - Low ARR/ARR frequency in the target populations when the BP-CS started (e.g. Spain and Slovakia).
  - Lack of national coverage of the BP-CS, and in particular regional heterogeneity (e.g. Italy).
  - Effectiveness of the BP-CSs manifested by the low number of genotyped rams in HGMF (e.g. Belgium and Spain), the limited rate of selection of rams within HGMF (e.g. Czech Republic, Spain and Italy), and the inefficacy of or the lack of rules for dissemination of resistant rams from HGMF to CF (e.g. Italy and Spain).
- In relation to surveillance and non-genetic control measures:
  - Variability and/or reduction of the surveillance pressure: due to changes in the EU requirements, countries with less than 750 000 small ruminants ceased the testing of SHC in 2007 (e.g. Belgium and Czech Republic), leading to a lack of confirmed cases afterwards. For example, in Belgium the disease has not been reported since 2008 after some years of an apparently decreasing trend. In this country three distinctive periods of different surveillance pressure are evident: until December 2003, 3 750 adult sheep/goats were tested within the SHC survey. At that point it was decided to stop testing because “*the number of animals that was tested was not representative of the Belgian sheep and goat population*”, as reported in the replies to the questionnaire ‘Sampling strategy and control measures’ (see Section 2.3 and Appendix A.1). Thus, between January 2004 and January 2006 there were no small ruminants tested within the SHC stream. From February/July 2006, all sheep/goats older than 18 months were tested, and since July 2007, no more testing of SHC has been conducted. Also the case of Czech Republic is not straightforward: an effective BP-CS may have contributed to the disappearance of observed cases of disease since 2009. However, as in Belgium, a major component of surveillance (SHC) has been interrupted since 2008.
  - Lack of representativeness of the selected animals for testing: over/under representation of certain flocks/herds in the SHC and NSHC surveys based on breed, age, geographical areas, rendering costs and subsidies (for example in Italy, Romania and Greece).
  - Limitation in data available preventing the detection of trends due to the lack of regular detection of cases: trends could not be estimated in countries not detecting cases consistently (e.g. Bulgaria, Germany, Hungary and Poland).
  - Short duration and/or the ineffective implementation of sanitary measures due to recent admission to the EU (e.g. Romania, Hungary, and Czech Republic), or implementation of measures below the EU minimum requirement. In other cases, alternative control measures



applied, such as enhanced surveillance in flocks with low CS prevalence instead of the cull (e.g. Greece), might have also played a role.

#### 4.5.5.2. Goats

In the case of CS in goats, the surveillance efforts are equal to those applied in sheep but with only five countries having a goat population larger than 750 000 and being required to test the maximum quota (i.e. Greece, Spain, France, Italy and Romania). However, it is not possible to compare the patterns of CS in sheep and goats for several reasons, which may also have influenced the absence of a favourable trend of CS in goats:

- Firstly, a difference in the husbandry systems in sheep and goats within countries where CS has been confirmed consistently in the goat population. In some cases (e.g. Greece, Spain and Italy) there is a high proportion of mixed sheep and goat holdings, which may explain the parallel evolution of the epidemic of CS in both species, whereas in other MS the two populations have little mixing, presenting different trends (e.g. United Kingdom).
- Secondly, absence of BP-CSs applied on a large or small scale, and the lack of resistant genotypes to be imposed for restocking in infected herds, result in the cull of infected flocks supported by surveillance for the detection of new cases, the only tools available to reduce the prevalence of CS. In the past, a lack of evidence about the availability of resistant alleles to promote breeding for resistance has precluded the implementation by breeders or breed associations of tailor-made initiatives to disseminate breeding animals holding resistant alleles or to apply selective culling in outbreaks. This was the case of sheep in some MSs well before national BP-CSs were put in place, e.g. for Swaledale, Suffolk, Charollais and Welsh Cheviots in Great Britain before the National Scrapie Plan was established in 2001. This situation could be reverted now with the mounting evidence of satisfactory levels of resistance identified in certain alleles of European breeds (see Section 1.2.2).
- Thirdly, variability of the control measures (which cannot include selective culling) applied in infected herds compared to sheep flocks. For example, the most affected MSs (e.g. Cyprus) were granted special dispensation from the culling and complete destruction of infected goat herds (the only option to apply to infected herds), which was replaced by the killing of animals with clinical signs, and the slaughter of healthy animals for human consumption (after testing negative). In other cases there was a delay in the implementation of the cull of infected herds (e.g. United Kingdom). The favourable evolution of the situation in France (in both sheep and goats) suggests the likely effectiveness of a high surveillance pressure in detecting and managing a large proportion of outbreaks, which may subsequently lead to a decline of the disease in the general population.

#### 4.5.6. Concluding remarks related to Terms of Reference 1 and 2

- BP-CSs, which satisfy the minimum requirements of EU legislation, have been implemented by 17 MSs. Other MSs did not implement BP-CSs, generally because of a favourable situation regarding the disease.
- There is a clear heterogeneity in the characteristics of the different BP-CSs implemented by the MSs. There is also heterogeneity within MSs according to different areas, ARR allele frequency in the general population prior to BP-CS implementation, breeds and production types.
- Generally BP-CSs in the EU focus on HGMPF and are carried out through three steps (genotyping, selection and dissemination), with the aim of increasing the frequency of the resistant alleles in the general sheep population.

- Such a strategy:
  - requires the dissemination of males from HGMF to CF as a major determinant of the effectiveness of the final BP-CS;
  - results in a lag of several years between the initiation of a BP-CS and an effective change in the genotype structure of the general population.
- Applying a BP-CS does not imply that a genetic control of the disease is achieved. This depends on the characteristics of the BP-CS and on its effectiveness, which needs to be assessed based on the evolution of the genetic structure of the population.
- The crucial parameter to assess the effectiveness of BP-CSs in MSs is the frequency of the resistant allele in the whole sheep population.
- In the absence of this parameter, which is generally not available, the potential effectiveness of BP-CSs has been assessed in this Opinion through a combination of qualitative expert assessment and modelling of the diffusion of CS resistant alleles from HGMF to CF through selected resistant reproducers.
- The result of the dissemination of CS resistant alleles to CF (in terms of the potential evolution of ARR/ARR frequency in CF) is not, by itself, a sufficient indicator to evaluate the effectiveness of a BP-CS in reducing CS prevalence.
- The impact of a national BP-CS on the epidemiological situation of CS may be influenced by: i) both the ARR allele frequency and the CS prevalence prior to the implementation of the BP-CS, and ii) their heterogeneous geographical distribution.
- Given the characteristics of each national BP-CS, a deterministic model was used to estimate the ARR/ARR frequency in the general sheep population over time. Subsequently, the outputs of the model were compared with the national CS situations. The results obtained suggest a favourable CS situation being linked to better-achieving BP-CSs.
- Cyprus and the Netherlands, countries in which the improvement in the epidemiological situation of CS is clear, applied their BP-CSs to the whole population, without any distinction between population tiers. This approach produced an effective change of the genetic structure of the whole sheep population, but required extensive genotyping efforts.
- A more detailed and reliable assessment of BP-CSs would need:
  - A detailed description of the breeding structure of the whole population, possibly by breed, with particular reference to the relative size of HGMF.
  - An explicit target for BP-CSs, in terms of time, to get a given ARR frequency in the whole population.
  - An information system able to collect:
    - Individual data on identification, genotype, selection, movements (e.g. number of genotyped reproducers moving from HGMF to CF).
    - Population data on the evolution of resistant allele frequencies, estimated through representative samples of the whole population. It would be preferable to have distinct estimations for each breed. If this is not possible, the sampling strategy must take into account breed size.

- Effective measures of the amount of reproducers moving from HGMF to CF.
- Although it is not possible to identify causes that can explain objectively the failure to improve the situation of CS, the assessment of the country-specific available data related to the implementation of surveillance, non-genetic control measures and BP-CSs may allow the formulation of some hypotheses.
- With regard to sheep, it is quite plausible that the causes of any unfavourable trend might be linked to ineffective implementation of either or both strategies. Based on data collected from MSs, a number of potential reasons to explain the lack of observation of a favourable trend in part of the countries can be envisaged in relation to:
  - The BP-CS: a short duration of implementation of a BP-CS, a low ARR/ARR frequency in the target populations when the BP-CS started, a lack of national coverage of the BP-CS, a low number of genotyped rams in HGMF, a limited rate of selection of rams within HGMF, and inefficiency of, or lack of, rules for the dissemination of resistant rams from HGMF to CF.
  - Surveillance and non-genetic control measures: variability and/or reduction of the surveillance pressure, a lack of representativeness of the animals selected for testing, a limitation in available data preventing the detection of trends due to the lack of regular detection of cases, a short duration and/or ineffective implementation of sanitary measures due to recent admission to the EU, or implementation of measures below the EU minimum requirement.
- With regard to goats, differences in the husbandry systems in sheep and goats within countries, absence of BP-CSs, and variability of the control measures (which cannot include selective culling) applied on infected herds compared to sheep flocks may explain the lack of observation of a favourable trend. The favourable evolution of the situation in France (in both sheep and goats) suggests the likely effectiveness of a high surveillance pressure in detecting and managing a large proportion of outbreaks, which may subsequently lead to a decline of the disease in the general population.

#### 4.6. Estimating a minimum ARR allele frequency to observe fading-out of Classical scrapie

Given the very strong resistance of the homozygote ARR genotype to CS, there is a conceptual parallel between the ARR homozygote proportion of a sheep population and the proportion immunized in a vaccinated population. As a result, in an analogy with the well-known concept of critical vaccination coverage (Anderson and May, 1992), one may expect that a minimum frequency of the ARR allele in a sheep population exists above which CS may be expected to fade-out.

##### 4.6.1. The concept of ‘fading-out’

It is a basic concept in modern infectious-disease epidemiology that the epidemic spread of an infection is under control as soon as the control measures taken reduce the ‘basic reproduction number’ (denoted by  $R_0$ ) to a value below one (Anderson and May, 1992). In this situation, each infection will cause less than one new infection, and as a result the epidemic will sooner or later come to an end (i.e. fade-out). The minimum frequency of the ARR allele in a sheep population above which CS may be expected to fade-out is that frequency level for which the  $R_0$  for CS in the population considered equals one.

It is important to note that once a sufficiently high ARR allele frequency is obtained (and thus the condition  $R_0 < 1$  has been met) in a MS or within a particular breed within a MS, this does not imply that after fade-out, if a sufficiently high frequency is maintained, CS cases will no longer occur. Rather, CS cases may still occur, either through introduction of infected animals from outside the population or from infectivity in the environment, but such cases will no longer pose a risk of causing prolonged epidemic spread of CS in the population.

In addition, it is important to note that the sheep population in a MS, or of a certain breed in a MS, is structured in flocks, and contacts between sheep residing within the same flock will be more intensive than that between animals residing in different flocks. This means that it is natural to consider two levels of transmission in the population: within-flock and between-flock transmission (Gubbins et al., 2010). From this point of view, a situation with  $R_0 < 1$  for CS may be interpreted as a situation in which isolated within-flock outbreaks of CS may occur but no major between-flock spread will be possible.

#### **4.6.2. ARR frequency and factors affecting its minimum level for fading-out of Classical scrapie**

An analysis of data for The Netherlands has shown that the relative decrease in the prevalence of CS in the period 2005-2008 exceeded the relative decrease of the sensitive population (non-ARR carrying genotypes) (Hagenaars et al., 2010). This observation indicates that the breeding programme generated 'herd immunity' – expected from the analogy with vaccination – and thus suggests that a minimum ARR frequency for CS fade-out indeed exists. In the years since these analyses were published, the Dutch CS prevalence found through surveillance has further declined (see Section 3.2.1). It is important to note that the minimum ARR frequency is a valid overall minimum level in the population as long as there are no large differences between frequency levels in sub-populations. Therefore, if a national population consists of several distinct large breeds with substantial differences in genotype frequency distribution, the minimum ARR frequency should be calculated for each breed separately.

The predicted minimum ARR frequency for CS fade-out is not universal, i.e. it is not the same for all sheep populations. The reason for this is that the transmissibility of any infectious disease (and thus CS in particular) in a given population is not only determined by properties of the agent itself and susceptibility properties of the population (i.e. genotype frequencies in the case of CS), but also by the prevalence of (further) risk factors which may well differ between populations. The level of lambing hygiene may be one such risk factor. A definite risk factor for transmission between flocks is the presence of relatively high contact rates between animals from different flocks (caused by co-grazing, or by keeping different flocks in relatively close geographic proximity). Likewise, a population in which there is a relatively frequent exchange of animals between flocks will be subject to higher CS transmission risks. To control these higher risks the predicted minimum ARR allele frequency level will be higher. Also, genetic risk factors different from the overall ARR allele frequency level are relevant. Firstly, differences in the level of heterogeneity between flock-level ARR allele frequency are expected to have an impact on the minimum ARR allele frequency (Melchior et al., 2010). Secondly, the susceptibility properties of the population are not only determined by the ARR allele frequency but also the frequency of other alleles in the population. For example, with the CS strain(s) encountered in Great Britain and The Netherlands, if sheep population A has a higher VRQ allele frequency than population B and all other properties are equal, than the predicted minimum ARR frequency for CS fade-out would be higher for A than for B. This is because the remaining non-ARR carrying animals in population A are on average more susceptible (VRQ/VRQ and VRQ/ARQ being more highly susceptible than ARQ/ARQ in Great Britain and The Netherlands (Hagenaars et al., 2010)), and thus their frequency is required to be lower to compensate for this. For similar reasons, for a given sheep population carrying the VRQ allele, a selective breeding strategy consisting of a combination of selecting ARR/ARR rams and selecting non-VRQ carrying ewes for breeding, will lead to a lower predicted minimum ARR allele frequency than a strategy based only on selecting ARR/ARR rams. For other countries and/or CS strains, the susceptibility pattern across non-ARR alleles may be different (Baylis and Goldmann, 2004), leading to a different dependence of the overall susceptibility of the population on the frequencies of certain alleles.

#### **4.6.3. Calculation of the minimum ARR frequency for fading-out of Classical scrapie**

In order to illustrate how the minimum ARR frequency for CS fade-out may be estimated and how it may differ between sheep populations, depending on VRQ frequency and between-sheep contact patterns, four case-study populations have been considered: sheep in The Netherlands, Sarda sheep in Sardinia (Italy), sheep in Cyprus, and sheep in Great Britain (GB).

Due to the dependence of the minimum ARR allele frequency on the various factors discussed above, data providing information on at least some of these factors is needed to enable a model estimation of this minimum level that is not rendered useless by a very wide confidence interval. In the case of The Netherlands in particular, the availability of a random flock genotyping sample from a random set of flocks in the field gave an insight into the heterogeneity between flock-level genotype distributions, and enabled scenario calculations of the minimum ARR allele frequency (Melchior et al., 2009). For the other three case study countries/breeds, no such random flock genotyping sample from the field was available, and therefore a (relatively) crude model analysis was used, based on input data provided by MSs (see Appendix A.4) and on published data (Ortiz-Pelaez et al., 2014a). The reference case prevalence was taken from SHC surveillance results for all three case study populations. The reference ARR allele frequency was taken from a sample from SHC for GB, and from a sample from NSHC for Cyprus (as for Cyprus the SHC sample was likely to suffer from a strong bias towards older animals, which have relatively low ARR allele frequencies). For Sarda the frequency of the ARR allele in rams genotyped from commercial flocks entering the BP-CS for the first time is used. The estimates obtained using the model, should not be considered as trustworthy estimates of the minimum ARR allele frequency. These estimates are presented solely for the purpose of illustrating the non-universality of the minimum ARR allele frequency, and of illustrating the influence of a number of the underlying determining factors mentioned above. In line with this, confidence bounds are not calculated. In order to illustrate how the model result depends on the assumed CS prevalence in the reference year/period, two scenarios are presented for each case study (scenario 1 and 2, see Table 4).

For the case of the Netherlands a report is available with model scenario calculations of the minimum ARR frequency (Melchior et al., 2009). For the purpose of this Opinion, consideration is given to the decline in CS prevalence observed in the Dutch active surveillance in recent years, concurrently with an increase in the ARR frequency in the Dutch sheep population. In 2013 the latter frequency reached a level of approximately 70 % according to random samples from the Dutch active surveillance. Recent very low prevalence (only 6 cases in the period 2009-2012 in close to 80 000 tested animals) suggests that the minimum ARR frequency for CS control has been reached. It is therefore plausible that in The Netherlands the minimum allele frequency required for CS control is in the range of 60 to 70 percent. As the mandate refers to a minimum allele frequency in the context where no control and eradication measure is being applied, there is a need to take into account the effect on the minimum allele frequency of cessation of the statutory control and eradication measures. As argued by Hagens et al. (2010), the effect of this on the reproduction number (and thereby on the minimum allele frequency) would be an increase of a few percent. On the basis of these observations and arguments it is assumed that the minimum allele frequency for CS control in The Netherlands, in a situation where no control and eradication measure is applied, is 70 %. The model calculation for the three non-Dutch case studies is based on calculating the  $R_0$  for a suitable starting year for which both scrapie surveillance data and random genotyping data are available, and subsequently evaluating at which minimum ARR allele frequency the  $R_0$  is reduced to below 1 when selecting homozygote ARR rams for breeding. The calculation is based on scenario assumptions for certain input parameter values, and for each of the case study populations two such scenarios are considered. In this crude modelling approach there is no explicit structuring of the population into flocks. Instead, the model is based on using the population level genotype distribution and the population level CS prevalence, which are both defined for a population of animals (and not a population of flocks), as input parameters; these are 'between-animal' parameters. Population-level random genotyping data in healthy slaughter and/or fallen stock and scrapie surveillance data in healthy slaughter are used to set these input parameters. The flock level is then included implicitly by using the case of The Netherlands to set a parameter that translates from the between-animal to the between-flock level. When this approach is applied to a country with a more stratified and/or segregated sheep population than the Dutch sheep population, it is likely to lead to underestimation of the between-flock  $R_0$ . This is because ignoring (part of) the structuring of a population will tend to bias the estimated  $R_0$  downward (Keeling and Rohani, 2008). A further possible difference between countries not taken into account in this model is in the contribution of 'control and eradication measures' to reducing  $R_0$ . Although for The Netherlands this contribution was estimated to be minor (Hagens et al., 2010), this might be different elsewhere. Cessation of these measures in that case leads to an increase in the minimum ARR



frequency required. A more detailed description of the modelling approach is provided in Appendix D.

Table 4 lists the model estimates for the non-Dutch case studies together with the input parameter values used that were derived from the surveillance and genotyping data. The results for GB suggest that only a relatively small increase in ARR frequency would be sufficient to obtain CS control. However, additional population heterogeneity due to between-breed differences in GB sheep, not taken into account in the model, may in reality lead to a higher minimum ARR frequency requirement. In comparison with the minimum level of approximately 70 % for The Netherlands, the predicted values of 56 % and 58 % of the two scenarios are low, and this is due to the input parameters which represent a lower reference prevalence in susceptible genotypes despite a lower reference ARR allele frequency, which may be explained by a combination of less intensive contact rates and lower relative abundance of the VRQ allele in GB as compared to NL.

The results for Sarda sheep in Sardinia also suggest a lower minimum ARR frequency than for NL. Here, on the other hand, the reference (starting point) value of CS prevalence in susceptible genotypes is higher than for NL (in both scenarios), such that a higher relative increase in ARR allele frequency is required to achieve CS control. On the other hand however, at the starting point the ARR allele frequency is much lower than for NL reference point.

The results for Cyprus suggest a minimum ARR frequency close to 100 %, i.e. much higher than for the other case study populations. A likely explanation is that contact rates are much higher between sheep in different flocks in Cyprus than elsewhere, due to the close geographic proximity of flocks located in farming areas on state-owned land. The lack of difference in result between the two scenarios for Cyprus illustrates that the input parameters for Cyprus are in a region of values for which the model is performing poorly.

#### 4.6.4. Limitations and uncertainties of the model

Model calculation of the minimum ARR frequency is used for four case-study populations, as a means to illustrate the non-universality of the minimum ARR allele frequency. The model used is crude, as lack of more detailed data precluded the use of more detailed models. Consequently, the results merely illustrate the influence of a number of the underlying determining factors on the minimum ARR frequency, and should not be regarded as trustworthy estimates of the minimum ARR allele frequency. One of the reasons why the model is crude is that it uses the case of The Netherlands to set a parameter that translates from the between-animal to the between-flock level. When this approach is applied to a country with a more stratified and/or segregated sheep population than the Dutch sheep population, it is likely to lead to underestimation of the between-flock  $R_0$ , and thereby of the minimum ARR allele frequency.

**Table 4:** Non-Dutch case study results: minimum ARR allele frequencies leading to CS fade-out.

Breed or country	Reference year/ period	Scenario <sup>(a)</sup>	Input: CS prevalence per 1 000 in surveillance in reference year/period	Input: ARR allele frequency in reference year/period	Result: model estimate for minimum ARR allele frequency
Cyprus	2012	1	2.0	0.706	Close to 100 %
		2	0.66		Close to 100 %
Great Britain	2006-2012 (case prev.)	1	0.186	0.523	56 %
	2012-2013 (ARR freq.)	2	0.256		58 %
Sarda in Sardinia	2005-2012	1	0.544	0.437	53 %
		2	0.777		58 %

(a) For scenario 1 the point estimate for the case prevalence in SHC is used. For scenario 2 either the upper bound of the 95 % confidence interval (GB and Sarda), or the 95 % lower bound (Cyprus) is used. Estimated relative abundance of VRQ amongst non-resistant alleles: 11.8 % for The Netherlands in 2008, 7.1 % for GB in 2012-2013.



#### 4.6.5. Concluding remarks related to Term of Reference 3

- Given the very strong resistance of the homozygote ARR genotype to CS, there is a conceptual parallel between the ARR homozygote proportion of a sheep population and the proportion immunized in a vaccinated population.
- A minimum frequency of the ARR allele in a sheep population can be estimated, above which CS may be expected to fade-out. The example of The Netherlands, where CS case prevalence in surveillance has become very low in recent years and the ARR frequency is (still) well below 100 %, provides evidence for the existence of the minimum ARR frequency for CS fade-out in the reality of the field.
- Once the ARR frequency in a MS has increased to above the minimum estimated requirement for fading-out of disease, this does not imply that CS cases will no longer occur. CS cases may still occur, either through introduction of infected animals from outside the population or from infectivity in the environment, but such cases will no longer pose a risk of causing prolonged epidemic spread of CS in the population.
- The concept of fade-out is different from eradication. Whereas eradication involves active interventions to remove remaining foci of transmission, fade-out is expected to occur naturally at some point in time once a sufficiently high ARR allele frequency is obtained and maintained.
- The minimum frequency of the ARR allele in the sheep population in a MS above which CS can be expected to fade-out, in a context where no control or eradication measure is being applied, is not universal and would have to be estimated for each particular national sheep population separately.
- Case studies illustrate the non-universality of the minimum ARR frequency; across the case studies it ranges between 53 % and close to 100 % according to a crude model. The case studies also provide some insight into how the minimum frequency depends on MS-specific parameters.
- Maintenance of an ARR allele frequency above the required minimum level needs to be monitored by taking representative genotyping samples at regular time intervals.

#### 4.7. Additional/alternative measures to control Classical scrapie

Two main operational tools for controlling and eradicating CS have been applied across the EU: the identification and management of infected flocks; and breeding for resistance taking into account the evolution of the genetic structure of the population (ARR allele in sheep / potential use of the K222 and S/D146 in goats) for preventing new cases and outbreaks. The combination of surveillance and control measures, together with increased genetic resistance at the population level must be ensured in order to achieve successful control of CS. Moreover an overall successful strategy will benefit from campaigns aimed at refreshing the knowledge of scrapie among the relevant stakeholders.

The evolution of the CS prevalence in the EU MSs in the goat and sheep population during the period covered by the Opinion (2002-2012) highlights the difficulties of efficiently controlling CS and ultimately eradicating it. The positive effect of the current statutory measures implemented across the EU has been recognised throughout this Opinion, as reflected in the significant decline in the prevalence of CS in sheep in six MSs where a combined strategy of surveillance/control and breeding for resistance has been in place. However there is room for improvement of the effectiveness of the control measures given the diversity of situations of EU MSs with regard to the size and structure of the small ruminant population, the breeding systems and the prevalence of CS.

#### 4.7.1. Surveillance and control

##### 4.7.1.1. Surveillance to detect cases of disease and facilitate its control

The detection of infected flocks, and the subsequent application of eradication measures, has likely played a role in reducing the prevalence and the number of outbreaks in most countries where such measures have been implemented (e.g. Iceland, France, UK, Ireland and Cyprus). The approach has had a rapid impact (within a few years) on the prevalence of the disease (e.g. Cyprus).

However, although these measures result in the reduction of prevalence, they are unlikely to result in the eradication of disease (i.e. the elimination of all infected cases from the sheep/goats populations of a country/region). The impact of these measures depends on their sustained and long term implementation, with the subsequent cost in human and physical resources. In a low prevalence situation, the number of tests required to detect a new outbreak increases substantially.

A risk-based approach, targeting subpopulations with a higher risk of disease, would improve the detection of new cases.

It is recommended that:

- The detection of infected flocks and subsequent disease control will be improved by additional risk-based targeted surveillance activities and/or a substantial increase in the tested fraction of the population.
- If additional targeted risk-based surveillance is implemented, data collection enables the discrimination between results generated through the continuation of the current surveillance and any additional targeted risk-based surveillance.
- Even after restrictions are lifted, infected flocks are considered at risk and targeted for enhanced surveillance, unless they are composed entirely of resistant animals.

##### 4.7.1.2. Surveillance to monitor the epidemiological evolution in time and space

The epidemiological evolution of scrapie in the EU is mainly based on data gathered from active surveillance. The current EU scrapie active surveillance program, is based on two sample-based surveys, with quotas assigned to MSs according to the size of each small ruminant population. Assessment of the compliance of MSs with the current EU regulations is beyond the scope of this mandate, and the information collated for this purpose had as its only target the assessment of the quality and completeness of the data used for the trend analysis. To optimise the efficiency of disease surveillance, it should be designed individually for each MS according to the estimated prevalence of disease, a predefined precision level, and specific national characteristics of the industry (e.g. population size, demography, number of breeds, breeding and production systems).

It is recommended that:

- TSE surveillance programmes in small ruminants:
  - are adapted to each MS (number of tests, targeted populations);
  - have a standardised audit/verification system to ensure the correct implementation of control measures;
  - include a data collection system facilitating the estimation of prevalence at both population and flock/herd level;

- systematically and accurately collect raw data regarding major confounding factors (i.e. age in years, stream).

#### 4.7.1.3. Preventing the reoccurrence of Classical scrapie in infected flocks

According to Regulation (EC) No 1915/2003, after the destruction of susceptible animals in infected flocks, only the following animals may be introduced: a) male sheep of the ARR/ARR genotype; and (b) female sheep carrying at least one ARR allele and no VRQ allele. The increase of the ARR allele frequency and its maintenance is a powerful measure to prevent the reoccurrence of disease despite the persistence of the agent in the environment.

It is recommended that:

- Long term/permanent compulsory use of ARR homozygous rams is required in the holdings where CS has been detected.

#### 4.7.1.4. Identification of animals and epidemiological investigation of infected flocks

Individual identification of sheep and goats is a crucial component of a reliable livestock traceability system, necessary for the management of outbreaks. The epidemiological investigations of cases, including the movement of animals from infected flocks, cannot be achieved if this is missing.

It is recommended that:

- Individual identification of small ruminants is implemented effectively, as part of a functional traceability system.

### 4.7.2. Breeding for resistance policies

A BP aims to permanently modify the genetic structure of the small ruminant population. This is a long term goal: it requires time to produce effects and remains the only currently known measure in sheep that is able to lead to permanent disease eradication.

The lack of official breeding for resistance policies in goats remains a major difficulty for disease control in this species.

A number of MSs have made substantial efforts to change the genetic structure of their sheep population through the implementation of breeding for resistance programmes as defined in Commission Decision 2003/100/EC. However, the diverse nature and variable quality of such programmes, in terms coverage, dissemination of resistant alleles, rules applied to members, and duration, have been highlighted in this Opinion.

BP-CSs have been effective in modifying the allele frequencies under two scenarios: compulsory use of ARR rams in the whole population (The Netherlands and Cyprus) and modification of the genotype structure in HGMF by eliminating alleles associated with higher susceptibility to the disease / selecting the ARR allele (e.g. France, United Kingdom, Slovenia, Slovakia).

The current rules for breeding for resistance, as established in the EU Regulation, are loose and do not set targets. This has contributed to a lack of effectiveness of the BP-CS observed in certain countries.

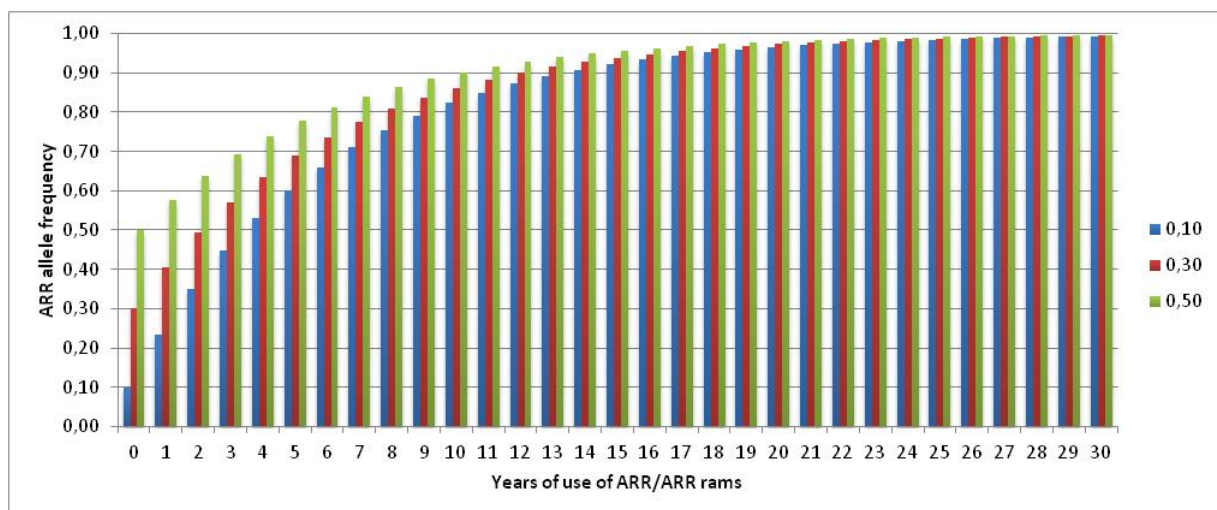
#### 4.7.2.1. Dissemination of the ARR allele in the general population

The effectiveness of BP-CSs is directly dependent on the dissemination of resistant alleles in the general population. This Opinion has highlighted the bottleneck that exists between the HGMF and the general population. The Cypriot, Sardinian and Dutch cases demonstrate that it is possible to overcome this gap by making the use of ARR rams in all flocks of a country or a region compulsory.

Such a measure could be temporarily envisaged, at least in certain populations (e.g. countries/regions with high prevalence, or specific breeds with a sufficient initial ARR frequency). The compulsory use of ARR/ARR rams over three or four generations would generate a major shift in the genetic structure of the entire sheep population. A graph representing the evolution of the ARR allele when just ARR/ARR male reproducers are used according to different starting frequencies is presented below (Figure 15). This shows that after 6 years, flocks with a starting frequency of 0.10, using only ARR/ARR rams, would reach at least an ARR frequency of 0.66 and a frequency of homozygous susceptible genotypes less than 0.10 (0.095).

Figure 15 provides an example of evolution of the ARR allele frequency over time with the use of ARR/ARR rams in flocks with different starting frequencies of the ARR allele. The evolution of the ARR frequency per year of use of ARR-ARR rams has been obtained by calculating:

- the expected frequency of the ARR allele in the female replacement at the year  $n$  by assuming a frequency of 100 % in the male parents and the frequency of the flock in the year  $n-1$  for the female parents;
- the expected frequency in the flock at the year  $n$  was obtained by calculating the weighed mean of the ARR allele frequency in the replacement (30 %) at the year  $n$  and the frequency in the flock at the year  $n-1$ .



**Figure 15:** Evolution of the ARR allele frequency over time with the use of ARR/ARR rams in flocks with different starting frequencies of the ARR allele (yearly replacement rate of females 30 %).

However it would be difficult to make this measure compulsory in large populations or in countries without BP-CSs or where there are insufficient, or no, rams available of resistant genotypes. Alternatively, rules for the dissemination of the resistant alleles from HGMF to CF must be specified in order to maximize the benefits of the BP-CSs.

The presence of incentives to farmers to breed for resistance would increase their compliance with the requirement to select for, and disseminate, resistant alleles. All initiatives intended to increase the ARR frequency should be implemented in parallel with measures preventing the introduction of susceptible alleles via the importation of live animals, embryos, ova and semen. Legislation recently introduced in the EU (Regulation (EU) No 630/2013) should ensure that this is the case. Measures to create incentives for the dissemination of resistant alleles may include the qualification of flocks composed entirely of ARR homozygous animals or the modulation of farming subsidies at national and EU level for the benefit of flocks carrying out BP-CSs.

However, the classification of flocks based on the genotype frequency in the whole flock is not always feasible in terms of efficiency and resources. Most BP-CSs are based on genotyping just male reproducers, which represent a small portion of the total population (average sex ratio of 1/40), and allows the detection of 50 % of the *PRNP* gene alleles of the next generation. To determine the remaining 50 % of the alleles, genotyping must be performed on about 50 times more animals. An alternative classification system could be based on the number of consecutive years of use of ARR/ARR reproducers. For example, a possible classification system might be as follows:

- level 1: flocks exclusively using ARR/ARR breeding rams for at least 10 years;
- level 2: flocks exclusively using ARR/ARR breeding rams for at least 6 years;
- level 3: flocks exclusively using ARR/ARR breeding rams;
- level 4: flocks exclusively using ARR carriers as breeding rams;
- level 5: flocks which participate in BP-CSs by genotyping breeding rams.

It is recommended that:

- BP-CSs, with an explicit temporal target and a fit for purpose information system, are compulsory in countries where CS is detected.
- The compulsory comprehensive use of ARR/ARR rams over three or four generations is considered, since it would generate a major shift in the genetic structure of the entire sheep population. Alternatively, BP-CSs should be:
  - reinforced in HGMF along with rules and incentives ensuring the dissemination of the resistant alleles from HGMF to CF;
  - extended to CF.
- Special status is granted to flocks with all ARR-homozygous animals and no history of CS, with incentives such as subsidies and export certification exceptions.
- An alternative classification system, based on the number of consecutive years of use of ARR/ARR breeding rams, is considered.

#### 4.7.2.2. Monitoring the *PRNP* allele frequency in the EU population

Beyond data that may be generated in the context of a BP-CS, there are two further sources of information on sheep prion protein genotypes regulated by the TSE Regulation: i) information on genotypes for each positive TSE case in sheep, and ii) information on genotypes to be gathered for a minimum sample of ovine animals, which should be representative of the entire ovine population.

The background for this 'minimum sample', de facto 100-600 samples per country, was previously outlined by EFSA (2006) in an Opinion. The appropriateness of the determination of these genotypes, in order to compare the prevalence of genotypes in TSE infected sheep with the genotypes in the general population, was also reviewed, and so was the ability, based on these figures, to monitor the evolution of genotypes over time. In this former Opinion it was recommended that, in order to serve the first purpose, the genotyping results from flocks where selective culling and TSE testing of a sample were applied can be used. Furthermore it was recognized that this 'random genotyping' cannot serve the second purpose to follow the evolution of genotypes in time, as the data generated might be biased due to the limited sample size in combination with the large variation of the *PRNP* genotype distribution between breeds. Subsequently EFSA (2006) recommended 'to increase the sample size to evaluate the genetic progress in sheep breeds, having regard to: - the minimum difference of interest

for progress in genotype prevalence, - the variance of genotype prevalence in sheep breeds, - acceptable levels of significance and power, - the current genotype distribution’.

It is acknowledged that the metadata currently available from the random genotyping (country, year, amount of samples and genotype) cannot be used in a meaningful way. This is because the information available is too limited for an *a posteriori* analysis, adjustment and monitoring of the sampling design, as it lacks of both information on: (i) the strategy followed by each MS to design the sampling frame in ‘representative sample’ of the entire ovine population as requested in the legislation, and (ii) the further metadata needed for adequate analysis of the results reported (e.g. breed, gender, age, flock identification, region).

It is recommended that:

- An appropriate system to monitor *PRNP* allele frequency evolution in the general sheep populations in MSs is designed and implemented, according to breed and birth cohort.

#### 4.7.2.3. Breeding for resistance in goats

There is a need to develop strategies for breeding for resistance in goats. Robust scientific evidence regarding the resistance of certain alleles in the goat breeds of the EU is already available and has been described earlier in this Opinion (Section 1.2.2). Some of the polymorphisms conferring resistance have worldwide distribution. This knowledge allows the definition of a set of aims for the goat species such as: i) building up data on the existence and distribution of bucks carrying resistant alleles, and ii) contributing to the eradication of the disease through measures based on selective culling of susceptible animals in outbreaks.

It is recommended that:

- Genotyping surveys of breeding bucks are performed.
- Selection activities and dissemination of resistant bucks are promoted.
- Formal breeding for resistance programmes, similar to those already implemented for sheep, are initiated for goats.
- The stamping out strategy is replaced with outbreak management based on the selective culling of susceptible animals, as is currently applied to sheep.
- Restocking following the application of control measures is done only with resistant animals, when they are available in sufficient number.

#### 4.7.3. Knowledge transfer on scrapie

Scrapie has lost the profile and visibility within the farming community that it had ten years ago when active surveillance started. It is important to remind all stakeholders that classical and AS are still present in many MSs and that a range of statutory actions are still in place. In parallel there have been advances in the knowledge of pathogenesis, transmission, genetics and epidemiology of TSEs and these may not have been effectively disseminated to stakeholders.

Moreover the experience gained by some MSs with regard to the effective implementation of control measures and BP-CSs has not been taken advantage of by other countries.

Any relaxation of the statutory measures in infected flocks/herds or the implementation of new control measures should be weighed against the expected effect (based on scientific evidence) on the risk of spread of the disease in the population.

It is recommended that:



- Training activities aimed at disseminating current knowledge to all stakeholders (farmers, veterinarians, policy makers) are promoted and implemented, at both EU and MS level.
- It is acknowledged that no single control policy is appropriate for all MSs due to the heterogeneity of the sheep and goat industries across the EU.

## CONCLUSIONS

### General conclusions

- TSEs in small ruminants have a long asymptomatic phase, during which animals can circulate in the population.
- With regards to Classical scrapie (CS):
  - Susceptibility is strongly influenced by different polymorphisms of the *PRNP* gene.
  - Horizontal transmission is considered to be the main route of transmission, through exposure to oral infection (e.g. mainly via placenta or milk from infected animals) or to contaminated environments. It is still unclear precisely which sources of infectivity and routes of transmission are possible, and which have the greatest effect on the spread and maintenance of infection in a population.
  - Animal movements, flock size and age are important risk factors, but are difficult to address by preventive measures.
  - The most powerful tools presently available for disease control in any flock which is not closed, and disease-free, are the effective identification and removal of infected animals and the control of the genetic composition of the flock, i.e. the methods covered (for sheep) by the current EU legislation.
- With regard to Atypical scrapie (AS):
  - The genotypes most resistant to CS occur frequently in the AS-infected population.
  - The late age at onset and the relative protease sensitivity of the atypical PrP limits the sensitivity of AS case detection through current surveillance programmes.
  - AS pathogenesis is poorly documented.
  - AS does not present, epidemiologically, like an infectious disease.
  - AS has been identified as occurring at a low but very consistent prevalence in every small ruminant population in which there has been screening.
  - AS has been found in populations in which CS has not been reported.
- Passive surveillance is very ineffective at identifying new affected flocks or herds, especially if the prevalence is low and if suspicion reporting may lead to socio-economical consequences such as animal movement restrictions or compulsory culling.
- Active surveillance, enforced since 2002 as a targeted sample-based monitoring of adult small ruminants, represents a major improvement of TSE surveillance in the EU.

- The EU TSE surveillance in small ruminants allows the monitoring of the evolution of the disease and the assessment of the effectiveness of control measures implemented by the MSs. It may not take into due account the heterogeneity of the sheep population and scrapie distribution in each MS: deviation from the representativeness of the sampled population may result in reduced validity of the prevalence estimate.
- The observed prevalence obtained through the EU TSE surveillance programme in small ruminants is affected by two main factors: i) the sensitivity of the detection method given the age of the tested animal, the stage of the incubation period and the sampling/testing procedures, and ii) the distribution of testing by surveillance stream. The availability of the relevant data allows the statistical control of their confounding effects.
- Surveillance programmes could be designed to ensure detection of as many scrapie cases and outbreaks as possible as a tool to facilitate the control or the eradication of the disease in a country.

### **Answer to Term of Reference 1:**

***“On the basis of the results of the TSE monitoring programme laid down in the TSE Regulation, what is the trend since 2002 of the situation of Classical scrapie and Atypical scrapie in sheep and in goats respectively, in the EU as a whole and in the 27 Member States individually?”***

- In order to analyse prevalence trends:
  - The statistical analysis was restricted to the MSs reporting scrapie cases in the framework of EU active surveillance.
  - Cases identified through passive surveillance, or as a result of culling animals from known infected flocks, were removed from the data sets prior to analysis.
  - In the case of CS, and in order to prevent the validity of the estimates of the prevalence rates being compromised by the confounding effect of surveillance stream, stream-adjusted estimates were systematically obtained through the application of ad hoc statistical techniques.
- Over the period 2002-2012, the overall average prevalence of CS in the sheep population was 8.7 cases per 10 000 rapid tests (considering the 4.7 million sheep tested in the 17 MSs where CS has been reported). The geographical distribution of CS cases in sheep shows great heterogeneity in the level of occurrence in MSs: in some cases only a few, or no, cases were detected, whereas Cyprus experienced a large epidemic.
- Country-specific temporal trends of CS in sheep are heterogeneous, preventing any meaningful interpretation of the overall temporal trend at the EU27-level. Therefore the analysis and interpretation of the temporal trends must be conducted at MS level. With regard to CS in sheep, the results of the analysis allow the classification of the EU27 MSs into four groups:
  - countries where CS has been detected, with a statistically significant decreasing trend (Cyprus, France, Ireland, The Netherlands, Slovenia and the United Kingdom);
  - countries where CS has been detected with an observed trend not statistically different from a flat one (Belgium, Czech Republic, Greece, Italy, Romania, Slovakia and Spain);
  - countries where CS has been reported only sporadically (Bulgaria, Germany, Hungary and Portugal);

- countries with no cases of CS during the period 2002-2012 (Austria, Denmark, Estonia, Finland, Latvia, Lithuania, Luxemburg, Malta, Poland and Sweden).
- Over the period 2002-2012, active surveillance enabled the detection of AS in sheep in 21 MSs. Since the ability to detect cases of AS is essentially restricted to few rapid tests, all the calculations were done with the 2.5 million (known) tests carried out with these rapid tests in the 21 MSs. This restricted the temporal analysis to the period 2006-2012. The overall prevalence of AS for this period was 5.8 cases per 10 000 rapid tests.
- Where detected, AS in sheep showed a similar prevalence over time and space: no large epidemics were reported and five countries detected AS in sheep only sporadically. Only two countries showed a statistically significant trend, with a reduction in the annual prevalence rates in France and an increase in the United Kingdom.
- CS in goats has been detected in eight MSs, where 1.4 million goats have been tested. The overall prevalence of CS in goats (9.8 cases per 10 000 rapid tests) is mostly explained by the unique epidemic in Cyprus which paralleled the epidemic in sheep. The prevalence in the remaining seven countries where CS in goats was detected was 2.2 cases per 10 000 rapid tests. Statistically decreasing trends were evident respectively for France over the entire period (2002-2012) and for Cyprus and the United Kingdom after 2007. The favourable French trend may reflect in part the impact of a strong surveillance pressure able to detect and manage a large proportion of outbreaks in small ruminants.
- AS in goats was reported by five countries, at a very low prevalence and with no statistically significant trend in any of them.

***“Where no favourable trend can be observed, what are the identifiable causes for failure to improve the situation of Classical scrapie?”***

- Although it is not possible to identify causes that can explain objectively the failure to improve the situation of CS, the assessment of the country-specific available data related to the implementation of surveillance, non-genetic control measures and BP-CSs may allow the formulation of some hypotheses.
- With regard to sheep, it is quite plausible that the causes of any unfavourable trend might be linked to ineffective implementation of either or both strategies. Based on data collected from MSs, a number of potential reasons to explain the lack of observation of a favourable trend in part of the countries can be envisaged in relation to:
  - The BP-CS: a short duration of implementation of a BP-CS, a low ARR/ARR frequency in the target populations when the BP-CS started, a lack of national coverage of the BP-CS, a low number of genotyped rams in HGMF, a limited rate of selection of rams within HGMF, and inefficiency of, or lack of, rules for the dissemination of resistant rams from HGMF to CF.
  - Surveillance and non-genetic control measures: variability and/or reduction of the surveillance pressure, a lack of representativeness of the animals selected for testing, a limitation in available data preventing the detection of trends due to the lack of regular detection of cases, a short duration and/or ineffective implementation of sanitary measures due to recent admission to the EU, or implementation of measures below the EU minimum requirement.
- With regard to goats, differences in the husbandry systems in sheep and goats within countries, absence of BP-CSs, and variability of the control measures (which cannot include selective culling) applied on infected herds compared to sheep flocks may explain the lack of observation of a favourable trend. The favourable evolution of the situation in France (in both sheep and goats) suggests the likely effectiveness of a high surveillance pressure in detecting and managing a large

proportion of outbreaks, which may subsequently lead to a decline of the disease in the general population.

**Answer to Term of Reference 2:**

*“Has the evolution of the Classical scrapie situation been statistically different in the MS which have implemented a breeding programme from 2004 to 2011 compared to the MS without a breeding programme in the same period?”*

- Since there were no MSs without a BP-CS with sufficient cases of CS in order to estimate a trend, the comparison requested in ToR 2 is not possible. Therefore, based on information collected from MSs, the potential effectiveness of BP-CS in the dissemination of resistance into the general sheep population was assessed for those countries for which the CS trend analysis was performed. Subsequently, the evolution of the CS situation between MSs, depending on the potential effectiveness of their BP-CS, was compared.
- BP-CSs, which satisfy the minimum requirements of EU legislation, have been implemented by 17 MSs. Other MSs did not implement BP-CSs, generally because of a favourable situation regarding the disease.
- There is a clear heterogeneity in the characteristics of the different BP-CSs implemented by the MSs. There is also heterogeneity within MSs according to different areas, ARR allele frequency in the general population prior to BP-CS implementation, breeds and production types.
- Generally BP-CSs in the EU focus on HGMF and are carried out through three steps (genotyping, selection and dissemination), with the aim of increasing the frequency of the resistant alleles in the general sheep population.
- Such a strategy:
  - requires the dissemination of males from HGMF to CF as a major determinant of the effectiveness of the final BP-CS;
  - results in a lag of several years between the initiation of a BP-CS and an effective change in the genotype structure of the general population.
- Applying a BP-CS does not imply that a genetic control of the disease is achieved. This depends on the characteristics of the BP-CS and on its effectiveness, which needs to be assessed based on the evolution of the genetic structure of the population.
- The crucial parameter to assess the effectiveness of BP-CSs in MSs is the frequency of the resistant allele in the whole sheep population.
- In the absence of this parameter, which is generally not available, the potential effectiveness of BP-CSs has been assessed in this Opinion through a combination of qualitative expert assessment and modelling of the diffusion of CS resistant alleles from HGMF to CF through selected resistant reproducers.
- The result of the dissemination of CS resistant alleles to CF (in terms of the potential evolution of ARR/ARR frequency in CF) is not, by itself, a sufficient indicator to evaluate the effectiveness of a BP-CS in reducing CS prevalence.
- The impact of a national BP-CS on the epidemiological situation of CS may be influenced by: i) both the ARR allele frequency and the CS prevalence prior to the implementation of the BP-CS, and ii) their heterogeneous geographical distribution.

- Given the characteristics of each national BP-SC, a deterministic model was used to estimate the ARR/ARR frequency in the general sheep population over time. Subsequently, the outputs of the model were compared with the national CS situations. The results obtained suggest a favourable CS situation being linked to better-achieving BP-CSs.
- Cyprus and the Netherlands, countries in which the improvement in the epidemiological situation of CS is clear, applied their BP-CSs to the whole population, without any distinction between population tiers. This approach produced an effective change of the genetic structure of the whole sheep population, but required extensive genotyping efforts.

### **Answer to Term of Reference 3:**

*“On the basis of the above analysis, can a minimum level of frequency of the ARR allele in the sheep population in a MS be defined or estimated above which Classical scrapie can be expected to fade-out, in a context where no control and eradication measure is being applied?”*

- Given the very strong resistance of the homozygote ARR genotype to CS, there is a conceptual parallel between the ARR homozygote proportion of a sheep population and the proportion immunized in a vaccinated population.
- A minimum frequency of the ARR allele in a sheep population can be estimated, above which CS may be expected to fade-out. The example of The Netherlands, where CS case prevalence in surveillance has become very low in recent years and the ARR frequency is (still) well below 100 %, provides evidence for the existence of the minimum ARR frequency for CS fade-out in the reality of the field.
- Once the ARR frequency in a MS has increased to above the minimum estimated requirement for fading-out of disease, this does not imply that CS cases will no longer occur. CS cases may still occur, either through introduction of infected animals from outside the population or from infectivity in the environment, but such cases will no longer pose a risk of causing prolonged epidemic spread of CS in the population.
- The concept of fade-out is different from eradication. Whereas eradication involves active interventions to remove remaining foci of transmission, fade-out is expected to occur naturally at some point in time once a sufficiently high ARR allele frequency is obtained and maintained.
- The minimum frequency of the ARR allele in the sheep population in a MS above which CS can be expected to fade-out, in a context where no control or eradication measure is being applied, is not universal and would have to be estimated for each particular national sheep population separately.
- Case studies illustrate the non-universality of the minimum ARR frequency; across the case studies it ranges between 53 % and close to 100 % according to a crude model. The case studies also provide some insight into how the minimum frequency depends on MS-specific parameters.
- Maintenance of an ARR allele frequency above the required minimum level needs to be monitored by taking representative genotyping samples at regular time intervals.

### **Answer to Term of Reference 4:**

*“In a context where no breeding programme is implemented, are the present mandatory measures in terms of active monitoring, eradication and control of Classical scrapie effective to achieve a decline of this disease and its eradication on the long term?”*

- As shown by the Iceland case-study, detection and eradication measures in affected flocks are effective in reducing the observed prevalence of CS in a population with a high prevalence of disease.
- The overall effectiveness of such a policy relies heavily on the detection rate of outbreaks in the population.
- However, because of the persistence of the agent in environment, repopulating scrapie infected farms with non-resistant genotype animals can lead to reoccurrence of the disease.
- Due to the pathogenesis and the epidemiological characteristics of CS, and to the high persistence of the CS agent in the environment, a CS eradication policy that relied solely on detection of infected flocks by *post-mortem* testing and subsequent depopulation would be unlikely to succeed.

### **Answer to Term of Reference 5:**

***“What additional measures can EFSA recommend in view of achieving the eradication of Classical scrapie in the MS?”***

- With regard to surveillance and control, it is recommended that:
  - The detection of infected flocks and subsequent disease control will be improved by additional risk-based targeted surveillance activities and/or a substantial increase in the tested fraction of the population.
  - If additional targeted risk-based surveillance is implemented, data collection enables the discrimination between results generated through the continuation of the current surveillance and any additional targeted risk-based surveillance.
  - Even after restrictions are lifted, infected flocks are considered at risk and targeted for enhanced surveillance, unless they are composed entirely of resistant animals.
  - TSE surveillance programmes in small ruminants:
    - are adapted to each MS (number of tests, targeted populations);
    - have a standardised audit/verification system to ensure the correct implementation of control measures;
    - include a data collection system facilitating the estimation of prevalence at both population and flock/herd level;
    - systematically and accurately collect raw data regarding major confounding factors (i.e. age in years, stream).
  - Long term/permanent compulsory use of ARR homozygous rams is required in the holdings where CS has been detected.
  - Individual identification of small ruminants is implemented effectively, as part of a functional traceability system.
- With regard to breeding for resistance policies in sheep, it is recommended that:
  - BP-CSs, with an explicit temporal target and a fit for purpose information system, are compulsory in countries where CS is detected.



- The compulsory comprehensive use of ARR/ARR rams over three or four generations is considered, since it would generate a major shift in the genetic structure of the entire sheep population. Alternatively, BP-CSs should be:
  - reinforced in HGMF along with rules and incentives ensuring the dissemination of the resistant alleles from HGMF to CF;
  - extended to CF.
- Special status is granted to flocks with all ARR-homozygous animals and no history of CS, with incentives such as subsidies and export certification exceptions.
- An alternative classification system, based on the number of consecutive years of use of ARR/ARR breeding rams, is considered.
- An appropriate system to monitor PRNP allele frequency evolution in the general sheep populations in MSs is designed and implemented, according to breed and birth cohort.
- With regard to breeding for resistance policies in goats, it is recommended that:
  - Genotyping surveys of breeding bucks are performed.
  - Selection activities and dissemination of resistant bucks are promoted.
  - Formal breeding for resistance programmes, similar to those already implemented for sheep, are initiated for goats.
  - The stamping out strategy is replaced with outbreak management based on the selective culling of susceptible animals, as is currently applied to sheep.
  - Restocking following the application of control measures is done only with resistant animals, when they are available in sufficient number.
- With regard to knowledge transfer on scrapie, it is recommended that:
  - Training activities aimed at disseminating current knowledge to all stakeholders (farmers, veterinarians, policy makers) are promoted and implemented, at both EU and MS level.
  - It is acknowledged that no single control policy is appropriate for all MSs due to the heterogeneity of the sheep and goat industries across the EU.

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## **APPENDICES**

### **Appendix A. Data requests to EU Member States in the framework of this Opinion**

#### **A.1. Questionnaire ‘Sampling strategy and control measures’**

##### **Questionnaire circulated to EU Member States**

A copy of the questionnaire ‘Sampling strategy and control measures’ circulated to MSs is included in the following pages.



**QUESTIONNAIRE SURVEY FOR THE ASSESSMENT OF TSE SURVEILLANCE AND CONTROL MEASURES IN EU MEMBER STATES**  
**EFSA WG “Scrapie situation in the EU after 10 years of monitoring and control in sheep and goats”**  
**August 2013**

**Section A**  
**Sampling strategy and testing protocol for SURVEILLANCE**

**A.1 - Healthy SMALL RUMINANTS slaughtered for human consumption – Abattoir survey (SHC)**

*Since the beginning of the implementation of the surveillance of healthy SMALL RUMINANTS slaughtered for human consumption (SHC) in your country (please reply also in case SHC have been sampled only for limited periods of time):*

1. Is the selection criterion of SHC for TSE testing based on:
- convenience sample (a strategy based on collecting easily accessible samples or any other non-random criteria)?
  - structured sampling strategy?
  - other (please specify in the box below)?

*click here to insert text*

2. Is there a description of the sampling strategy available?
- YES
  - NO
  - Unknown

**If YES, please provide documentation (enclosed) or describe in detail in the box below**

*click here to insert text*

3. Is a formal random selection of SMALL RUMINANTS for sampling and TSE testing carried out?
- YES
  - NO
  - Unknown

**If YES, please explain how randomisation of samples is ensured in the box below**

*click here to insert text*

4. Are SHC selected for TSE testing representative of the adult (>18 months of age) SMALL RUMINANT slaughtered population of your country, i.e. their characteristics represent as accurately as possible the adult SMALL RUMINANT slaughtered population of your country?
- YES
  - NO
  - Unknown
5. Are adult sheep (>18 months of age) slaughtered for human consumption representative of the general adult SMALL RUMINANT population of your country, i.e. their characteristics represent as accurately as possible the general adult SMALL RUMINANT population of your country?
- YES
  - NO
  - Unknown
6. **If NO to Q4 and/or Q5, what are the potential reasons?**
- Over-representation of some particular flocks/herds (please explain in the box below)
  - Over-representation of some age bands (please explain in the box below)
  - Over-representation of some breeds (please explain in the box below)
  - Over-representation of some geographical locations (please explain in the box below)
  - Non-random testing (please explain in the box below)
  - Differences in sheep health status (please explain in the box below)
  - Other (please specify in the box below)

*click here to insert text*

7. Has the selection criterion of SMALL RUMINANTS for TSE testing in the SHC changed over the years?
- YES
  - NO
  - Unknown

**If YES, please provide details of the changes in chronological order in the box below**

*click here to insert text*

8. Has the testing protocol (screening test) changed over the years?
- YES
  - NO
  - Unknown

**If YES, please provide details of the changes in chronological order in the box below**

*click here to insert text*

9. If there have been substantial differences in the implementation of the surveillance of SHC between SHEEP and GOATS in your country, please describe them in chronological order in the box below

[click here to insert text](#)

10. Are there any risk-based strategies to enhance surveillance sensitivity in SHC?

- Minimum sample size per flock  
 Targeting animals of specific age bands  
 Other (please specify in the box below)

[click here to insert text](#)

11. Are there specific monitoring measures in place for emergency slaughtered animals?

- YES  
 NO  
 Unknown

If YES, please provide details of the measures in place in the box below

[click here to insert text](#)

#### A.2 - SMALL RUMINANTS not slaughtered for human consumption – Fallen stock (NSHC)

*Since the beginning of the implementation of the surveillance of SMALL RUMINANTS not slaughtered for human consumption (NSHC) in your country (please reply also in case NSHC have been sampled only for limited periods of time):*

12. Is the selection criterion of NSHC for TSE testing based on:

- convenience sample (a strategy based on collecting easily accessible samples or any other non-random criteria)?  
 structured sampling strategy?  
 other (please specify in the box below)?

[click here to insert text](#)

13. Is there a description of the sampling strategy available?

- YES  
 NO  
 Unknown

If YES, please provide documentation (enclosed) or describe in detail in the box below

[click here to insert text](#)

14. Is a formal random selection of SMALL RUMINANTS for sampling and TSE testing carried out?

- YES  
 NO  
 Unknown

If YES, please explain how randomisation of samples is ensured in the box below

[click here to insert text](#)

15. Are NSHC selected for TSE testing representative of the adult (> 18 months of age) SMALL RUMINANT fallen stock population of your country, i.e. their characteristics represent as accurately as possible the adult SMALL RUMINANT fallen stock population of your country?

- YES  
 NO  
 Unknown

If NO, what are the potential reasons?

- Important subpopulations of fallen stock not collected for rendering (please explain in the box below)  
 Owners avoiding TSE testing (please explain in the box below)  
 Over-representation of some particular flocks/herds (please explain in the box below)  
 Over-representation of some breeds (please explain in the box below)  
 Over-representation of some geographical locations (please explain in the box below)  
 Remote location of sampling sites (please explain in the box below)  
 Non-random testing (please explain in the box below)  
 Other (please specify in the box below)

[click here to insert text](#)

16. Has the selection criterion of SMALL RUMINANTS for TSE testing in the NSHC changed over the years?

- YES  
 NO  
 Unknown

If YES, please provide details of the changes in chronological order in the box below

[click here to insert text](#)

17. Has the testing protocol (screening test) for NSHC changed over the years?

- YES  
 NO  
 Unknown

If YES, please provide details of the differences in chronological order in the box below

[click here to insert text](#)

18. If there have been substantial differences in the implementation of the surveillance of NSHC between SHEEP and GOATS in your country, please describe them in chronological order in the box below

[click here to insert text](#)

19. Are there any risk-based strategies to enhance surveillance sensitivity in NSHC?

- Minimum sample size per flock  
 Targeting animals of specific age bands  
 Other (please specify in the box below)

[click here to insert text](#)

#### Section B

#### Applied measures for the control/eradication of CLASSICAL SCRAPIE in SMALL RUMINANTS

20. When were statutory actions after confirmation of a case of scrapie first introduced in the legislation of your country (year)?

[click here to insert text](#)

21. Are any control/eradication measures for Classical scrapie implemented in flocks/herds where Classical scrapie is confirmed in your country (currently or in the past)?

- YES  
 NO  
 Unknown

If NO/Unknown to Q21, please go to Section C (Q36)

22. When did control/eradication measures for Classical scrapie complying with EU legislation start to be applied in your country (year)?

[click here to insert text](#)

23. Are all confirmed cases of Classical scrapie since the implementation of active surveillance based on rapid testing traced back resulting in the confirmation of infected flocks/herds and the implementation of control/eradication measures?

- YES  
 NO  
 Unknown

If NO, what is the proportion (%) of confirmed cases of Classical scrapie since the implementation of active surveillance based on rapid testing that resulted in the implementation of control/eradication measures in infected flocks/herds?

[click here to insert text %](#)

24. What is the total number of flocks/herds in which control/eradication measures have been applied following to the confirmation of Classical scrapie through the implementation of active surveillance based on rapid testing?

Number of sheep flocks [click here to insert text](#)

Number of goat herds [click here to insert text](#)

25. Which measures are or have been applied to SHEEP in infected flocks?

- Killing of all animals, regardless of the genotype  
 Killing of animals with a genotype susceptible to Classical scrapie only  
 Both the two previous options, with the following proportion (for example, 50%:50%):

Killing all [click here to insert text %](#)

Killing susceptible only [click here to insert text %](#)

- Other (please specify in the box below)

[click here to insert text](#)

26. Which measures are or have been applied to GOATS in infected herds?

- Killing and complete destruction  
 Other (please specify in the box below)

[click here to insert text](#)

27. In infected flocks/herds, after control/eradication measures are implemented, are restrictions enforced according to the EU TSE regulation or to national legislation?

- EU TSE regulation  
 National legislation  
 Unknown

If "National legislation", please explain how it differs from the EU TSE regulation in the box below

[click here to insert text](#)

28. In the infected flocks/herds, during the restriction period, is the TSE testing (enhanced surveillance) conducted according to the EU TSE regulation or to national legislation?

- EU TSE regulation  
 National legislation  
 Unknown

If "National legislation", please explain how it differs from the EU TSE regulation in the box below

[click here to insert text](#)

29. Have any of the derogations to control measures foreseen by EU legislation been enforced in the implementation of the control/eradication measures for scrapie in your country?

- Derogation to the killing of small ruminants in infected holdings and of parents, embryos, ova and last progeny of diseased female animals  
 Replacement of the killing of some animals with the slaughtering for human consumption  
 Delay of the killing of some animals  
 Derogation to the restriction of movements of animals from infected holdings during the enhanced surveillance period after outbreaks  
 Other (please specify in the box below)

[click here to insert text](#)

30. If any derogation has been enforced, please describe in which circumstances derogations have been applied in the box below

[click here to insert text](#)

31. When identifying an outbreak of Classical scrapie, do you systematically carry out standardised epidemiological investigations?

- YES  
 NO  
 Unknown

32. If carried out, are those epidemiological investigations aimed at investigating the possible origin of the disease and at confirming officially the disease in a particular flock/herd?

- YES  
 NO  
 Unknown

33. If carried out, are those epidemiological investigations aimed at investigating the possible spread of the disease to other flocks/herds?

- YES  
 NO  
 Unknown

34. Are any of the following measures applied in the event of an outbreak of Classical scrapie?

- Disinfection procedures?  
 Grazing restrictions to avoid mixing with other flocks?  
 Compulsory enrolment in breeding programmes or in qualification schemes?  
 Other (please specify in the box below)

[click here to insert text](#)

35. Have the control/eradication measures applied to infected flocks/herds changed over the years?

- YES  
 NO  
 Unknown

If YES, please provide details of the changes in chronological order in the box below

[click here to insert text](#)

### Section C

#### Applied measures for the control/eradication of ATYPICAL SCRAPIE in SMALL RUMINANTS

36. Are all confirmed cases of Atypical scrapie traced back and resulting in the confirmation of infected flocks/herds?

- YES  
 NO  
 Unknown

37. Are any control/eradication measures for Atypical scrapie implemented in flocks/herds where Atypical scrapie is confirmed in your country (currently or in the past)?

- YES  
 NO  
 Unknown

#### If NO/Unknown to Q37, END OF THE QUESTIONNAIRE

38. When did control/eradication measures for Atypical scrapie complying with EU legislation start to be applied in your country (year)?

[click here to insert text](#)

39. Are all confirmed cases of Atypical scrapie since the implementation of active surveillance based on rapid testing traced back resulting in the confirmation of infected flocks/herds and the implementation of control/eradication measures?

- YES
- NO
- Unknown

If NO, what is the proportion (%) of confirmed cases of Atypical scrapie since the implementation of active surveillance based on rapid testing that resulted in the implementation of control/eradication measures in infected flocks/herds?

[click here to insert text %](#)

40. Which measures are or have been applied to flocks/herds infected with Atypical scrapie?

- Killing, with the following proportion of infected flocks/herds where animals have been killed: [click here to insert text %](#)
- Monitoring via enhanced surveillance (TSE testing)
- Trade restrictions
- Other (please specify in the box below)

[click here to insert text](#)

41. In infected flocks/herds, after control/eradication measures are implemented, are restrictions enforced according to the EU TSE regulation, or to national legislation?

- EU TSE regulation
- National legislation
- Unknown

If "National legislation", please explain how it differs from the EU TSE regulation in the box below

[click here to insert text](#)

42. In the infected flocks/herds, during the restriction period, is the TSE testing (enhanced surveillance) conducted according to the EU TSE regulation, or to national legislation?

- EU TSE regulation
- National legislation
- Unknown

If "National legislation", please explain how it differs from the EU TSE regulation in the box below

[click here to insert text](#)

43. Have the control/eradication measures applied to infected flocks/herds changed over the years?

- YES
- NO
- Unknown

If YES, please provide details of the changes in chronological order in the box below

[click here to insert text](#)

## Summary of the replies to the questionnaire ‘Sampling strategy and control measures’

A questionnaire survey was delivered by EFSA to all 27 MSs, plus Croatia, during 2013 in order to obtain additional information on the implementation of surveillance activities and control measures for scrapie in sheep and goats for the mandate period: 2002-2012. The aim of this survey was to gather information and knowledge beyond the statutory requirements for reporting that would allow a better assessment of the observed trends of scrapie over the same period. Responses were collated and summarised. Croatia was not included in the summary since admission to the EU as a full MS occurred after the end of the mandate period. In the summary below, and after references to the answers for specific questions, the initials (see Abbreviation section at the end of the Opinion) of some countries are presented in brackets. These are examples of countries for which the answer applies. However they are not exhaustive lists of all MS applying the measure, since not all countries participated in the survey, nor is there certainty on whether countries that did not answer that question actually implemented it as well.

Twenty-five of the 27 pre-2013 EU MSs responded to the questionnaire survey. At the time of writing this opinion, data from Bulgaria and Lithuania were not available.

Immunohistochemistry (IHC) and Western blotting (WB) were the tests initially used for TSE surveillance until the additional ELISA-based rapid tests were approved by the EU. Changes in the testing protocols implemented by MSs for financial or logistic reasons occurred in parallel with the inclusion and deletion of authorised tests for screening and confirmation across the period covered by the Opinion, as per EU legislation. Some rapid tests applied in the early 2000s, such as Prionics Check WB (FI, NL, FR), Bio-Rad Platelia (UK) and Enfer TSE (IE, IT, SK, FR) were replaced by or used in parallel with other tests, more popular in the later years, for example Bio-Rad TeSeE (FI, SK, SE, UK) and lately the IDEXX Herdcheck BSE scrapie (DK, IE, NL, SI, SE, FR, IT).

### Small ruminants slaughtered for Human Consumption (SHC)

There have been changes in the EU legislation over the years with regards to the TSE surveillance in small ruminants in sheep and goats slaughtered for human consumption. MSs with small sheep populations (< 750 000) were required to test a small sample between 2002 and 2006, to test all SHC between 2006 and 2007, and since 2007 there has been no obligation to test any apparently healthy animals slaughtered for human consumption. In MSs with large sheep populations, testing requirements have been based on a quota. This route of active surveillance is independent of, and additional to, the testing of apparently healthy animals slaughtered for human consumption from infected flocks/herds under restrictions.

Most of the countries subject to a quota during all or part of the mandate period applied a structured sampling strategy, based on distribution of the quota. The sampling procedures at abattoirs varied by country: some allocated numbers by counties, regions, provinces, prefectures (HU, IE, ES, RO, EL) while others used throughput data at national level to allocate sub-quotas to each abattoir (PT, FR) or region (IT) or to a number of them with throughputs above a threshold (UK). In some cases, quotas were also distributed by month or season (IE, UK, IT, FR). A more stringent approach was followed by SK whereby as part of the national programme, a minimum number of holdings were selected for sampling based on geographical location, age groups (minimum 18 months), breed and season. The ultimate approach was applied by CY where “*an annual monitoring sampling programme is prepared. Circulars [...] with instructions for collecting the appropriate samples, with information about the farm code, the status of the flock/herd, the animal population and the samples expected to be sampled are sent to the slaughter houses. Periodically checks for the sampling, reporting and registration are performed*”.

Some countries exceeded the quota by testing 10 % of all SHC sheep > 18 months (NL, FR), with a minimum of 1 per slaughterhouse (NL), two sheep per flock (CY), or by testing all SHC (PL). Others stopped the testing because the number of animals that were tested was not representative of the sheep and goat population (e.g. BE between 2004 and 2006).



Some countries that are not required to test SHC decided to continue with the testing of high-risk groups, such as emergency slaughtered animals and those > 18 months of age showing signs of wasting or neurological illness (FI).

The sampling strategy and the selection of individual animals for testing proved to be a challenge for many MSs and it is the weakest point of this surveillance route, based on the responses to the questionnaire survey. The random selection of animals is not universally applied. It is recognized that some flocks/herds are overrepresented in the survey (IT), for example larger flocks/herds (SE) and/or certain breeds, like Texel (NL). In other cases the apparently healthy slaughter population is underrepresented because old animals are rendered rather than sent to abattoir (SE) or due to the selection for testing of animals submitted for slaughter at abattoirs but categorised as unhealthy during *ante-mortem* inspection (SI, IT). Additionally, certain geographical areas are underrepresented due to their remote location and/or inaccessibility (EL).

There is not much information about the selection of individual animals within abattoirs. Some countries applied a systematic random selection by abattoir personnel (every  $x^{\text{th}}$  sheep) (SE) or randomly selection of batches arriving at the abattoir (RO). A few others delegate the implementation rules to local or regional authorities (PT).

Some measures are applied to increase the representativeness, such as sampling a maximum of one animal per holding (RO), two sheep per holding (CY), sampling a maximum of one sample/year from a holding tested the previous year (RO), minimum/maximum sample size per flock (CY, DE, EL, SK) or avoiding repeated sampling from the same flock/herd (PT). Equally some risk-based strategies aimed to enhance the sensitivity of the SHC survey are applied by some MSs: selection of flocks based on their TSE history (EL), targeting animals of specific age bands (CY, ES, MT, LV), targeting emergency slaughtered sheep and goats (HU, ES, FI) or identified sick animals during *ante-mortem* (SI) or on farm (MT). This is also applied in goats where in some countries only emergency slaughtered animals are selected for testing (HU).

#### Small ruminants not Slaughtered for Human Consumption (NSHC)

The selection of animals for testing is usually carried out at rendering plants. Sixteen out of the 25 respondents (64 %) declared themselves to be applying a structured sampling strategy, 5 countries (20 %) a convenience sampling and four other type(s) (16 %). Among those countries with a quota assigned over the mandate period that declared a structured sampling strategy, several methods are described: minimum/maximum number of sheep found dead per flock selected for testing (CZ, DE), specific sampling days in which one animal is selected at random from each holding (DK), assigned areas for sampling selected at random where animals are individually selected, based on available data (birth holding, date sampling, weekday) (DK). The allocation of sub-quotas proportional to the small ruminant population in each region, province, county, throughput of rendering plants, etc., is also widely applied for fallen stock (EL, ES, HU, IT, PT). In some cases quotas are distributed by month or season (UK, FR, NL, PT, SK), although some seasons are over-represented due to the weather conditions or the peak of lambing (UK, SI). In some cases the sampling design is even included in national legislation that “foresees sampling according to number from each holding, nature of holding (breeding, reproductive, breeding-experimental, commercial), age groups (minimum 18m), even testing throughout the year” (SK).

It is not clear in the answers provided by MSs how the implementation of this survey ensures the representativeness of the NSHC sheep selected for TSE testing. Voluntary notification of found dead animals, or cost-sharing policies for the collection and testing of fallen stock can introduce inherent bias to the selection of animals for testing (UK). Countries do not specify how the figures for killed or found dead animals are collated at either a central or regional level. An isolated example is IE where “an analysis of data is conducted every quarter to ascertain how representative of season and region the sampling is”. Then sampling is adjusted in terms of number of sampled animal and flocks and frequency of sampling at rendering plants. Setting upper limits, for example no more than two eligible

animals from same flock/day (IE), selecting every  $x^{\text{th}}$  carcass at the rendering plant until assigned monthly quotas are met (UK), targeting at least one dead animal from each flock per year (CY, SK) or at least animals from large flocks (SI) are strategies aimed to improve representativeness at the time of selecting individual animals for testing.

Some countries exceeded the quota by testing all fallen sheep/goats > 18 months, during certain periods, in the entire country (DK, FR, IT, RO, SE, SI). The compliance of full testing can be challenging (IT) and some countries with small sheep or goat populations struggle to meet their own targets (IE). Being a small country could otherwise be an advantage when all fallen stock is channelled to a single central collection centre (MT) or it is easier to set maximum number of tested animals per flock/herd (goats in UK). Although attempts are made to distribute the number of samples evenly across regions, breeds, production type and age (SI, SK), the implementation of such measures is difficult to carry out due to the extra cost.

Yet again derogations in accordance with EU Regulation (EC) No 999/2001 ('TSE Regulation') are applied, mainly to some areas due to their remote location and/or inaccessibility (EL, IE) or low farm density (SE).

All the factors described in the sampling strategy lead to the recognition that important subpopulations may be over/under-represented in the NSHC population, based on different factors like breed (FI), age (LV), geographical areas (FI, IE, IT, RO, SE), catchment areas of rendering plants (FI), seasons (UK, RO, SI), voluntary/compulsory notification (FI, UK), rendering costs and subsidies (IT, RO) and eradication measures in place (SE). In other cases bias occurs in areas where there is no organized collection of found dead sheep and the selection of animals to be submitted for testing is left to the discretion of farmers (FI).

Although this surveillance route is in itself an exercise of risk-based strategy since it targets high-risk sub-populations (found dead) excluding the age where infected animals are not likely to be detected yet (< 18 months), certain criteria are applied by MSs to target sections of the fallen stock population with known risk factors for CS, for example even older age groups (ES, MT) and larger flocks (IE).

### Control measures for CS

Most of the MSs started to apply statutory actions to individual cases after confirmation of a case of CS, as early as 1970 (SE), 1980 (FI), 1987 (CY), 1988 (DK), 1990 (LU, BE), 1991 (IT), 1993 (UK, NL), 1996 (PT) and 1997 (FR, EL). Other MSs enforced control measures against scrapie following the enactment of the TSE Regulation (IE, LV), as amended by Regulation (EC) No 1915/2003 or when they became members of the EU (HU, CZ).

Most of the countries stated that all confirmed cases of CS are traced back with the aim of identifying the natal flock and the most likely location where the animal could have been infected, resulting in the confirmation of infected flocks/herds and the implementation of control/eradication measures. All countries stated that detected cases result in the application of control measures, except two: NL (29.2 %) and UK (no % provided). Fourteen countries reported the number of sheep flocks on which control/eradication measures have been applied. There is a wide range, as expected, given the different sizes of the susceptible populations and the prevalence of scrapie: from 529 (IT), 495 (EL, mixed sheep and goats), 358 (FR), 83 plus 221 mixed sheep/goat flocks (CY), 144 (IE), 75 (NL), 74 (RO), less than 50 (DE, CZ, BE, HU, PT, SI) (ES did not report figures and UK only since 2008). Eight countries reported to have applied control/eradication measures on goat herds: 495 (EL mixed sheep and goat farms), 58 plus 221 mixed sheep/goat flocks (CY), 27 (IT), 25 (FR), 5 (FI), 2 (RO), 1 (SI) (ES did not report figures and UK only since 2008).

Among the 17 countries that took action on sheep flocks with confirmed cases of CS, five apply or have applied the cull of all animals, regardless of the genotype (BE, CZ, DK, FI, SE), in some cases beyond the requirements by "*stamping out, cleaning and disinfection and 7-year restriction period for sheep/goats since 1970*" (SE), three countries apply the cull of animals with a genotype susceptible to

CS only (FR, NL, SI), and nine apply both the total and selective cull, with different ratios: 1 % (total cull) / 99 % (selective cull) (IE), 2/98 (CY), 8/92 (DE), 16/84 (IT), 40/60(EL), 43/57 (HU), 90/10 (PT). Some did not provide ratios (RO, ES, UK). Some MSs have applied only enhanced surveillance to infected flocks since 2011 (UK, DE). Additional measures are applied by some countries, such as the cleaning and disinfection of premises (AT, DK, FI, DE, EL, HU, RO, SK, SI, ES, SE), grazing restrictions based on epidemiological assessments (AT, DK, FI, DE, EL, IE, PT, RO, SK, SI, ES, SE), and compulsory enrolment in BP-CSs or qualification schemes (CY, IT, SK).

Eighteen of the 25 countries participating in the survey carry out systematic standardised epidemiological investigations when an outbreak of CS is identified, aimed at confirming disease in a particular flock or herd and at investigating the possible origin of the disease. All except one (IE) stated that those epidemiological investigations are aimed at elucidating the possible spread of the disease to other flocks/herds.

In countries applying control measures to goat herds, all of them apply the ‘cull and complete destruction’ approach. Only one country applies temporary measures to contact herds, beyond the requirements of the EU legislation (FI) and another MS (CY), given the high level of scrapie in the ovine and caprine populations, was granted formal dispensation from the cull and complete destruction, which was replaced by the killing of animals with clinical signs, and the slaughter of healthy animals for human consumption (after testing negative).

All countries participating in the survey conduct enhanced surveillance during the restriction period, according to the EU TSE Regulation, and in one country the cleaning of areas surrounding infected holdings is also undertaken (FI).

The use of the powers granted by the legislation to derogate the implementation of the legislation is widely applied by numerous MS. The most common derogation applied is the delay of the cull of animals from infected flocks/herds (CY, IT, DE, EL, ES, RO, SI, UK); the second is the replacement of the killing of some animals with the slaughtering for human consumption (IT, ES, UK, SK), followed by the derogation of the killing of small ruminants in infected holdings and of parents, and the destruction of embryos, ova and last progeny of diseased female animals (ES, IT, UK). The derogation of the restriction of movements of animals from infected holdings during the enhanced surveillance period after outbreaks has been also applied by one MS (UK). Special mention must be made in the case of CY for which transitional measures in relation to eradication measures in scrapie infected holdings were granted in January 2004 due to the high level of scrapie in the ovine and caprine population, the low level of genetically determined resistance in the ovine population, and the nature of farming in the country. Comprehensive animal identification (ruminal EID bolus) and traceability systems were put in place covering the entire sheep and goat population, in parallel with a compulsory BP-CS only for sheep.

### Control measures for AS

In countries where AS has been confirmed, in general all confirmed cases are traced back resulting in the confirmation of infected flocks/herds with some control/eradication measures implemented currently or in the past. MS started to apply measures with regards to AS when the first case was confirmed (FI, DE), when they joined the EU (HU, CZ), or well after the first case of AS was reported (UK, NL).

The measures applied included the cull of a percentage of all affected flocks ranging from 17 % of infected flocks (DK, IT), 37 % (AT), 40 % (FI) to 98 % (HU) (SE, DE, PT, ES and IE did not provide a figure) and the selective killing and destruction of rams carrying AHQ or AF<sub>141</sub>RQ alleles (IT). All the MSs that reported AS and participated in the survey (17 of them) also apply trade restrictions, except ES. One MS temporarily applied (until 2006) measures in excess of the EU requirements, by “*depopulation and the restrictions with no repopulation allowed for 7 years; after that enhanced*

*surveillance and trade restrictions*”, and currently “*only enhanced surveillance, according to changes in EU legislation*” (SE).

Some derogations have been applied to flocks/herds with AS. For example, since July 2007 animal owners are allowed “*to apply derogation from killing animals in infected flocks. And since July 2013 infected flock are subject to movement restrictions and monitoring, but no animals are killed*” (FI). The substitution of the kill by enhanced surveillance has been applied by various MSs at different points in time (FI, IE, SE, SI, UK).

## **A.2. Additional information on breeding programmes received from Member States**

A questionnaire “*on the implementation of breeding programmes for genetic resistance to TSEs in sheep in accordance with Article 6(a) of Regulation (EC) No 999/2001*” was circulated by the European Commission to all MSs in 2010, gathering information about the implementation of BP-SCs in the EU. According to the replies collected by the Commission, 16 MSs declared to have a BP-SC in place. In 2013 the European Commission requested all MSs having a BP-SC in place to provide, requirements for BP-SCs and annual reports, in accordance with the TSE Regulation.

Information from the above sources was made available to EFSA, and was used, in addition to information described in Appendix A.3, to inform the assessment of BP-SC in EU MSs in the framework of the mandate for this Opinion.

## **A.3. Questionnaire ‘Breeding programmes’**

### **Questionnaire circulated to EU Member States**

A copy of the questionnaire ‘Breeding programmes’ circulated to MSs is included in the following pages.



2 **GENOTYPING** [RANGE FROM 2.1. TO 2.11., PLEASE ATTEMPT TO ANSWER ALL QUESTIONS]

2.1. **Regarding farget flocks for the genotyping activity in the breeding plans (BPs) for scrapie:**

**FOR High Genetic Merit Flocks (HGMF):** **REPLY** [unit/possible answers]

2.1.1.	What percentage of total HGMF have been genotyped, i.e. "active flocks" within HGMF?		[percentage]
2.1.2.	In HGMF, is it a pure <b>ram ONLY</b> genotyping scheme?		[yes/no/unknown]

**FOR commercial flocks (CF):** **REPLY** [unit/possible answers]

2.1.1.	What percentage of total CF have been genotyped, i.e. "active flocks" within CF?		[percentage]
2.1.2.	In CF, is it a pure <b>ram ONLY</b> genotyping scheme?		[yes/no/unknown]

**REPLIES** [unit/possible answers]

2.2.	When (year) did <b>start</b> the scrapie genotyping in <b>HGMF</b> ?		[year]
2.3.	When (year) did <b>end</b> the scrapie genotyping in <b>HGMF</b> ?		[year/ongoing]

**REPLIES** [unit/possible answers]

2.4.	When (year) did <b>start</b> the scrapie genotyping in <b>CF</b> ?		[year]
2.5.	When (year) did <b>end</b> the scrapie genotyping in <b>CF</b> ?		[year/ongoing]

2.6. Does an **ad hoc data management system** exist to collect, collate and analyse the BPs genotyping data?

**REPLY** [possible answers]

		[yes/no/unknown]
--	--	------------------

2.7. How many **genotype analysis** (heads tested) have been carried out **annually**?

		<b>REPLY</b> [unit]
Annual average on the entire period of the BP		[average all period]
Minimum number carried out		[minimum number on one year]
Maximum number carried out		[maximum number on one year]

**REPLY** [unit]

in what year was it?		[year]
in what year was it?		[year]

2.8. What is the **proportion of genotypes** been from:

		<b>REPLY</b> [unit/possible answers]
<b>HGMF</b>		[%/unknown]
<b>CF</b>		[%/unknown]



2.9. Regarding both HGM and CF, what is the **proportion of genotypes** been from:

**REPLY**

<b>rams</b>		[%/unknown]
<b>ews</b>		[%/unknown]

2.10. What is the **reason to genotype ews**, if done?

**REPLY** [unit/possible answers]

To qualify flocks		[yes/no/unknown/blank]
To plan matings		[yes/no/unknown/blank]
Other reason:		[describe with text/unknown/blank]

2.11. Which is the **breed share in the genotyping?**

**REPLY**

<b>BREED NAME</b>	share as active HGMF	share as active commercial or estimated?	calculated or estimated?	[unit/possible answers]
				[breed name] [%/unknown] [%/unknown] [cacluated/estimated/blank]
				[breed name] [%/unknown] [%/unknown] [cacluated/estimated/blank]
				[breed name] [%/unknown] [%/unknown] [cacluated/estimated/blank]
				[breed name] [%/unknown] [%/unknown] [cacluated/estimated/blank]
				[breed name] [%/unknown] [%/unknown] [cacluated/estimated/blank]
				[breed name] [%/unknown] [%/unknown] [cacluated/estimated/blank]
				[breed name] [%/unknown] [%/unknown] [cacluated/estimated/blank]
				[breed name] [%/unknown] [%/unknown] [cacluated/estimated/blank]
				[breed name] [%/unknown] [%/unknown] [cacluated/estimated/blank]
				[breed name] [%/unknown] [%/unknown] [cacluated/estimated/blank]
				[breed name] [%/unknown] [%/unknown] [cacluated/estimated/blank]
				[mixed breeds] [%/unknown] [%/unknown] [cacluated/estimated/blank]

3 **SELECTION OF RAMS** [RANGE FROM 3.1. TO 3.7., PLEASE ATTEMPT TO ANSWER ALL QUESTIONS]

3.1. With regards to genotypes in **rams ONLY**, what are the results of the genotyping activity within the BP in terms of:

3.1.1. Frequency of **ARR/ARR** genotype

**REPLY**

			Year	in HGMFs	in CFs	[unit/possible answers]
in first year of genotyping						[year] [%/unknown] [%/unknown]
in last year of genotyping						[year] [%/unknown] [%/unknown]

3.1.2. Frequency of **ARR** allele

**REPLY**

			Year	in HGMFs	in CFs	[unit/possible answers]
in first year of genotyping						[year] [%/unknown] [%/unknown]
in last year of genotyping						[year] [%/unknown] [%/unknown]

3.2. Has the BP a **ram classification system** based on genotypes for scrapie resistance?

**REPLY** [possible answers]

[yes/no/unknown]

If "yes", please briefly describe key principles

**REPLY** [describe details in box below]

3.3. Do **rules of ram use** exist, based on the above classification?

**REPLY** [possible answers]

[yes/no/unknown]

If "yes", please briefly describe key principles

**REPLY** [describe details in box below]

3.4. If existing, the rules were **issued as:**

**REPLY** [possible answers]

[compulsory from official authorities/advice-guidelines]

3.5. Does an **auditing system** monitoring rules compliance exist?

**REPLY** [possible answers]

[yes/no/unknown]

If "yes", who is in charge of running and coordinating the system?

**REPLY** [possible answers]

[official authorities/breeder associations]

3.6. What **proportion of flocks** participating to the BP may be estimated to comply with those rules

**REPLY** [answer by introducun an "X" in the box that applies]

0-20%	
21-40%	
41-60%	
61%-80%	
81%-100%	
unknown	

3.7. What **proportion of rams** kept for replcement were born from active flocks that complied with the rules?

**REPLY** [answer by introducun an "X" in the box that applies]

0-20%	
21-40%	
41-60%	
61%-80%	
81%-100%	
unknown	

4 **DISSEMINATION OF RAMS** [RANGE FROM 4.1. TO 4.8., PLEASE ATTEMPT TO ANSWER ALL QUESTIONS]

4.1. Has the BP a **flock qualification system** based on genotypes for scrapie resistance?

**REPLY** [possible answers]

[yes/no/unknown]

If "yes", please briefly describe the flock qualification system

**REPLY** [describe details in box below]

4.2. Do **rules of ram dissemination** exist, based on the above qualification system or on the ram genotype?

**REPLY** [possible answers]

[yes/no/unknown]

If "yes", please briefly describe the rules

**REPLY** [describe details in box below]

4.3. What **proportion of flocks** participating to the BP is estimated to **comply** with those rules?

**REPLY** [answer by introduc in an "X" in the box that applies]

0-20%	<input type="checkbox"/>
21-40%	<input type="checkbox"/>
41-60%	<input type="checkbox"/>
61%-80%	<input type="checkbox"/>
81%-100%	<input type="checkbox"/>
unknown	<input type="checkbox"/>

4.4. Do **rules to avoid/reduce the circulation of susceptible rams** exist?

**REPLY** [possible answers]

[yes/no/unknown]

If "yes", the rules were issued as:

**REPLY** [possible answers]

[compulsory from official authorities/advice-guidelines]

4.5. What **proportion of flocks participating to the BP may be estimated to comply** with those rules?

**REPLY** [answer by introducun an "X" in the box that applies]

0-20%	<input type="text"/>
21-40%	<input type="text"/>
41-60%	<input type="text"/>
61%-80%	<input type="text"/>
81%-100%	<input type="text"/>
unknown	<input type="text"/>

4.6. Does an **auditing system** monitoring rules compliance exist?

**REPLY** [possible answers]

[yes/no/unknown]

If "yes", who is in charge of runnig it?

**REPLY** [possible answers]

[official authorities/breeder associations]

- 4.7. Based on the original list of breeds, can you indicate **how strict the dissemination rules are in the individual breeds?** Please fill the table below indicating with a cross in the corresponding cell if the rules are 'very strict', 'less strict', 'no dissemination rules at all' or last 'Unknown, unable to answer' depending on the case

**REPLY** [answer by introducing an "X" in the box that applies]

Breed	Highly strict	Less strict	No dissemination rules at all	Unknown

- 4.8. **OVERALL**, what proportion of flocks from the general population (i.e. involved and not involved by the BP) may be estimated to have introduced animals originating from the active flocks?

**REPLY** [answer by introducing an "X" in the box that applies]

0-20%	
21-40%	
41-60%	
61%-80%	
81%-100%	
unknown	



**Summary of the replies to the questionnaire ‘Breeding programmes’**

**Table 5:** Main information from the questionnaire ‘Breeding programmes’ (BE, CY, CZ, FR).

	Belgium	Cyprus	Czech Republic	France
<b>Sheep population and structure</b>				
Number total flocks	26337	2032	13246	59961
Number HGMF flocks	885	unknown	485	1940
Number CF flocks	25452 <sup>(a)</sup>	2032	12761	58021
Total sheep population	185624	304894	159324	5541149
HGMF population	32573	unknown	23217	536466
CF population	153051 <sup>(a)</sup>	304894	136107	5004683
<b>Genotyping</b>				
% HGMF flocks genotyped	unknown	-	100	98
Pure ram genotyping in HGMF?	no	0	No	no
% CF flocks genotyped	unknown	100	0	0
Pure ram genotyping in CF?	unknown	no	n/a	n/a
First year of genotyping in HGMF	2005	n/a	2003	2002
Last year of genotyping in HGMF	ongoing	n/a	ongoing	ongoing
First year of genotyping in CF	n/a	1999	2003	n/a
Last year of genotyping in CF	n/a	ongoing	ongoing	n/a
Ad hoc data management system exists?	yes	yes	yes	yes
Annual average genotypings	733	86750	4297	70300
Minimum annual genotypings	455	33391	1283	51100
Year with minimum genotypings	2007	2004	2003	2011
Maximum annual genotypings	1395	139016	8626	91600
Year with maximum genotypings	2005	2010	2006	2003
% genotypings performed in HGMF	100.0	0.0	100.0	100.0
% genotypings performed in CF	0.0	100.0	0.0	0.0
% genotypings performed in rams	58.0	100.0	33.6	50.0
% genotypings performed in ewes	42.0	100.0	66.4	50.0
Ewes genotyped to qualify flocks?	yes	yes	no	yes
Ewes genotyped to plan matings?	yes	yes	yes	yes
Ewes genotyped for other reasons?	-	-	no	yes
<b>Selection</b>				
First year of genotyping	2005	1999	2003	2002
Last year of genotyping	2012	2012	2012	2012
% ARR/ARR HGMF first year	42.3	n/a	21.5	26.0
% ARR/ARR HGMF last year	78.8	n/a	50.5	72.0
% ARR/ARR CF first year	unknown	1 ram	unknown	unknown
% ARR/ARR CF last year	unknown	76.4	unknown	unknown
% ARR HGMF first year	87.3	n/a	53.2	49.0
% ARR HGMF last year	98.0	n/a	85.1	85.0
% ARR CF first year	unknown	1 ram	unknown	unknown
% ARR CF last year	unknown	99.0	unknown	unknown
Ram classification system exists?	yes	yes	yes	yes
Rules of ram use exist?	yes	yes	yes	yes
Type of rules of ram use	compulsory (BA)	compulsory (OA)	yes	OA, advice/guidelines
Auditing system to monitor compliance exists?	yes	yes	yes	yes
Who is auditing?	OA, BA	OA	BA	OA, BA, TRI
% flocks complying with rules	81-100	81-100	81-100	81-100
% rams born from flocks complying with rules	81-100	81-100	81-100	81-100
<b>Dissemination</b>				
Flock qualification system exists?	yes	yes	yes	yes
Rules of ram dissemination exist?	yes	yes, ram gen	yes	yes
% flocks complying with rules	81-100	81-100	81-100	81-100
Rules to avoid circulation susceptible rams exist?	yes	yes	yes	yes
Type of rules of avoiding susceptible rams	compulsory (OA)	compulsory (OA)	yes	compulsory (OA)
% flocks complying with rules	81-100	81-100	81-100	81-100
Auditing system to monitor compliance exists?	yes	yes	yes	yes
Who is auditing?	OA	OA	BA	OA
% flocks general population introducing animals from active flocks	unknown	81-100	81-100	41-60

BA: breeder associations; n/a: not applicable; OA: official authorities; TRI: technical and research institutes

(a): Calculated assuming that number CF flocks (CF population) = number total flocks (total sheep population) - number HGMF flocks (HGMF population)

**Table 6:** Main information from the questionnaire ‘Breeding programmes’ (IE, IT, NL, SK).

	Ireland	Italy	The Netherlands	Slovakia
<b>Sheep population and structure</b>				
Number total flocks	32176	95507	28354	3684
Number HGMF flocks	1200	3806	2835 <sup>(a)</sup>	124
Number CF flocks	30976	91701	25519 <sup>(a)</sup>	3560
Total sheep population	3430300	7310739	1334252	416952
HGMF population	unknown	529741	66713 <sup>(a)</sup>	27184
CF population	unknown	6780998	1267539 <sup>(a)</sup>	389768 <sup>(b)</sup>
<b>Genotyping</b>				
% HGMF flocks genotyped	66	81.82	90	100
Pure ram genotyping in HGMF?	no	no	no	no
% CF flocks genotyped	1.04	16.43	unknown	0
Pure ram genotyping in CF?	no	no	unknown	n/a
First year of genotyping in HGMF	2004	2004	1998	2004
Last year of genotyping in HGMF	ongoing	ongoing	ongoing	ongoing
First year of genotyping in CF	2004	2005	1998	n/a
Last year of genotyping in CF	ongoing	ongoing	ongoing	n/a
Ad hoc data management system exists?	yes	yes	yes	yes
Annual average genotypings	9418	34734	26900	3590
Minimum annual genotypings	488	20533	4300	1787
Year with minimum genotypings	2011	2008	2009	2005
Maximum annual genotypings	26718	62889	70800	8615
Year with maximum genotypings	2004	2009	2004	2012
% genotypings performed in HGMF	97.3	41.8	unknown	100.0
% genotypings performed in CF	2.7	58.2	unknown	0.0
% genotypings performed in rams	53.2	56.2	unknown	100.0
% genotypings performed in ewes	46.8	41.9	unknown	3.9
Ewes genotyped to qualify flocks?	yes	yes	yes	yes
Ewes genotyped to plan matings?	yes	yes	unknown	yes
Ewes genotyped for other reasons?	to facilitate trade	-	unknown	-
<b>Selection</b>				
First year of genotyping	2004 <sup>(c)</sup>	2005	2005	2004
Last year of genotyping	2012 <sup>(c)</sup>	2013	2013	2012
% ARR/ARR HGMF first year	62.4 <sup>(d)</sup>	22.5	17.4 <sup>(e)</sup>	18.7
% ARR/ARR HGMF last year	83.7 <sup>(d)</sup>	49.8	52.4 <sup>(e)</sup>	51.3
% ARR/ARR CF first year	see above <sup>(d)</sup>	11.5	see above <sup>(e)</sup>	unknown
% ARR/ARR CF last year	see above <sup>(d)</sup>	25.4	see above <sup>(e)</sup>	unknown
% ARR HGMF first year	85.7 <sup>(d)</sup>	46.5	37.5 <sup>(e)</sup>	39.5
% ARR HGMF last year	99.8 <sup>(d)</sup>	69.8	70.4 <sup>(e)</sup>	40.2
% ARR CF first year	see above <sup>(d)</sup>	26.7	see above <sup>(e)</sup>	unknown
% ARR CF last year	see above <sup>(d)</sup>	47.3	see above <sup>(e)</sup>	unknown
Ram classification system exists?	no	yes	yes	yes
Rules of ram use exist?	no	yes	unknown	yes
Type of rules of ram use	n/a	advice/guidelines	compulsory first, then voluntary	compulsory (OA)
Auditing system to monitor compliance exists?	n/a	yes	unknown	yes
Who is auditing?	n/a	OA	unknown	OA
% flocks complying with rules	n/a	41-60	unknown	81-100
% rams born from flocks complying with rules	n/a	21-40	unknown	81-100
<b>Dissemination</b>				
Flock qualification system exists?	no	yes	yes	yes
Rules of ram dissemination exist?	no	yes	no	yes
% flocks complying with rules	n/a	61-80	61-80	81-100
Rules to avoid circulation susceptible rams exist?	no	yes	unknown	yes
Type of rules of avoiding susceptible rams	n/a	advice/guidelines	unknown	compulsory (OA)
% flocks complying with rules	n/a	21-40	unknown	81-100
Auditing system to monitor compliance exists?	n/a	no	unknown	yes
Who is auditing?	n/a	n/a	unknown	OA
% flocks general population introducing animals from active flocks	unknown	41-60	81-100	81-100

n/a: not applicable; OA: official authorities

(a): calculated based on the approximate percentage provided

(b): calculated assuming that number CF population = total sheep population - HGMF population

(c): not provided but assumed from information provided in previous section of the questionnaire

(d): values provided for the whole population included in the BP-CS, without distinction between HGMF and CF. However, since 97.3 % of the genotypings were performed in HGMF, it may be assumed that the values provided represent the genotypic and allelic frequencies in HGMF

(e): values provided for the whole population included in the BP-CS

**Table 7:** Main information from the questionnaire ‘Breeding programmes’ (SI, ES, UK(GB/NI)).

	Slovenia	Spain	United Kingdom (GB)	United Kingdom (NI)*
<b>Sheep population and structure</b>				
Number total flocks	5203	111787	52500	13417
Number HGMF flocks	221	3607	14000	unknown
Number CF flocks	4982	108180	38500	unknown
Total sheep population	86535	16609069	15200000	1969000
HGMF population	13111	2157070	7690000	unknown
CF population	73424	14451999	7510000	unknown
<b>Genotyping</b>				
% HGMF flocks genotyped	85	95	85	unknown
Pure ram genotyping in HGMF?	no	no	yes	no
% CF flocks genotyped	unknown	1	0	0
Pure ram genotyping in CF?	unknown	no	n/a	n/a
First year of genotyping in HGMF	2006	2003	2001	2003
Last year of genotyping in HGMF	ongoing	ongoing	2009	2010
First year of genotyping in CF	2010	2003	n/a	n/a
Last year of genotyping in CF	ongoing	ongoing	n/a	n/a
Ad hoc data management system exists?	yes	yes	no <sup>(a)</sup>	yes
Annual average genotypings	3636	355214	700000	16822
Minimum annual genotypings	2159	34785	unknown	6967
Year with minimum genotypings	2011	2003	unknown	2010
Maximum annual genotypings	5281	621893	unknown	27953
Year with maximum genotypings	2007	2007	unknown	2004
% genotypings performed in HGMF	100.0	90.6	100.0	100.0
% genotypings performed in CF	0.0	9.4	0.0	0.0
% genotypings performed in rams	95.0	8.8	20.0	44.0
% genotypings performed in ewes	80.0	91.3	80.0	56.0
Ewes genotyped to qualify flocks?	yes	yes	unknown	no
Ewes genotyped to plan matings?	yes	yes	yes	yes
Ewes genotyped for other reasons?	unknown	-	cost	sample size
<b>Selection</b>				
First year of genotyping	2006	2003	2002	2003
Last year of genotyping	2011	2012	2008	2010
% ARR/ARR HGMF first year	4.3	11.3	28.8	23.8
% ARR/ARR HGMF last year	13.4	25.1	53.9	59.2
% ARR/ARR CF first year	unknown	unknown	unknown	unknown
% ARR/ARR CF last year	unknown	30.3	unknown	unknown
% ARR HGMF first year	38.3	28.0	50.4	45.9
% ARR HGMF last year	55.4	48.4	68.8	75.9
% ARR CF first year	unknown	unknown	unknown	unknown
% ARR CF last year	unknown	47.9	unknown	unknown
Ram classification system exists?	yes	yes	yes	yes
Rules of ram use exist?	yes	yes	yes	yes
Type of rules of ram use	compulsory (BA)	compulsory (OA)	compulsory (NSPm)	compulsory (OA)
Auditing system to monitor compliance exists?	yes	no	yes	yes
Who is auditing?	OA, BA	n/a	NSP administration	OA
% flocks complying with rules	81-100	81-100	81-100	81-100
% rams born from flocks complying with rules	81-100	81-100	81-100	81-100
<b>Dissemination</b>				
Flock qualification system exists?	yes	yes	no	no
Rules of ram dissemination exist?	yes	yes	no	no
% flocks complying with rules	61-80	61-80	n/a	n/a
Rules to avoid circulation susceptible rams exist?	yes	yes	yes	yes
Type of rules of avoiding susceptible rams	compulsory (OA)	compulsory (OA)	compulsory (NSPm)	compulsory (OA)
% flocks complying with rules	61-80	81-100	81-100	unknown
Auditing system to monitor compliance exists?	yes	no	unknown	no
Who is auditing?	OA	n/a	n/a	n/a
% flocks general population introducing animals from active flocks	21-40	0-20	61-80	unknown

\* Northern Ireland not included in the assessment of BP-SC because of the lack of some data (see Section 4.5.4.1).

BA: breeder associations; GB: Great Britain; NI: Northern Ireland; NSPm: NSP members; n/a: not applicable; OA: official authorities

(a): data management system existed, but is considered unfriendly for large data extractions and manipulation

**Table 8:** Main information from the questionnaire ‘Breeding programmes’ (EE, DE, HU).

	Estonia*	Germany*	Hungary*
<b>Sheep population and structure</b>			
Number total flocks	1549	299130	6700
Number HGMF flocks	34	1375	200
Number CF flocks	1515	10600	6500
Total sheep population	77265	1799700	890000
HGMF population	3779	5323	40000
CF population	73486	1741000	850000
<b>Genotyping</b>			
% HGMF flocks genotyped	100	unknown <sup>(a)</sup>	100
Pure ram genotyping in HGMF?	yes	unknown	yes
% CF flocks genotyped	0	unknown	2
Pure ram genotyping in CF?	n/a	unknown	unknown
First year of genotyping in HGMF	2006	2006	2004
Last year of genotyping in HGMF	ongoing	ongoing	ongoing
First year of genotyping in CF	n/a	2002	2004
Last year of genotyping in CF	n/a	ongoing	ongoing
Ad hoc data management system exists?	yes	no	unknown
Annual average genotypings	286	46179	4500
Minimum annual genotypings	160	4747	2400
Year with minimum genotypings	2012	2009	2004
Maximum annual genotypings	448	13458	4776
Year with maximum genotypings	2006	2006	2012
% genotypings performed in HGMF	100.0	unknown	43.8
% genotypings performed in CF	0.0	unknown	56.2
% genotypings performed in rams	78.0	unknown	40.0
% genotypings performed in ewes	22.0	unknown	60.0
Ewes genotyped to qualify flocks?	no	yes	yes
Ewes genotyped to plan matings?	yes	-	no
Ewes genotyped for other reasons?	no	reporting	unknown
<b>Selection</b>			
First year of genotyping	2006	2006	2004
Last year of genotyping	2012	2012	2012
% ARR/ARR HGMF first year	20.5	unknown <sup>(b)</sup>	16.1
% ARR/ARR HGMF last year	41.3	unknown <sup>(c)</sup>	46.8
% ARR/ARR CF first year	unknown	unknown <sup>(d)</sup>	unknown
% ARR/ARR CF last year	unknown	unknown <sup>(e)</sup>	22.2
% ARR HGMF first year	41.5	unknown	42.9
% ARR HGMF last year	69.1	unknown	66.4
% ARR CF first year	unknown	unknown	unknown
% ARR CF last year	unknown	unknown	46.5
Ram classification system exists?	yes	no	yes
Rules of ram use exist?	yes	yes	unknown
Type of rules of ram use	compulsory (OA)	compulsory (Smon)	n/a
Auditing system to monitor compliance exists?	yes	no	yes
Who is auditing?	OA	n/a	BA
% flocks complying with rules	81-100	41-60	81-100
% rams born from flocks complying with rules	81-100	unknown	81-100
<b>Dissemination</b>			
Flock qualification system exists?	yes	yes	no
Rules of ram dissemination exist?	yes	no	yes
% flocks complying with rules	81-100	21-40	81-100
Rules to avoid circulation susceptible rams exist?	yes	no	yes
Type of rules of avoiding susceptible rams	compulsory (OA)	n/a	advice/guidelines
% flocks complying with rules	81-100	unknown	81-100
Auditing system to monitor compliance exists?	yes	no	yes
Who is auditing?	OA	n/a	BA
% flocks general population introducing animals from active flocks	61-80	unknown	81-100

\* Estonia, Germany and Hungary not included in the assessment of BP-SC because analysis of the epidemiological trend was not performed (see Section 4.5.4.1). BA: breeder associations; OA: official authorities; n/a: not applicable; Smon: scrapie monitoring

(a): in total, 7518 farms genotyped (b): percentage not provided; absolute number of genotypings provided (5661) (c): percentage not provided; absolute number of genotypings provided (3340) (d): percentage not provided; absolute number of genotypings provided (4801) (e): percentage not provided; absolute number of genotypings provided (371)

#### **A.4. Request for data to model the minimum frequency of the ARR allele to observe fading-out of Classical scrapie in some case studies**

A request for data was made to some Member States (i.e. Cyprus, Italy and the United Kingdom) in order to obtain information allow the calculation of the minimum frequency of the ARR allele to observe fading-out of CS for some case-studies. Case-studies included sheep in Cyprus, Sarda sheep in Sardinia (Italy), and sheep in Great Britain.

Information requested included:

- Qualitative information on the type of selective breeding strategy. This information was needed to inform the construction of relevant breeding strategy scenarios for model extrapolation.
- Data from active surveillance, in particular in relation to: i) total number of animals tested by year and by stream; ii) number of positive cases by year, by stream and by genotype. These data were needed for the calculation of the CS transmission level (quantified by  $R_0$ ) at a given year, for which random genotyping data are also available (see below).
- Data on frequency of genotypes, based on a random genotyping sample from the population of interest. for at least one year, for which data on tested and positive animals are also available (see above). These data were needed for the calculation of allele and genotype frequencies for the given year.

## Appendix B. Analysis of scrapie trends (2002-2012): materials and methods

### Materials

With regards to the two species of interest, i.e. sheep and goats, the European Commission made available two different sources of data covering the 2002-2012 period:

- Testing data by species from both passive and active surveillance and from the eradication cull of the animals in outbreaks. The individual record was based on aggregated data given by the combination of sampling year, sampling month, target group (i.e. the surveillance stream) and type of test by country, providing the total number of tested animals, and of positive and negative/inconclusive results. Due to the large proportion of missing data, some of the original variables (i.e. age, regional location, flock status) were skipped. Data on the type of test were largely missing for the 2002-2005 period.
- Individual case data that includes information on each individual scrapie case: country, month and year of sampling, case type (Classical vs. Atypical), species, surveillance stream and type of test.

The available data were used for the estimation of prevalence of scrapie over time, separately by species and country and after taking into account the potential effect of confounding factors (e.g. the surveillance stream).

Data collation, manipulation, cleaning and analyses were conducted using Stata (v11.2; Stata Corp, College Station, TX).

#### *Animals tested data (2002-2012): the source of denominators*

The initial extraction of the EU dataset included 5 356 057 tested sheep and 1 745 335 tested goats.

In the case of sheep 5 049 092 were tested in the frame of active surveillance, 17 578 in passive surveillance and the remaining mostly in activities linked to outbreak management.

In the case of goats 1 697 775 were tested in the frame of active surveillance, 14 204 in passive surveillance and the remaining mostly in activities linked to outbreak management.

It was decided to focus the data analysis on the active surveillance data only. Data obtained from passive surveillance and from eradication culls following an outbreak were not considered fit for purpose due to their inherent limitations and biases.

Therefore only the data relating to the two main streams 'slaughtered for human consumption' (SHC), i.e. healthy slaughtered animals, and 'not slaughtered for human consumption' (NSHC), i.e. fallen stock, were kept in the dataset, aggregated by year.

The final testing datasets available for statistical analysis for each species include the following variables: country, year, stream, type of rapid test, and total tested animals. As it was not possible to discriminate the type of scrapie among the cases, only denominator figures were used from these datasets.

Finally, with regard to AS, all testing results referring to rapid tests not able to consistently detect the disease (i.e. other than Bio-Rad and IDEXX rapid tests) were excluded from the denominators. As a result the prevalence estimation was possible only for the period 2006-2012.

#### *Individual case data (2002-2012): the source of numerators*

The datasets included detailed data on each individual case allowing the discrimination between CS and AS cases by country, year, stream and type of rapid test used to confirm the disease.



Before any data analysis, as for the testing datasets, all cases detected in categories other than those of active surveillance (i.e. NSHC and SHC) were dropped.

The case type category was largely missing in the period before 2006 when an EU system of automatic upload was set up. Therefore it was decided to consider as CS cases also those cases that before 2006 were categorised as unknown or with the case type variable missing.

Finally a certain number of duplicate entries were detected and dropped: they originated through the automatic multiplication of records in the EU information system when more than one rapid test had been used in the case detection process.

## Methods

Data analysis was split to consider separately species (sheep vs. goats) and disease (CS vs. AS). In each individual subset, descriptive frequency tables were produced to show the breakdown of animals tested and cases by country, year, stream and rapid test. The potential confounding effect of stream in the case of CS, in both sheep and goats, was observed when comparing the stream-specific prevalence and the different distribution of the number of tests carried out in each stream by country or by year. No significant difference in prevalence by stream was evident in case of AS. Moreover the effect of the type of rapid test on the prevalence estimation has been excluded.

## Space and time analysis

A brief spatial description of the epidemiological impact of the diseases (scrapie types) on the two populations of small ruminants was carried out by mapping at the European level the reporting of CS and/or AS by species. After that, the European geographical distribution of each disease by species was obtained through proportional symbol mapping of the national stream adjusted (for CS) or crude (for AS) prevalences over the 2002-2012 period. The adjustment on stream was carried out by means of a direct standardization using as standard population the European proportion of tests carried out in NSHC vs. SHC in sheep and goats respectively over the same period.

## Time trends analysis

Before any multivariate analysis, EU-wide and national stream-specific annual prevalences (cases per 10 000 rapid tests) with 95 % CIs were obtained and plotted for the 2002-2012 period. Individual plots were produced separately by scrapie type, species and surveillance stream.

Temporal trends of annual prevalence for CS and AS by species were tested by fitting Poisson and negative regression models. The negative binomial regression is applied to estimate count models when the Poisson estimation is inappropriate due to over-dispersion. While in a Poisson distribution the mean and the variance are equal, negative binomial regression should be applied when the variance is greater than the mean. The models were compared with the Akaike Information Criterion (AIC). This criterion is calculated using the log-likelihood penalized by the number of parameters in the model. For models with the same dataset, the best model is the one with the lowest AIC.

The number of cases confirmed by year was used as a dependent variable, the year as an independent variable and the annual number of animals tested as an offset of the model. Two different models using year as a categorical or a continuous variable, respectively were fitted for the EU as a whole and for each country. When year was used as a categorical variable, an estimate of the expected prevalence was obtained for each individual year, whereas year was used as a continuous variable to describe the annual linear change in the prevalence over time and to test its statistical significance (see below). To account for confounding, surveillance stream (SHC vs. NSHC) was entered in the models as a covariate (internal reference).

The models served to obtain the annual stream-adjusted prevalence rates over the period 2002-2012 and the adjusted annual prevalence ratios (PRs). In models where year was entered as a continuous variable, the PR provided an estimate of the average relative change in prevalence per year. A PR

larger than one indicates an increasing trend whereas a PR less than one is associated with a decreasing trend. The PR is statistically significant when its 95 % CI does not include one (that would indicate a flat trend).

**Appendix C. Information on the control of Classical scrapie in Iceland**

Following to a request for information from EFSA to Iceland, a comprehensive report was kindly provided by the Institute for Experimental Pathology of the University of Iceland (IEP, S. Thorgeirsdottir) and the Icelandic Food and Veterinary Authority (MAST, L. Arnthorsdottir). A copy of such report, including historical information on the control of CS in Iceland, is provided in the following pages.

## Classical scrapie control program in Iceland

1. Eradication program
  - a. Historical review and milestones
  - b. Current eradication plan and accompanying measures
  - c. Current rules for atypical/Nor98 scrapie
2. Demography of small ruminant population
  - Goats
  - Sheep
    - a. Breed
    - b. Number of animals
    - c. Feeding and transport
    - d. Genotypes
3. Epidemiology
  - a. Evolution
  - b. Current situation
4. Testing
  - a. Type of tests
  - b. Category of tested animal
  - c. Results of testing
  - d. Genotypes
5. References

**1. Eradication program**

**a. Historical review and milestones**

In 1978 the first scrapie eradication plan was established in Iceland, but the disease is believed to have been brought to the country 100 years earlier (1878) by imported sheep. The first scrapie survey in 1953 showed that the disease had spread from the Northern part of the country to other parts and in the following years initial plans were made to combat the disease but those efforts were not effective apparently due to non-compliance by farmers. In the beginning, the aim was only to try to stop the spread of the disease, but in 1978 rigorous methods were adopted with eradication of the disease as the final goal. Stamping out of scrapie flocks in regular manner began, at that time only in newly infected areas, but after the revisions in 1986, all scrapie flocks detected were culled. In 1993 further enhancements of the program were made, mainly in the practical aspects of handling scrapie cases. That plan is still in effect for classical scrapie, but in 2012 different measures for atypical or Nor98 cases were adapted. See further descriptions of the development of scrapie control in table 1.

In the past the fight against other diseases in sheep has undoubtedly affected the control as well as epidemiology of scrapie in Iceland. This explains why two milestones are listed in the table below which originated from the fight against another disease than scrapie. In the late 1930's the country was divided into 36 movement restriction zones in an effort to stop the spread of the so called Karakul diseases (maedi/visna and paratuberculosis) brought to Iceland in 1933 with imported Karakul sheep. These areas are either marked by man-made fences or natural boundaries such as rivers and glaciers. In the following decade stamping out was used to eradicate these diseases, with a success in case of maedi/visna. During that time total culling of sheep within the endemic scrapie area did take place, but scrapie did reappear there as well as in new areas. At that time (late 1940's) the scrapie endemic area in Iceland only included a few restriction zones in the Northern part of the country.

**Table 1. Important dates and milestones in the history of scrapie control in Iceland**

<b>Dates</b>	<b>Milestone</b>
1878	Scrapie believed to be introduced to Northern Iceland.
1930's	Restriction zones by fences, rivers and glaciers (to fight Karakul diseases).
1945-7	Total culling of sheep in scrapie endemic area in Northern Iceland (to eradicate maedi/visna).
1953	Scrapie surveying begins; shows spread of disease to other parts of country.
1978	Eradication plan established to stop the spread of disease with the final goal of eradicating scrapie from the country. Cooperation of authorities with farmers. Scrapie sheep culled in already infected areas, and whole scrapie flocks culled in newly infected areas. Disinfection of farm houses and restrictions set on transport of sheep and hay. Active surveillance begins with testing of abattoir samples.

	Ban on MBM import and feeding MBM from scrapie-infected areas to ruminants.
1986	Eradication plan enhanced with more intensive culling of sheep. <i>All</i> scrapie flocks detected were culled in addition to those already detected in previous five years within infected areas. Culling of apparently healthy neighbouring flocks in heavily infected areas. Cooperation with and compensation to farmers.
1993	Further enhancement of eradication program. A period without sheep for a minimum of 2 years. Restocking only from scrapie free areas. Thorough cleaning and disinfection of premises.
2012	Changes regarding atypical/Nor98 scrapie; no more culling of atypical scrapie flocks, but increased surveillance and testing.

## b) Current eradication plan and accompanying measures

### 1) Notification and testing

Scrapie is a notifiable disease: Suspicious animals have to be reported and eventually inspected by a District Veterinary Officer (DVO). If deemed necessary he/her takes samples for diagnosis, reports to the Chief Veterinary Officer (CVO) and is responsible for instructing the owner and supervising control measures in the area. Visits and testing because of suspect animals is without cost to the farmer.

### 2) Slaughter and burial

All sheep in scrapie-affected flocks are culled and occasionally apparently healthy neighbouring/contact flocks as well. This is done as soon as possible after diagnosis. The sheep are buried 1,5 m down in the earth on a site approved by municipal environmental authorities. Incineration facilities are used if available within reasonable distance.

### 3) Epidemiological inquiry

The farmer is questioned about circumstances with the aim of identifying potentially infected sheep and herds in addition to the index case. This can lead to preventive culling of additional flocks consisting of apparently healthy sheep but suspected of possible infection. The categories for culling (and testing) include all sheep and goats originating from the infected farm as well as sheep which have been in close contact.

### 4) Burial of manure and hay

The manure is buried and usually also the hay, but occasionally it is sold for horse feed within the same movement restriction zone and where there is no contact to sheep.

### 5) Cleaning and disinfection of premises and equipment

Out-houses on affected farms, such as stables, sheds and barns and various equipments and machines are cleaned by high-pressure soap washing (150 Bar/cm<sup>2</sup>) followed by disinfection in steps with the following chemicals: 500 ppm hypochlorite; drying; 300 ppm iodophor; drying; insecticide (hay mites). Wood from the interior of sheep houses, even whole houses,



which cannot be cleaned or disinfected in a proper manner, is removed and destroyed. Stone walls, difficult to clean, are burned off by flame. Afterwards surfaces are sealed off up to 1,5 m high, with creosote (wood) or oil-based paint (concrete and iron). Soil around the buildings is removed and replaced with clean soil, gravel or asphalt. The disinfection process must be finished at least one year before restocking (usually within a year from scrapie incidence).

#### **6) Restocking**

The farm can be restocked with 5-6 months old lambs from scrapie-free zones (scrapie never found) after a minimum of two winters (usually 2-3 years) of depopulation. The first 3-5 years the farmers are advised to buy replacement lambs and avoid breeding their own.

Feeding of hay wrapped in plastic (big bales) is recommended for new stock.

#### **7) Movement of sheep and equipment**

Within infected restriction zones trading of sheep (or cattle) is banned (or strictly controlled). Breeding rams used for insemination are only bought from non-infected areas. Movement of sheep (and goats) between zones has always been prohibited (or strictly controlled) and sheep straying over boundaries are killed and tested for scrapie. Transport of hay and farming equipment between zones is only allowed under certain circumstances.

#### **8) Compensation**

The farmer is compensated for the loss of sheep and lost production for up to three years after culling. In addition, animals culled for diagnostic purposes are compensated. Cleaning of farmhouses and transport of new stock is supported.

#### **9) Surveillance by/cooperation with farmers**

The farmers themselves were involved in establishing and designing the scrapie eradication plan and were made in part responsible for keeping it in force.

### **c) Current rules for atypical/Nor98 scrapie**

Since the first case of atypical scrapie/ Nor98 case was detected in Iceland in 2004, these cases have undergone the same procedure as classical scrapie cases (see above). In the year 2012 changes were made regarding handling of Nor98 scrapie flocks in Iceland. This was done following the change in definition of Nor98 by OIE; that it did not fit under the definition of classical scrapie, although it was not classified as a specific disease (OIE Terrestrial Animal Health Code, 2009). Since atypical/Nor98 scrapie was not classified as an A disease anymore, it was not necessary to follow the stringent rules of the eradication plan. Recent epidemiological information indicating that Nor98 scrapie might not be an infectious disease or at least not as infectious as classical scrapie, did also play an important role when these changes in regulations were decided.

The change in the eradication plan in regard to atypical scrapie includes; restricted culling of older sheep, testing for scrapie of all culled sheep and intensive surveillance of farm for five years. In 2012 there was one case of Nor98, where these new regulations were implemented for the first time. All sheep five years and older were culled and tested for scrapie. The farm will be under close surveillance for five years and all adult sheep that are slaughtered will be tested.

## 2. Demography of small ruminant population

The small ruminant population in Iceland consists mainly of sheep, while less than 1% consists of the very small goat population. The focus of this report is therefore on sheep and all numbers presented as well as discussion refer to sheep only, except for a short description of Icelandic goats below.

### Goats

The goat population in Iceland has always been small and is currently less than one thousand animals; in 2011 there were 818 goats on 76 farms. The numbers have fluctuated in the past; at the time of the Second World War there were around 3000 animals, but at that time keeping goats became a symbol of poverty. With increasing affluence the numbers decreased and in the 1960's the total number of goats went under 100 animals, which encouraged the authorities to establish stipends for farmers for keeping goats.

The goats are mainly kept as pets rather than for commercial purposes, although in recent years experiments have been made with cheese production from goat milk. Scrapie has never been detected nor suspected in Icelandic goats, but testing of goats for scrapie has not been systematic and therefore very few animals have been tested over the years.

### Sheep

#### a. Breed

There is only one breed of sheep in Iceland, *Ovis brachyura pall*, a short-tailed breed which is believed to be related to old Norwegian breeds (Figure 1). It was imported from Norway by the viking settlers more than 1000 years ago and because of Iceland's geographical isolation it is believed that it has not mixed to any extent with other sheep breeds through the centuries. It is mostly bred for the meat, but the sheep are usually sheared once a year and the wool is collected and sold. The wool is special in that it is made up of two types of hair; outer hair (Icelandic: tog) which is long, rough and waterproof, and inner hair (Icelandic: þel) which is fine and soft. The main color is white, besides a variety of rare colors, like black, grey and brown.



Figure 1. Icelandic sheep

**b. Number of animals**

The number of winter fed sheep in Iceland is now roughly half a million; in 2011 there were a total of 474.759 sheep on 2684 farms. Around 60% of the farms have more than 50 winter fed sheep, 35% have more than 200 sheep, but a large portion are hobby farms with less than 50 winter fed sheep (Table 2).

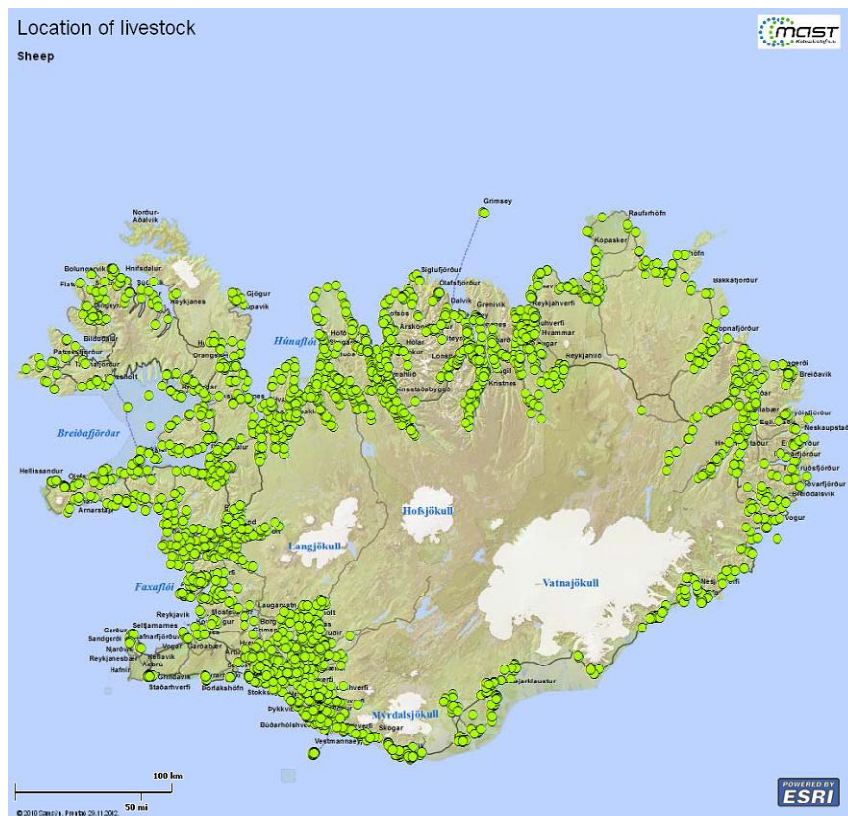
**Table 2. Size of sheep farms in Iceland<sup>†</sup>**

Winter fed sheep	Sheep farms	%
1 - 50	1044	38,90
51 - 100	317	11,81
101 - 200	414	15,42
201 - 400	528	19,67
> 400	381	14,20
<b>Total</b>	<b>2684</b>	<b>100</b>

<sup>†</sup>Numbers are from 2011

The numbers of sheep have now stabilized following a steady decrease in the last few decades. The sheep population peaked in the 1980's at around one million winter fed sheep, which at that time was four times the human population in Iceland. The number of sheep farmers has decreased as did the number of sheep, and culling of whole flocks as a way to control scrapie and other diseases has most definitely played a role in that.

The sheep farms are distributed along the coast of Iceland and in the lowlands and valleys (Figure 2). Parts of the unpopulated highlands are used for pasture in the summer.



**Figure 2. Distribution of sheep farms in Iceland**



### c. Feeding and transport

During the winter the sheep are kept in sheds at the farm where they are fed and the lambing usually takes place indoors in May. Outdoor feeding and grazing during the winter is rare. In the spring and fall the sheep graze in pastures close to the farms, while during the summer the bulk of the sheep population graze freely in common pastures in the highlands. Sheep from many farms within one particular zone can graze together but the boundaries of the movement restriction zones keep them together and away from sheep from other areas. In the fall the farmers gather the sheep together, often by thousands, to the lowlands where they are sorted by farms in special made pens (Icelandic: réttir).

Marketing with live sheep is very limited, mostly from zones considered free of scrapie to other zones. Within scrapie affected zones transport of sheep between farms is prohibited. Import of live sheep from abroad has been banned since middle of last century.

### d. Genotypes

Studies on prion protein (PrP) gene polymorphism show that Icelandic sheep are more homogeneous in regard to genotype diversity compared to foreign breeds but they show some new polymorphism. A comparison of scrapie areas and scrapie free areas in Iceland showed a comparable frequency of the PrP allelic variant, VRQ (amino acids at codons 136, 154 and 171), but revealed a small but significant increase in AHQ within the latter areas.

Genotyping of the PrP gene in Icelandic sheep in regard to scrapie incidence showed that VRQ is highly associated with susceptibility to classical scrapie; similar to what has been found for many other breeds. ARR, strongly associated with scrapie resistance, was not found in Icelandic sheep, since no polymorphism was found at codon 171 (only Q). Novel polymorphisms were detected at codons 138 (S/N) and 151 (R/C) and the latter diversity might matter in regards to scrapie sensitivity i.e. as a protective factor by affecting the structure and stability of the protein. The AHQ allelic variant seems to confer some resistance to the classical scrapie disease in Iceland, but only one case of classical scrapie has been detected in an AHQ sheep, besides a few cases of atypical/Nor98 scrapie. AHQ has been associated with a decrease in susceptibility to classical scrapie infection although it appears to differ between sheep breeds as well as scrapie variants, as is apparent in atypical scrapie cases, which are often found in animals carrying the AHQ allele.

During a period of 25 years (1980-2005), scrapie was detected for the second (or third) time on 30 farms in scrapie endemic areas, while the total number of scrapie farms in that period was ten times higher. These flocks were studied in relation to PrP genotype, and it was found that the defined risk allelic variant, VRQ, did not increase the risk of recurrence of scrapie, which may instead be influenced by the infection load of the environment as well as other unknown factors.

### 3. Epidemiology

#### a. Evolution

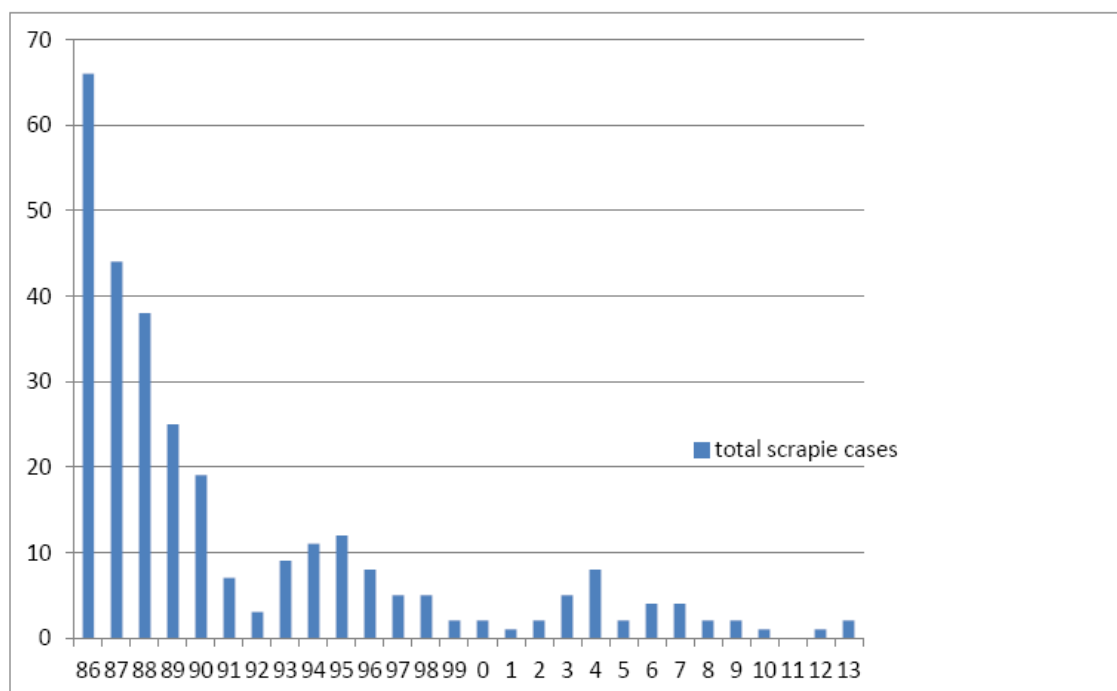
The history of scrapie in Iceland is overlapping 135 years, which can be divided into a few periods based on changes in prevalence, which again were affected by changes in control measures (Table 3). For the first 75 years (1878-1953), the cases were few and the disease stayed within a relatively small area in Northern Iceland, but at the mid of the last century the disease began to spread to other parts of the country. At the same time the disease became more progressive and showed other clinical symptoms than in the beginning, i.e. pruritus instead of ataxia or tremor. The name of scrapie in Icelandic; rida, means ataxia and refers to the clinical picture found in most scrapie affected sheep in Iceland.

During the next period (1953-1986) number of cases increased and the disease spread. In 1978 the disease had spread to most sheep raising districts of the country, causing considerable losses of sheep. Usually 10-15% of the breeding sheep were affected, but on a small number of farms that number could go up to 50%. This situation prompted the establishment of an eradication plan, which from the beginning included culling all scrapie flocks in newly affected areas (see chapter 1). The scrapie epidemic peaked in 1986, when there were 104 infected farms (66 new) located in 25 of the movement restriction zones (2/3 of country) while 13 zones were still scrapie free.

After enhancements of the eradication program in 1986 the number of scrapie cases started to drop and during the next period (1986-1993) there was a steep decrease in the number of newly affected flocks as there were in the total number of sheep because of the heavy culling. During the last two decades or so (1993-2013) the number of scrapie cases per year have been very low, sometimes only one or two (Figure 3).

**Table 3. Historical review of scrapie in Iceland**

Period	Description
1878-1953	Scrapie is imported to Northern Iceland, where it stays and becomes prevalent within a limited area for 75 years.
1953-1986	The disease spreads to other parts of the country, including most sheep raising districts and losses of sheep became high. Height of epidemic in 1986, a few years after establishment of the scrapie eradication plan in 1978.
1986-1993	Number of scrapie cases drop drastically over few years, while the control measures become even more intensive.
1993-2013	Number of scrapie cases down to a few per year.

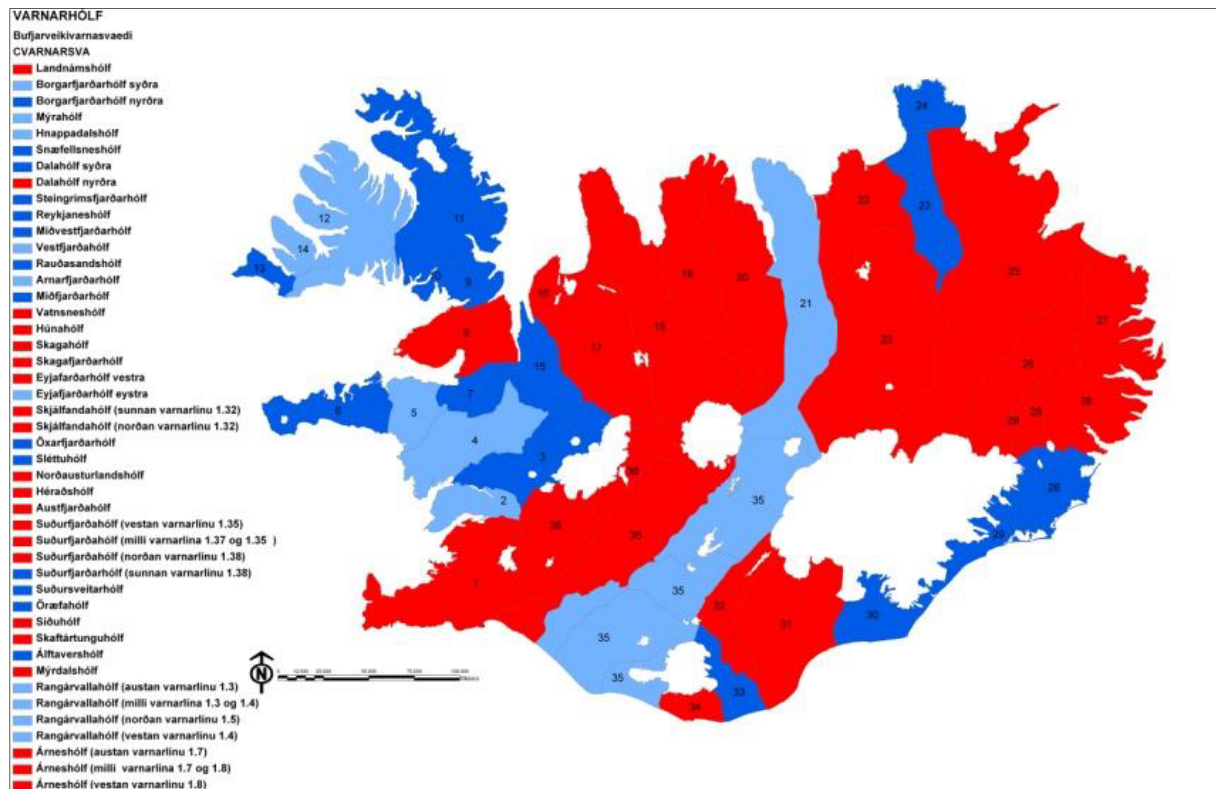


**Figure 3. Total scrapie cases in Iceland 1986-2013**

**b. Current situation**

Since 1986, the goal of the Icelandic government has been to eradicate the disease from the country and the means followed to reach that goal have in short been culling and cleaning. The goal of eradication has not yet been reached completely although yearly incidence has been lowered considerably and is down to a few cases per year. An important accomplishment is that the original scrapie-free zones are still free of scrapie. The quarantine fences, originally built in the 1930's for eradication of slow virus diseases and paratuberculosis, divided the country into movement restriction zones with the help of natural boundaries like rivers and glaciers. Besides rigorous culling of sheep, this establishment managed to eliminate maedi-visna (not paratuberculosis), but in addition it has most definitely prevented scrapie from entering into six of the 36 (now 23) movement restriction zones. The scrapie free areas are distributed around the country, but the main scrapie free areas are Strandir (North-West), Snæfellsnes (peninsula in the West), Örfæfi (South-East) and Þistilfjörður (North-East) (Figure 4). Areas where no cases of scrapie have occurred in 20 years are considered scrapie free but areas where scrapie has been detected are kept under special surveillance for 10 years.

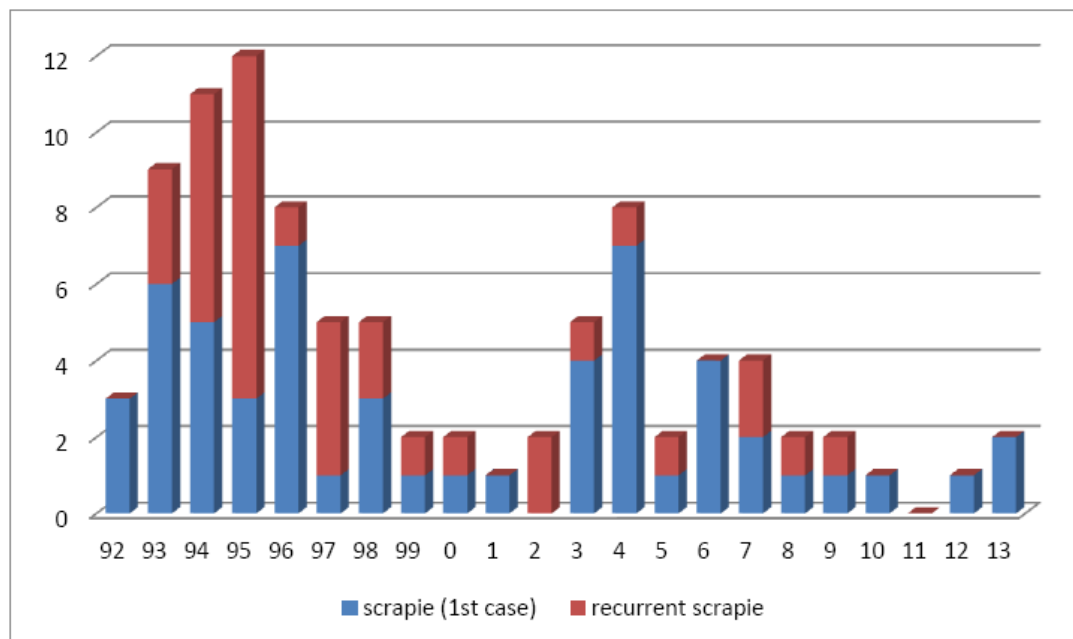




**Figure 4. Distribution of scrapie in Iceland**

Dark blue areas: Scrapie has never been detected. Light blue areas: Scrapie has not been detected for the last 20 years. Red areas: Scrapie has been detected within the last 20 years. Numbers represent different movement restriction zones.

It is of great interest that scrapie is detected on some of the farms in a repeated manner, i.e. the disease is reoccurring on farms although extensive cleanup work has been done and the sheep flock renewed after previous culling of the flock. In the last twenty years, 40% of detected cases were at a farm with a history of scrapie (Figure 5). The infectious agent is known to be extremely resistant and is believed to persist for a long time in soil. In one particular scrapie incident in Iceland, where 18 years passed from the first case until the second case was diagnosed, it was speculated that the infectious agent had persisted in one animal house which had not been cleaned after the first incident.



**Figure 5. Recurrence of scrapie in Iceland 1992-2013**

#### 4. Testing

Active surveillance of scrapie has been carried out in Iceland since 1978 and in the past up to ten thousand sheep samples have been tested per year. The surveillance is controlled by the Icelandic Food and Veterinary Authority, while the testing is carried out at the Institute for Experimental Pathology (IEP) at Keldur, which has been designated as the national reference laboratory for (TSEs).

##### a. Type of tests

The main tests used for the first 25 years were histopathology (HP) and immunohistochemistry (IHC). In 2004 rapid testing replaced the more tedious histopathological studies and now the routine scrapie surveillance is performed by elisa (Bio-Rad TeSeE, sheep/goat). Confirmation is done by Western blotting (Bio-Rad TeSeE WB) and in case of clinical suspects (CS), samples are also taken for HP and IHC if possible. Normally fresh brain samples (medulla and cerebellum) are collected from adult sheep during slaughtering season and kept frozen until tested.

##### b. Category of tested animal

Screening of healthy slaughter (HS) from the abattoir is making up the bulk of scrapie testing in Iceland. The numbers of tested sheep in the category of fallen stock (FS) is very low. Inspection of sheep is performed during the gathering of sheep in the fall and once

during the winter. Sheep showing nervous symptoms or other unknown or suspect symptoms are inspected and if deemed necessary killed and tested. Scrapie is a notifiable disease in Iceland and testing suspected animals is of no cost to the farmer.

Currently three to four thousand adult sheep are tested each year, originating mostly from healthy slaughter, but to a minor extent from fallen stock as well as clinical suspects. Around one hundred bovine samples are tested for BSE every year and occasionally samples from other animals like goats and reindeer are tested for TSEs (Table 4).

**Table 4. Rapid testing 2004-2013**

<b>Samples tested</b>	<b>Total</b>	<b>Positive</b>
<b>Sheep</b>	<b>34321</b>	<b>127</b>
<i>Categories of sheep:</i>		
Healthy slaughter	31785	11
Fallen stock/clinical suspects	302	22
Zone crossovers	246	0
Classical scrapie flocks	1011	93
Atypical/Nor98 flocks	977	1
<b>Other animals</b>	<b>757</b>	<b>0</b>
Goats	32	0
Bovines	709	0
Reindeer	16	0

**c. Results of testing**

Currently the majority of classical scrapie cases in Iceland originate from passive surveillance (Table 5). Although we have had active surveillance of scrapie since 1978, no cases were detected among healthy slaughter until 2004, when rapid testing was implemented. The same year the first atypical/Nor98 scrapie case was detected in Iceland, but a total of four atypical/Nor98 scrapie cases have been found in Iceland. Those cases came equally from passive and active surveillance. In the first Nor98 flock detected in 2004, one additional case was detected after the flock was culled.

**Table 5. Scrapie cases detected by rapid testing (2004-2013)**

<b>Index cases</b>	<b>Total</b>	<b>Classical scrapie</b>	<b>Atypical/Nor98</b>
Clinical suspects	10 (20 pos samples)	8	2
Fallen stock	2	2	0
Healthy slaughter	9 (11 pos samples)	5	4
<b>Total</b>	<b>21 (33 pos samples)</b>	<b>15</b>	<b>6</b>
<b>Additional cases</b>	<b>94</b>	<b>93 (9 flocks)</b>	<b>1 (1 flock)</b>

BSE has never been found in Iceland, probably because of strict regulations regarding importations of live animals and animal feed, which were implemented several years before BSE was first detected in the UK.

#### **d. Genotypes**

In Iceland genotyping has been used for breeding to a very limited extent, the obvious reason being the lack of ARR; the allelic variant associated with scrapie resistance. Rams from insemination centres have been genotyped since 1997, and chosen in regard to their genotype since 2004; rams carrying the allelic variant associated with risk, VRQ, are excluded from use. At that time the frequency of VRQ was unusually high at the insemination centres (30%), compared to the general population (15%).

At IEP, Keldur genetic testing is currently performed on scrapie-affected sheep and selected samples from scrapie-affected flocks.

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## Appendix D. Approach used to model the minimum frequency of ARR allele to observe fading-out of Classical scrapie: methods

### Modelling strategy

In the case studies discussed below the minimum ARR frequency for CS fade-out in a sheep population is defined as that value for which the basic reproduction number  $R_0$  for CS in that population equals one. For the case of The Netherlands, the model explicitly models the heterogeneities of mixing as well as genetic heterogeneities at the between-flock level, thus producing a between-flock  $R_0$ . The modelling strategy for this case is described in more detail below. For the other case studies, in the absence of data on the genetic differences between flocks in the MS/breed of interest, population level data is used to parameterize the model: the population level genotype distribution and the population level scrapie prevalence. As these are both defined for a population of animals (and not a population of flocks), these are ‘between-animal’ parameters. We define a parameter that extrapolates from between-animal input parameters to the between-flock  $R_0$  quantifying the transmission intensity of CS in a context where no control or eradication measure is being applied. This parameter is calculated based on a scenario assumption for the true minimum ARR allele frequency for The Netherlands and on between-animal input parameters for the Netherlands.

### Mathematical details: the model used for the non-Dutch case studies

For a suitable reference year or period an initial  $R_0$  is calculated. A reference year or period is suitable when for that year both scrapie surveillance data and random genotyping data are available. Subsequently we use a simple model to extrapolate the  $R_0$  in new situations (to subsequent years) with a higher ARR allele frequency, and calculate for which minimum ARR allele frequency the  $R_0$  is reduced to one.

In the standard SI (susceptible, infectious) model for a homogeneously susceptible and homogeneously mixing population, the following relationship holds between  $R_0$  and the endemic infection prevalence  $i^*$  :

$$R_0 = \frac{1}{1 - i^*}$$

It is assumed that the case prevalence in the healthy slaughter (HS) surveillance at any given moment in time approximately represents an endemic equilibrium prevalence corresponding to the genotype frequencies in the population at that moment in time. In a population in which a proportion  $n_R$  of animals have resistant genotype, the above relationship generalizes to:

$$R_0 = \frac{1}{1 - \frac{i^*}{1 - n_R}}$$

In analogy with a vaccination campaign, the BP-CS reduces  $R_0$  by a factor equalling the remaining susceptible proportion of the population. In this case this factor is

$$\frac{1 - n_R(y)}{1 - n_R(Y)}$$

Here  $Y$  is the reference year for which  $R_0$  was estimated, and  $y$  is the year of interest. Thus:

$$R_0(y) = \frac{1 - n_R(y)}{1 - n_R(Y)} R_0(Y)$$

The approximation is used that this relation also holds for the between-flock reproduction number  $R_0^{bf}$ . For this quantity  $R_0^{bf}$  the model used is:



$$R_0^{bf} = \frac{1}{1 - \frac{\theta i_{HS}^*}{1 - n_R}}$$

where  $i_{HS}^*$  is the case prevalence in HS and  $\theta$  is a proportionality factor that translates the ‘between-animal’ ratio  $\frac{i_{HS}^*}{1 - n_R}$  into a between-flock equivalent. Assuming Hardy-Weinberg equilibrium (which is appropriate to model the transmission risk in a situation in which application of control and eradication measures are no longer being applied) and assuming that ARR heterozygote animals, if infected, do not transmit CS to other animals, then:

$$1 - n_R = (1 - f_{ARR})^2$$

A minimum ARR allele frequency for CS fade-out  $f_{ARR}^{cr}$  for The Netherlands can be defined. If we assume that as  $R_0=1$  for  $f_{ARR} = f_{ARR}^{cr}$ , then:

$$R_0(Y) = \frac{(1 - f_{ARR}(Y))^2}{(1 - f_{ARR}^{cr})^2}$$

The factor  $\theta$  can now be calculated using  $f_{ARR}^{cr,NL}$  for The Netherlands and using Dutch reference input parameters  $i_{HS}^*(Y_{NL})$  and  $f_{ARR}(Y_{NL})$ , as follows:

$$\theta = \frac{i_{HS}^*(Y_{NL})}{(1 - f_{ARR}(Y_{NL}))^2 - (1 - f_{ARR}^{cr,NL})^2}$$

Setting  $R_0^{bf}(y) = 1$  now leads to the following expression for the minimum ARR frequency for the non-Dutch population of interest:

$$(1 - f_{ARR}^{cr})^2 = \left( (1 - f_{ARR}(Y))^2 - \frac{i_{HS}^*(Y)}{i_{HS}^*(Y_{NL})} \left( (1 - f_{ARR}(Y_{NL}))^2 - (1 - f_{ARR}^{cr,NL})^2 \right) \right)$$

In case the right-hand side is smaller than (or equal to) zero a minimum ARR allele frequency of ‘close to 100 %’ is reported.

## Appendix E. Selection for resistance to Classical scrapie in the Sarda dairy sheep breed

### Description of the Sarda breed population

The Sarda breed is the largest Italian dairy sheep breed (51 % of the national stock) with approximately 2.5 million ewes bred in Sardinia on 11 000 farms<sup>8</sup>. The selection scheme for the Sarda breed is based on a pyramidal management of the population (Barillet, 1997). The high genetic merit population, consisting of around 240 000 ewes in 800 HGFMF, forms the top of the pyramid (Carta et al., 2009). Selection tools are applied in HGFMF to generate genetic progress that is successively transferred to the CF, mainly by rams for natural mating. The time-lag between HGFMF and CF is proportional to the relative size of HGFMF and the flow of breeding animals. An artificial insemination (AI) programme is used in HGFMF with three main purposes: to create genetic links, to progeny test young males, and to enable planned matings between elite rams and elite ewes. Only 8-10 % of replacement ewes were born from AI rams. In the selection scheme, AI is coupled with controlled natural mating. Breeders manage natural mating by grouping ewes with a single ram ('mating group') during the reproduction period (Salaris et al., 2008). This management strategy makes it easy to determine the correct sire of a lamb based on the lambing date.

Milk yield is the main selection goal. CS resistance (Salaris et al., 2007) and udder morphology (Casu et al., 2006) selection criteria have only been implemented recently. Evolution in the number of flocks and population demographic patterns across years were reported in Salaris et al. (2008).

### The Sarda breed selection plan for CS resistance

#### Implementation

Genotyping of AI rams at the *PRNP* locus started in 2000. Successively, in order to estimate allelic frequency, 2 075 rams and 6 424 ewes of HGFMF (Salaris et al., 2004) and 5 677 rams of CF (Mura et al., 2004) were genotyped. The official selection plan for CS resistance started in 2005<sup>9</sup>, according to the European and national guidelines.

The Sarda Plan aims at reducing the risk of CS in sheep flocks by increasing the frequency of the ARR allele and eliminating the VRQ one, and this HGFMF plan is managed with the scientific and technical support of an advisory group including experts from of all the involved industry and government institutions. The HGFMF plan is managed, with the scientific support of the Sardinian Agency for Research in Agriculture (AGRI Sardegna), by the National Sheep Breeders Association (ASSONAPA) and the Regional Ministry of Health. The data is centralised within the National database of the Italian BP-CS, based in Turin. The CF activity is managed by the regional and national Health Authorities with the scientific support of both the Istituti Zooprofilattici of Sardinia and Piemonte (the latter as National Reference Laboratory for animal TSEs).

Two steps were planned. In the first (until 2009), genotyping and selection of rams was applied mainly in HGFMF to increase the availability of ARR carrier rams while preserving the genetic merit for production traits. The selection plan established that all the breeding males and the young ewes with high pedigree genetic merit for milk yield, and therefore candidates to generate male reproducers, had to be genotyped. To preserve production traits, in the early stages of the plan even homozygous susceptible rams with high genetic merit for production traits were used for breeding. These rams were preferably mated to ARR homozygous ewes. With the same goal, no distinction was made between homozygous and heterozygous ARR carriers and both were selected according to the genetic merit for production traits. On the other hand, young ARR homozygous rams without any progeny test for production traits were licensed to generate male reproducers when their pedigree index was beyond a

<sup>8</sup> See [www.istat.it](http://www.istat.it)

<sup>9</sup> Ministry of Health, 2005. DM n.17, December 2004. Piano nazionale di selezione genetica per la resistenza alle encefalopatie spongiformi negli ovini. Gazzetta Ufficiale n. 51, 03.03.2005.

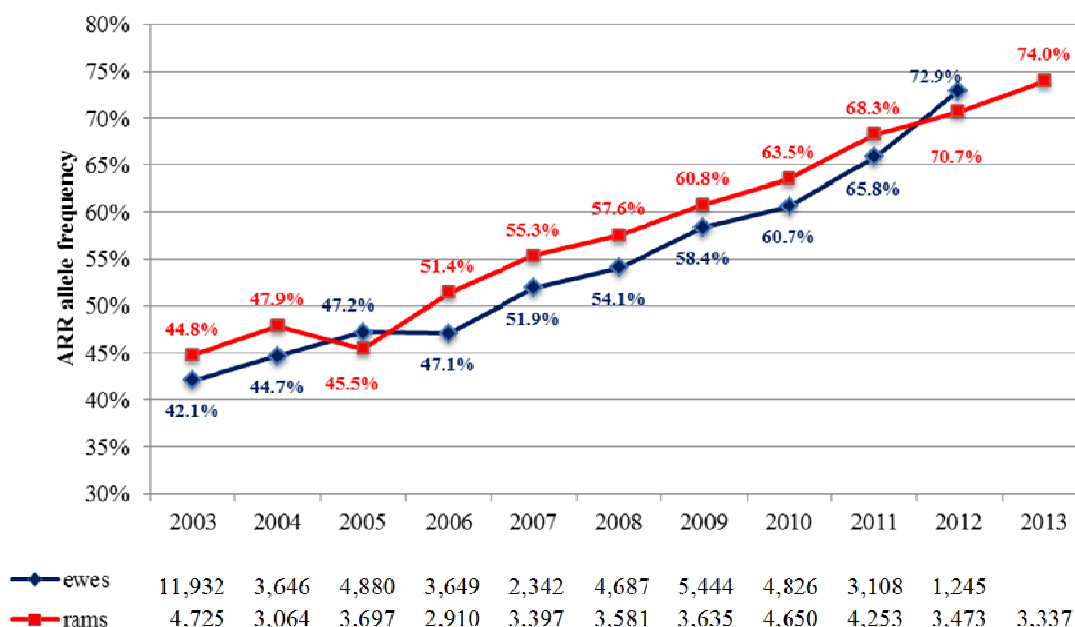
specific threshold. Furthermore a publicly-funded flock was established, to produce homozygous ARR rams to supply outbreaks.

In the second step (since 2009), genotyping was extended to the whole population while making the use of ARR carrier rams compulsory. Flocks involved in the CS plan qualify yearly according to the number of years of use of ARR homozygous or ARR carrier rams. Rams with the VRQ allele must be slaughtered within 30 days of the date of genotyping.

#### 4.7.3.1. Evolution of the distribution of genotypes and alleles

The frequency of the ARR allele was 42 % in HGMF and 40 % in the CF in 2004. Over the duration of the plan in HGMF, *PRNP* genotyping has been carried out on 40 722 rams and 45 750 ewes.

Finally, focussing on rams, the ARR frequency in HGMF increased from 46 % in rams born before 2005, to 74 % in rams born in 2013, with an annual gain of about 3 %. A similar pattern was observed in genotyped ewes (Figure 16).



**Figure 16:** ARR allele frequency and number of genotyped animals according to sex and year of birth

The frequency of the ARR/ARR genotype increased from 21.7 % to 54.1 % and the frequency of ARR carriers (both homozygous and heterozygous) increased from 69.3 % to 93.9 % in rams born in 2005 and 2013 respectively.

The ARR allele frequency of rams used in the 2002 AI programme was 36.4 %. It increased to 49.2 % at the start of the regional plan in 2005 and reached 100 % in 2013. The percentage of replacement from ARR homozygous rams used in HGMF increased from 25 % in 2005 to 70 % in 2011.

The ARR frequency in CF was steady in rams born by 2008 and increased up to 55 % in rams born 2012 (Table 9).

**Table 9:** Genotype and ARR frequency in rams of the commercial population.

Birth year	Susceptible	Heterozygous resistant	Resistant	Total	%ARR
2004	4 280	6 137	2 458	12 875	42.9 %
2005	2 174	3 249	1 206	6 629	42.7 %
2006	3 042	4 413	1 747	9 202	43.0 %
2007	3 283	5 015	2 038	10 336	44.0 %
2008	3 451	5 048	2 098	10 597	43.6 %
2009	3 324	5 127	2 255	10 706	45.0 %
2010	1 866	3 392	1 599	6 857	48.1 %
2011	1 554	2 890	1 655	6 099	50.8 %
2012	659	1 580	965	3 204	54.8 %

The CF comprises 2.5 million ewes. For this population, approximately 50 000 males are used every year (sex ratio 1 to 50). Assuming a replacement rate of 0.33, 16 666 new rams are needed yearly. HGMF are able to produce 18 000 rams per year by using 40 % of ARR homozygous lambs selected for the best production traits. Tools to monitor the flow of rams from HGMF to CF should be implemented.

The introduction of selection for CS resistance in the BP did not produce any decline in the genetic trend for milk yield (Salaris et al., 2007).

#### *Overall considerations of the case-study*

The Sarda selection programme for production traits is based on a pyramidal management of the population with HGMF at the top and CF at the bottom. This breeding structure has been exploited also for selection for CS resistance. The basic idea was to create the genetic progress in HGMF and then transfer it to CF. A two-step plan combined with rules for preserving genetic merit for production traits and facilitating the use of ARR/ARR rams has produced good results in HGMF. These results are now being quickly transferred to CF. However, further tools to precisely monitor the ram flow from HGMF to CF should be implemented.

## Appendix F. Assessment of breeding programmes for resistance to Classical scrapie in EU Member States

### Description of the model

A deterministic model, written in Fortran language, was developed to: i) compare the potential and observed evolution of *PRNP* genotype distribution in the HGMF; and ii) evaluate the dissemination of resistant breeders towards the CF. Classifications of resistance (*R* or *S*) were considered rather than the different *PRNP* alleles. Thus, the *PRNP* gene was simplified as being biallelic with one resistant allele (*R*) and one susceptible allele (*S*) which includes all susceptible polymorphisms. With this model a single animal can be at the resistant homozygous state (*RR*), at the heterozygous state (*RS*) or at the homozygous susceptible state (*SS*). If it is assumed that the resistant allele is dominant, *RR* and *RS* are phenotypically equivalent, but the breeding value of *RS* animals equals one half of *RR* since they transmit the *S* allele to one half of their progeny. The diagram in Figure 17 summarises the structure of the model.

The model is based on a number of hypotheses and in particular assumes that:

- Males were used for three years, without mortality, giving a replacement rate  $RRM = 1/3$ .
- Females were used 5 years, without mortality, giving a replacement rate  $RRF = 0.20$ .
- On average a female gave birth to  $FtoF = 0.34$  young replacement candidate ewe lamb /year (prolificacy 1.4, survival 0.9, sex ratio 0.5, elimination of ewe lambs for standard defects 1/3, no replacement from 1 year old females).
- On average a female gave birth to  $FtoM = 0.17$  young replacement candidate ram lamb /year (prolificacy 1.4, survival 0.9, sex ratio 0.5, elimination of ram lambs for standard defects 2/3, no replacement from 1 year old females).
- One ram could mate  $SR = 40$  ewes/year.
- Only two generic alleles were considered for *PRNP* : *R* for resistant allele, *S* for susceptible.

All those parameters could be modified to better fit the observation in the presence of data derived from actual observations, and to allow the estimation of the robustness of the results given the uncertainties about their values (by sensitivity analysis). These hypotheses are optimistic concerning the evolution of *PRNP* allele frequencies. The resulting model is a starting point from which better fits are sought, to take into account selection for other traits.

The available data for the HGMF part were:

- |  |         |
|--|---------|
| • Number of females                              | NfH     |
| • Number of <i>PRNP</i> genotypes measured/ year | NG      |
| • Proportion of genotyping for HGMF              | propH   |
| • Proportion of genotyping for males             | propm   |
| • First year <i>R</i> allele frequency           | prr     |
| • Duration of the selection scheme               | maxtime |
| • Selection rate applied on other traits         | rate    |

The last parameter ('rate') was set to 1 in a first run of the model. The  $R$  frequency in HGMF given by the model was compared to the frequency observed (from the questionnaire 'Breeding programmes', see Appendix A.3) in 2012, or at the end of the BP-CS if it was stopped before this date. When a large difference was observed (more than 20 %), a value for the selection rate on traits other than the resistance to CS (rate < 1) was estimated by trial and error to force the model to fit the observation. The model run corresponding to this fitted selection rate gave the expected evolution of the  $R$  frequency in the commercial flocks. It must be emphasized that, given that improving resistance to CS is only one amongst, possibly many, other selection objectives, intense selection rates on other traits are to be expected in well-designed BP-CSs.

From these parameters, it was estimated that:

- Number of males in HGMF  $N_{mH} = N_{fH}/SR$
- Number of needed new males / year  $N_{nmH} = N_{mH} \cdot RRM$
- Number of needed new females / year  $N_{nfH} = N_{fH} \cdot RRF$
- Number of males produced in HGMF / year  $N_{pmH} = N_{fH} \cdot FtoM$
- Number of available new males for commercial flocks  $N_{pmHtoC} = N_{pmH} - N_{nmH}$
- Number of genotyped rams in HGMF  $N_{GmH} = N_g \cdot propH \cdot propM$
- Number of available genotyped rams for CF  $N_{gmHtoC} = N_{GmH} - N_{nmH}$

The model manipulated genotype and allele frequencies of classes of individuals defined by sex ( $s$  = male/female), age ( $a$  = 1 to 5) and tier ( $l$  = HGMF/CF).

It was assumed that a selection on *PRNP* genotype was operated on the young rams within their first year and that matings were at random. Following responses to the questionnaire, which never mentioned selection of ewes for the replacement of the female reproducers, selection on the female side was not incorporated into the model.

Let  $p_{salg}(t)$  the genotype  $g$  (*RR,RS,SS*) frequency in class  $[s,a,l]$ .

The ageing process was simply described by  $p_{salg}(t) = p_{sa-1lg}(t-1)$ .

The  $R$  allele frequencies in the reproducers flocks were given by  $f_{sl}(t) = \text{mean}_a(p_{salRR}(t) + 1/2 p_{salRS}(t))$ .

Assuming random matings, genotype frequencies in the newborns were  $h_{0IRR}(t) = f_{ml}(t-1) \cdot f_{fl}(t-1)$ ,  $h_{0IRS}(t) = f_{ml}(t-1) \cdot (1-f_{fl}(t-1)) + (1-f_{ml}(t-1)) \cdot f_{fl}(t-1)$  and  $h_{0ISS}(t) = (1-f_{ml}(t-1)) \cdot (1-f_{fl}(t-1))$  for *RR, RS* and *SS*, respectively. As no selection was assumed for the females,  $p_{f0IRR}(t) = h_{0IRR}(t)$ .

The model was run considering allele  $R$  initial frequency ( $f_{mH}(0) = prr$ ) as given in the questionnaire. The Hardy-Weinberg Equilibrium (HWE) was first checked comparing the initial *RR* frequency as given in the questionnaire to the square of  $prr$ . A large discrepancy between these data points is an indication that the data may be of poor quality.

It was assumed that the initial genotype frequencies  $p_{salg}(1)$  were identical in all classes (whatever  $m$ ,  $a$  and  $l$ ) and followed the HWE ( $p_{sal1}(1) = prr \cdot prr$ ;  $p_{sal2}(1) = 2 \cdot (1-prr) \cdot prr$ ;  $p_{sal3}(1) = (1-prr) \cdot (1-prr)$ ).

Depending on the values of the parameters, different hypotheses were made concerning the selection and diffusion of rams in the BP-CS.



### Selection in HGMF

TypeBP1: When  $NGmH > NnmH/rate$  (the number of genotyped males is higher than the number of males needed for the replacement of the rams in the flock), a selection rate  $qmH = \inf(1, NnmH/(rate \cdot NGmH))$  was applied to the genotyped males. This creates a change in genotype frequencies. The following rules of selection were applied:

If  $qmH < h_{0HRR}(t)$  (*i.e.* all replacement rams may be *RR*)  $\Rightarrow$

$$p_{m0HRR}(t) = 1$$

$$p_{m0HRS}(t) = 0$$

$$p_{m0HSS}(t) = 0$$

If  $h_{0HRR}(t) < qmH < h_{0HRR}(t) + h_{0HRS}(t)$  (*i.e.* all replacement rams may be *R* carriers)  $\Rightarrow$

$$p_{m0HRR}(t) = h_{0HRR}(t)/qmH$$

$$p_{m0HRS}(t) = 1 - p_{m0HRR}(t)$$

$$p_{m0HSS}(t) = 0$$

If  $h_{0HRR}(t) + h_{0HRS}(t) < qmH$  (*i.e.* it is needed to use some *SS* rams for the replacement)  $\Rightarrow$

$$p_{m0HRR}(t) = h_{0HRR}(t)/qmH$$

$$p_{m0HRS}(t) = h_{0HRS}(t)/qmH$$

$$p_{m0HSS}(t) = 1 - (p_{m0HRR}(t) + p_{m0HRS}(t))$$

TypeBP2: When  $NGmH < NnmH/rate$  (the number of genotyped males is lower than the number of males needed for the replacement of the rams in the flock), it was (possibly suboptimally) assumed that the genotyping was only used to discard *SS* rams. The quantity  $frac = 1/qmH = rate \cdot NGmH / NnmH$  represents the proportion of genotyped rams amongst the newborns and the rules give the following genotype frequencies

$$p_{m0HRR}(t) = frac \cdot h_{0HRR}(t) / (h_{0HRR}(t) + h_{0HRS}(t)) + (1 - frac) \cdot h_{0HRR}(t)$$

$$p_{m0HRS}(t) = frac \cdot h_{0HRS}(t) / (h_{0HRR}(t) + h_{0HRS}(t)) + (1 - frac) \cdot h_{0HRS}(t)$$

$$p_{m0HSS}(t) = (1 - frac) \cdot h_{0HSS}(t)$$

### Dissemination from HGMF to CF

The available data for the CF part were:

- Number of females in CF NfC
- Dissemination mode TypeDI
- Maximum proportion of young males in the CF born in HGMF Difrate

This dissemination mode was inferred from the responses to the questionnaire ‘Breeding programmes’ (questions Q4.2 to Q4.7, see Appendix A.3) which gave an idea about the dissemination rules, their compulsory or voluntary bases and the way they were controlled: TypeDI1 when only *RR* males were disseminated, TypeDI2 when only *R* carriers males were disseminated, TypeDI3 corresponded to the fixed proportion of rams coming from HGMF, with a selection classifying *RR* rams before *RS* rams and *RS* rams before *SS* rams, and TypeDI4 when rams sent from the HGMF to the CF were not selected on their *PRNP* genotype.

Different numbers of rams born in HGMF and available for CF were needed to describe the dissemination:

- Number of *RR* rams  $RRHtoC = \max[0, NGmH \cdot h_{0HRR}(t) - NnmH \cdot p_{m0HRR}(t)]$
- Number of *RS* rams  $RSHtoC = \max[0, NGmH \cdot h_{0HRS}(t) - NnmH \cdot p_{m0HRS}(t)]$
- Number of *R* carrier rams  $RcarHtoC = RRHtoC + RSHtoC$
- Number of *SS* rams  $SSHtoC = NGmH \cdot h_{0HSS}(t) - NnmH \cdot p_{m0HSS}(t)$

Whatever the BP type (1 or 2), the dissemination rules depend on the relative value of the CF replacement needs potentially covered by the HGFMF ( $difrate \cdot NnmC$ ) and the availability of *RR* and/or *RS* rams ( $DispHtoC = RRHtoC, RSHtoC, RcarHtoC$ ). In TypeDI1 and TypeDI2, the maximum proportion of new CF rams born in HGFMF ( $DispHtoC/NnmC$ ) may be less than  $difrate$ . In these situations, the dissemination rate was adjusted accordingly:  $difrate = \min[difrate, DispHtoC/NnmC]$ .

TypeDI1: Only *RR* males are sent from the HGFMF to the CF

The number of *RR* rams available for diffusion  $RRHtoC$  was compared to the number of new HGFMF rams needed in the CF:  $difrate \cdot NnmC$ . When the number of available *RR* rams was insufficient to cover the needs, the diffusion rate was adjusted accordingly:  $difrate = \min[difrate, RRHtoC/NnmC]$ .

$$p_{m0CRR}(t) = difrate + (1 - difrate) \cdot h_{0CRR}(t)$$

$$p_{m0CRS}(t) = (1 - difrate) \cdot h_{0CRS}(t)$$

$$p_{m0ICSS}(t) = (1 - difrate) \cdot h_{0ICSS}(t)$$

When the number of *RR* rams available for diffusion ( $RRHtoC$ ) was negative, the previous equation was used, with  $difrate=0$ .

TypeDI2: Only *R* carriers (*RR* or *RS*) are sent from the HGFMF to the CF.

When the number of *RR* rams available for diffusion ( $RRHtoC$ ) is higher than the replacement need ( $difrate \cdot NnmC$ ), only homozygous rams are sent to the CF

$$p_{m0CRR}(t) = difrate + (1 - difrate) \cdot h_{0CRR}(t)$$

$$p_{m0CRS}(t) = (1 - difrate) \cdot h_{0CRS}(t)$$

$$p_{m0ICSS}(t) = (1 - difrate) \cdot h_{0ICSS}(t)$$

When the inequality  $RRHtoC < difrate \cdot NnmC < RcarHtoC$  holds, all available *RR* rams and only a fraction of *RS* rams are sent to the CF, giving:

$$p_{m0CRR}(t) = RRHtoC/NnmC + (1 - difrate) \cdot h_{0CRR}(t)$$

$$p_{m0CRS}(t) = (difrate \cdot NnmC - RRHtoC)/NnmC + (1 - difrate) \cdot h_{0CRS}(t)$$

$$p_{m0ICSS}(t) = (1 - difrate) \cdot h_{0ICSS}(t)$$

When the number of *R* carrier rams available for diffusion ( $RcarHtoC$ ) is lower than the replacement need ( $difrate \cdot NnmC$ ), the previous equations are used after adjustment of  $difrate$ :  $difrate = \min[difrate, RcarHtoC/NnmC]$ .

TypeDI3: Rams sent from the HGFMF to the CF are selected on their *PRNP* genotype with a decreasing preference from *RR* to *SS*.

When the number of *RR* rams available for diffusion ( $RRHtoC$ ) is higher than the replacement need ( $difrate \cdot NnmC$ ), only homozygous rams are sent to the CF

$$p_{m0CRR}(t) = difrate + (1 - difrate) \cdot h_{0CRR}(t)$$

$$p_{m0CRS}(t) = (1 - difrate) \cdot h_{0CRS}(t)$$

$$p_{m0ICSS}(t) = (1 - difrate) \cdot h_{0ICSS}(t)$$

When the inequality  $RRHtoC < difrate \cdot NnmC < RcarHtoC$  hold, all available *RR* rams and only a fraction of *RS* rams are sent to the CF, giving:

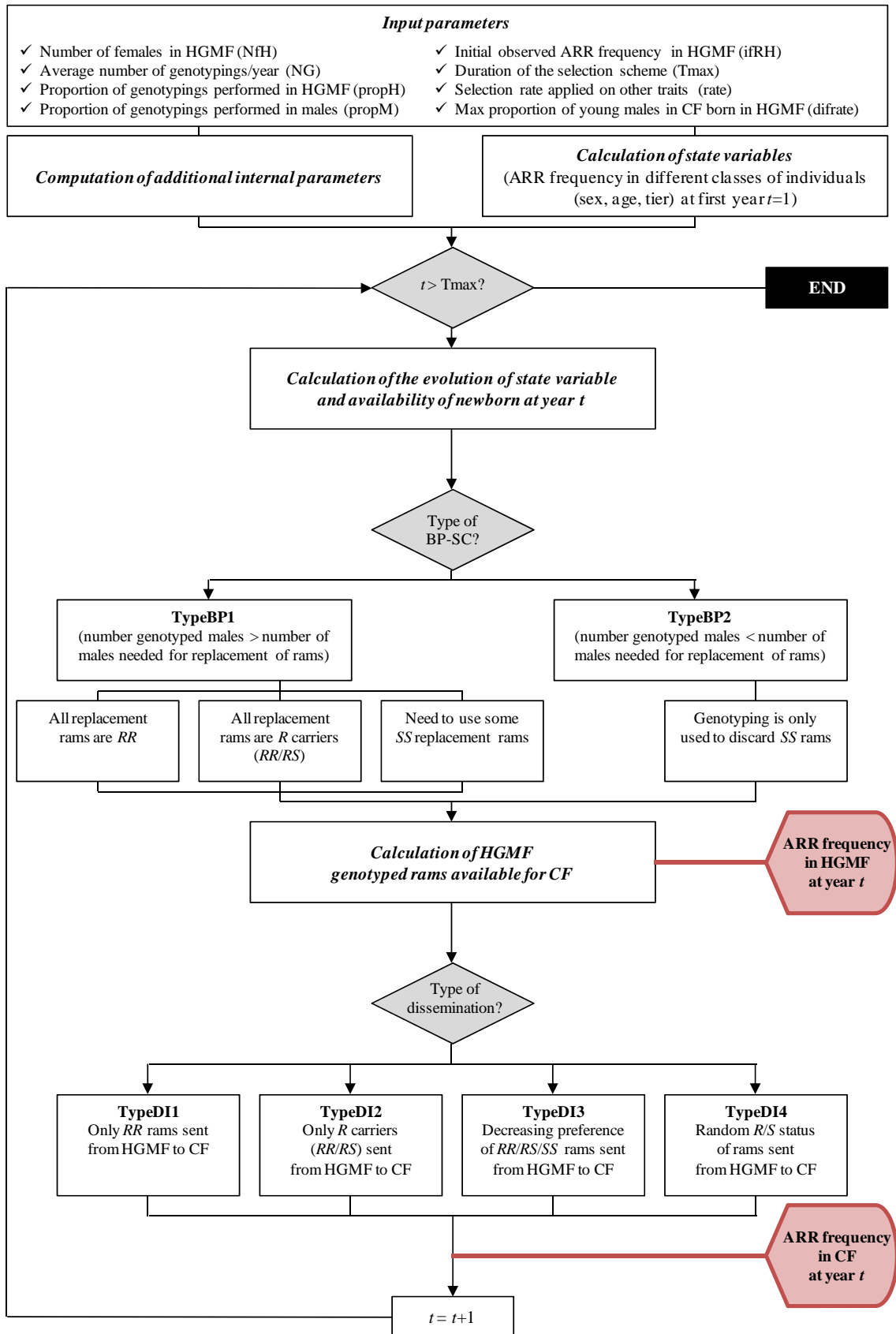
$$\begin{aligned} p_{m0CRR}(t) &= RRHtoC/NnmC+(1-difrate) \cdot h_{0CRR}(t) \\ p_{m0CRS}(t) &= (difrate \cdot NnmC - RRHtoC)/NnmC+(1-difrate) \cdot h_{0CRS}(t) \\ p_{m0ICSS}(t) &= (1-difrate) \cdot h_{0ICSS}(t) \end{aligned}$$

When the number of *R* carrier rams available for diffusion (*RcarHtoC*) is lower than the replacement need ( $difrate \cdot NnmC$ ), *SS* individuals are sent to the CF and the equations become:

$$\begin{aligned} p_{m0CRR}(t) &= RRHtoC/NnmC+(1-difrate) \cdot h_{0CRR}(t) \\ p_{m0CRS}(t) &= RSHtoC/NnmC+(1-difrate) \cdot h_{0CRS}(t) \\ p_{m0ICSS}(t) &= difrate \cdot RcarHtoC/NnmC+(1-difrate) \cdot h_{0ICSS}(t) \end{aligned}$$

TypeDI4: Rams sent from the HGMF to the CF were not selected on their *PRNP* genotype but randomly chosen according to this criteria.

$$\begin{aligned} p_{m0CRR}(t) &= difrate \cdot RRHtoC/NpmHtoC+(1-difrate) \cdot h_{0CRR}(t) \\ p_{m0CRS}(t) &= difrate \cdot RSHtoC/NpmHtoC+(1-difrate) \cdot h_{0CRS}(t) \\ p_{m0ICSS}(t) &= difrate \cdot SSHtoC/NpmHtoC+(1-difrate) \cdot h_{0ICSS}(t) \end{aligned}$$



**Figure 17:** Diagram summarising the structure of the deterministic model. Boxes in red represent the main outputs of the model. After a first run of the model, the parameter ‘rate’ is estimated by trial and error to force the model to fit the observation, and the model is run again (see text for details).

## Results of the model

This section reports detailed results of the deterministic model, when applied to the eleven MSs for which an analysis was done to compare the effectiveness of their BP-CSs to the trend of CS from 2002 to 2012.

Tables 10 and 11 report the input and output parameters of the deterministic model for the different MSs.

A short discussion of the results of the model is provided for each country, together with information on some aspects of the BP-CSs. Figures 18 and 19 show the evolution of the ARR frequency in HGMF and CF as estimated by the deterministic model.

**Table 10:** Parameters used and results estimated by the deterministic model (countries with a statistically significant decreasing trend of CS). Parameters in bold are obtained from the answer by MSs to the questionnaire ‘Breeding programmes’ (see Appendix A.3), or estimated based on information provided in the questionnaire. Parameters preceded by \* are outputs of the model.

Parameter	Abbreviation	Calculation	CY	FR	IE	NL	SI	UK (GB)
<b>Number of females in HGMF<sup>a</sup></b>	NfH		304894	536466	127933	66713	13111	7690000
Number of males in HGMF	NmH	NfH/SR	7622	13412	3198	1668	328	192250
Number of needed new males in HGMF / year	NnmH	NmHxRRM	2541	4471	1066	556	109	64083
Number of needed new females in HGMF / year	NnfH	NfHxRRF	60979	107293	25587	13343	2622	1538000
Number of males produced in HGMF / year	NpmH	NfHxFtoM	51832	91199	21749	11341	2229	1307300
Number of available males for CF	NpmHtoC	NpmH-NnmH	-	86728	20683	10785	2120	1243217
<b>Average number of genotypings / year</b>	NG		86750	70300	9418	26900	3649	700000
<b>Proportion of genotypings performed in HGMF</b>	propH		1	1	0.97	1	1	1
<b>Proportion of genotypings performed in males</b>	propM		0.6	0.5	0.53	0.42	0.15	0.2
Number of genotyped HGMF males / year	NGmH	NGxpropHxpropM	52050	35150	4842	11298	547	140000
Number of genotyped HGMF females / year	NGfH	NGxpropHx(1-propM)	34700	35150	4294	15602	3102	560000
Number of genotyped males available for CF	NGmHtoC	NGmH-NnmH	-	30679	3776	10742	438	75917
Ratio of new males genotyped to males needed for replacement	rnmG	NGmH/NnmH	20.48	7.86	4.54	20.32	5.02	2.18
Ratio of new females genotyped to females needed for replacement	rnfG	NGfH/NnfH	0.57	0.33	0.17	1.17	1.18	0.36
Rate	rate		1.00	0.22	0.28	1.00	0.50	1.00
Males selection rate without extra selection		NnmH/NGmH	0.049	0.13	0.22	0.049	0.20	0.46
Males selection rate considering extra selection	qmH	NnmH/(NGmHxrate)	0.049	0.58	0.79	0.049	0.40	0.46
<b>Number of females in CF<sup>b</sup></b>	NfC		-	5004683	3430300	1267500	73424	7510000
Population size ratio	HtoC	NfH/NfC	-	0.11	0.04	0.053	0.18	1.02
Number of needed new males in CF / year	NnmC	NfC/120	-	41706	28586	10563	612	62583
Max proportion of replacement males from HGMF / year	propnmHtoC	NpmHtoC/NnmC	-	2.08	0.72	1.02	3.46	19.87
Max proportion of dissemination new males genotyped		NGmHtoC/NnmC	-	0.74	0.13	1.02	0.72	1.21
<b>Initial observed ARR/ARR frequency in HGMF (year)</b>	ifRRH		0.16 (2004) <sup>c</sup>	0.26 (2002)	0.62 (2004)	0.17 (2005) <sup>d</sup>	0.14 (2006) <sup>e</sup>	0.29 (2002)
<b>Initial observed ARR frequency in HGMF (year)</b>	ifRH		0.40 (2004) <sup>c</sup>	0.49 (2002)	0.86 (2004)	0.38 (2005) <sup>d</sup>	0.38 (2006)	0.50 (2002)
<b>Final observed ARR/ARR frequency in HGMF (year)</b>	ffRRH		0.76 (2012) <sup>c</sup>	0.72 (2012)	0.84 (2012)	0.52 (2013) <sup>d</sup>	0.13 (2011)	0.54 (2008)
<b>Final observed ARR frequency in HGMF (year)</b>	ffRH		0.99 (2012) <sup>c</sup>	0.85 (2012)	0.998 (2012)	0.70 (2013) <sup>d</sup>	0.55 (2011)	0.69 (2006)
*Final adjusted potential ARR/ARR frequency in HGMF (year)	affRRH		- <sup>c</sup>	0.77 (2013)	0.87 (2013)	0.88 (2013) <sup>d</sup>	0.46 (2013)	0.76 (2013)
*Final adjusted potential ARR/ARR frequency in CF (year)	affRRC		0.75 (2013) <sup>c</sup>	0.50 (2013)	0.62 (2013)	0.50 (2013) <sup>d</sup>	0.20 (2013)	0.083 (2013)
Relative estimated increase of ARR/ARR frequency in HGMF	rifRRH	(affRRH/ifRRH)-1	- <sup>c</sup>	1.96	0.4	0.45 <sup>d</sup>	2.29	1.62
Relative estimated increase of ARR/ARR frequency in CF	rifRRC	(affRRC/ifRRH)-1	3.69 <sup>c</sup>	0.92	0.00	3.03 <sup>d</sup>	0.43	-0.71

a: Approximated as total HGMF population. b: Approximated as total CF population. c: In the case of CY, parameters ifRRH and ifRH have been obtained from answers from 2010 Commission questionnaire (see text section for CY for details). In CY there is no distinction of HGMF and CF, and the population is organised as a single-tier with respect to the BP-CS. Parameters affRRC and rifRRC are therefore referred to the whole population (HGMF+CF). d: In the case of NL, parameters ifRRH, ifRH, ffRRH, ffRH refer to the whole population (HGMF+CF), as reported in the answer to the questionnaire ‘Breeding programmes’. The initial (2005) values for ARR/ARR frequency in HGMF and CF have been estimated as being 0.61 and 0.13 respectively (see text section for NL for details), which have been used to obtain parameters rifRRH and rifRRC. e: Value estimated from parameter ifRH because of a lack of consistency of the data provided with HWE.



**Table 11:** Parameters used and results estimated by the deterministic model (countries with a trend of CS not different from a flat one). Parameters in bold are obtained from the answer by MSs to the questionnaire ‘Breeding programmes’ (see Appendix A.3), or estimated based on information provided in the questionnaire. Parameters preceded by \* are outputs of the model.

Parameter	Abbreviation	Calculation	BE	CZ	IT	SK	ES
<b>Number of females in HGMF<sup>a</sup></b>	NfH		32573	23217	529741	27184	2157070
Number of males in HGMF	NmH	NfH/SR	814	580	13244	680	53927
Number of needed new males in HGMF / year	NnmH	NmHxRRM	271	193	4415	227	17976
Number of needed new females in HGMF / year	NnfH	NfHxRRF	6515	4643	105948	5437	431414
Number of males produced in HGMF / year	NpmH	NfHxFtoM	5537	3947	90056	4621	366702
Number of available males for CF	NpmHtoC	NpmH-NnmH	5266	3754	85641	4394	348726
<b>Average number of genotypings / year</b>	NG		733	4297	34734	3590	355214
<b>Proportion of genotypings performed in HGMF</b>	propH		1	1	0.42	1	0.91
<b>Proportion of genotypings performed in males</b>	propM		0.58	0.34	0.56	0.66	0.087
Number of genotyped HGMF males / year	NGmH	NGxpropHxpropM	425	1461	8169	2369	28122
Number of genotyped HGMF females / year	NGfH	NGxpropHx(1-propM)	308	2836	6419	1221	295122
Number of genotyped males available for CF	NGmHtoC	NGmH-NnmH	154	1268	3754	2142	10146
Ratio of new males genotyped to males needed for replacement	rnmG	NGmH/NnmH	1.57	7.57	1.85	10.44	1.56
Ratio of new females genotyped to females needed for replacement	rnfG	NGfH/NnfH	0.05	0.61	0.06	0.22	0.68
Rate			0.66	0.22	0.90	0.180	0.90
Males selection rate without extra selection		NnmH/NGmH	0.64	0.13	0.54	0.096	0.64
Males selection rate considering extra selection	qmH	NnmH/(NGmHxrate)	0.97	0.60	0.60	0.53	0.71
<b>Number of females in CF<sup>b</sup></b>	NfC		153051	136107	6781000	391768	14451999
Population size ratio	HtoC	NfH/NfC	0.21	0.17	0.08	0.07	0.15
Number of needed new males in CF / year	NnmC	NfC/120	1275	1134	56508	3265	120433
Max proportion of replacement males from HGMF / year	propnmHtoC	NpmHtoC/NnmC	4.13	3.31	1.52	1.35	2.90
Max proportion of dissemination new males genotyped		NGmHtoC/NnmC	0.12	1.12	0.07	0.66	0.08
<b>Initial observed ARR/ARR frequency in HGMF (year)</b>	ifRRH		0.76 (2005) <sup>c</sup>	0.22 (2003)	0.23 (2005)	0.19 (2004)	0.11 (2003)
<b>Initial observed ARR frequency in HGMF (year)</b>	ifRH		0.87 (2005)	0.53 (2003)	0.47 (2005)	0.40 (2004)	0.28 (2003)
<b>Final observed ARR/ARR frequency in HGMF (year)</b>	ffRRH		0.79 (2012)	0.51 (2012)	0.50 (2013)	0.51 (2011)	0.25 (2012)
<b>Final observed ARR frequency in HGMF (year)</b>	ffRH		0.98 (2012)	0.85 (2012)	0.70 (2013)	0.40 (2011)	0.48 (2012)
*Final adjusted potential ARR/ARR frequency in HGMF (year)	affRRH		0.80 (2013)	0.57 (2013)	0.50 (2013)	0.59 (2013)	0.29 (2013)
*Final adjusted potential ARR/ARR frequency in CF (year)	affRRC		0.78 (2013)	0.44 (2013)	0.22 (2013)	0.32 (2013)	0.08 (2013)
Relative estimated increase of ARR/ARR frequency in HGMF	rifRRH	(affRRH/ifRRH)-1	0.05	1.59	1.17	2.11	1.64
Relative estimated increase of ARR/ARR frequency in CF	rifRRC	(affRRC/ifRRH)-1	0.03	1	-0.07	0.68	-0.27

a: Approximated as total HGMF population. b: Approximated as total CF population. c: Value estimated from parameter ifRH because of a lack of consistency of the data provided with HWE.

## A. Countries with a statistically significant decreasing trend in the prevalence of Classical scrapie

### Cyprus

#### *The population*

The sheep population in Cyprus consists of about 305 000 sheep (about 2 000 flocks). 18.7 % of the animals are pure Chios, while most of the other animals are crossbred.

#### *The breeding programme*

The Cyprus situation is very particular since all flocks are involved in the BP-CS, without any distinction between HGMF and CF. The programme started on a small scale in 1999 with the creation of two governmental nucleus units which produced ARR animals for the industry. Since 2004, the BP-CS was organized on a large scale, involving all flocks. Since 2008, it has been restricted to all rams only.

No precise information about the repartition of genotype data between males and females was available, even if it was clear that females were genotyped both for flock qualification and mating purposes. With a mean of 86 750 genotypings performed each year since 2004, there was clearly the opportunity to genotype females. The worst scenario was considered in the model (i.e. genotyping all available males, meaning 59.8 % of the genotyping effort).

The genotyping programme allows a very strong selection of the replacement males on their *PRNP* genotype ( $qmH = 0.050$ ), and a weak selection of females (neglected in the modelling).

As described in the questionnaire, breeders are obliged to genotype all their rams, and only use ARR/ARR as reproducers. An auditing system exists, and the rules are considered as fully followed.

From the information provided by Cyprus to the European Commission in 2010, 15.9 % ( $n = 5308$ ) of the genotyped animals in 2004 were ARR/ARR, 48.6 % ( $n = 16\ 240$ ) were ARR/X, giving an ARR allele frequency of 40.2 %. The HWE is therefore respected.

From this information, the model predicts 100 % of ARR allele and 68 % of ARR/ARR genotypes in 2012. The first figure is in agreement with the evolution observed and reported in the questionnaire 'Breeding programmes'. Nevertheless, the 68 % genotype frequency is less than the 76.4 % observed in practice. This last reported frequency is surprising since it leads to a strong divergence from HWE (even if all non-ARR/ARR animals were ARR/X, this would give an ARR allele frequency of 88 %). The numbers of analyses performed each year are very large, and a sampling error seems very improbable. Globally the results obtained suggest that the rules of the BP-CS, as described in the responses to the questionnaire 'Breeding programmes', were fully implemented, with an optimal effect at population level.

### France

#### *The population*

The total number of ewes is more than 5.5 million. There are a large number of breeds (54), five of them being bred for milk production, and half of the others being of very limited population size.

#### *The breeding programme in HGMF*

Selection for CS resistance started in 2002 and was organized on a large scale with the help of the state. It is still running. The BP-CS focuses on HGMF, on which a very large proportion of the genotyping was performed. Compulsory rules were established for the replacement of HGMF reproducers, and more recently for dissemination to the CF.

All the males used for replacement in the HGMF are genotyped, and the possible selection rate on *PRNP* genotype is large. According to the rules, only ARR/ARR rams are selected in HGMF, classifying the BP-CS as TypeBP1.

The initial allelic and genotypic frequencies are consistent with HWE. The observed frequency after 11 years of selection is below the predicted one. An extra selection rate of 22 % gives the correct adjustment.

#### *Dissemination to the whole population*

The relative size of the two tiers only allowed a full dissemination of genes from HGMF to the CF in a selection of 50 % of flocks. From the questionnaire, 50 % of the flocks introduced rams from the HGMF. The model was run with this hypothesis, considering a selection rate of 0.22 on unknown traits in HGMF, and assuming that half of the replacement males are ARR/ARR rams from HGMF. The evolution of the ARR/ARR frequency in the CF is very limited.

### **Ireland**

#### *The population*

The sheep population is large (3.4 million ewes), shared between 25 breeds, with 1 200 HGMF and 31 176 CF. The number of HGMF ewes was not given in the responses to the questionnaire 'Breeding programmes', and was assumed to be proportional to the number of flocks, giving around 130 000 individuals.

#### *The breeding programme in HGMF*

Selection for CS resistance started in 2004 on a voluntary basis, with subsidies from the Department of Agriculture Fisheries and Food (DAFF) regardless of whether flocks were HGMF or CF. The DAFF contribution was cancelled in 2006 or 2007, with a concomitant large decrease of genotyping effort.

The number of sheep being genotyped has fallen from 26 178 in 2005 to 601 in 2012. Even if limited in some years, the number of genotypes produced/year in HGMF was sufficient to largely cover the needs for replacement.

Thus, all the males used for replacement in the HGMF were genotyped, and the possible selection rate on *PRNP* genotype was large. As no compulsory rules were imposed on the breeders, the hypothesis of a real selection on *PRNP* (TypeBP1) is optimistic.

Genotypic frequencies were 62 % ARR/ARR at the beginning (2004) and 84 % at the end (2012), while allelic frequencies were 86 % (2004) and 99.8 % (2012). These frequencies were provided for the whole population included in the BP-CS, without distinction between HGMF and CF. However, since over 97 % of the genotypings were performed in HGMF vs. less than 3 % in CF, it was assumed that the starting frequencies could represent the situation in HGMF, and these values were therefore used as inputs of the model. The model results are roughly consistent with this observation, an extra selection rate of 0.28 giving a very good fit.

#### *Dissemination to the whole population*

The relative size of the two tiers did not allow a complete replacement of commercial rams by HGMF animals. As a consequence, there is a lag between the two tiers. The replies to the questionnaire do not provide specific information about the rate of dissemination (proportion of new rams in the CF born in HGMF).

The model was run assuming an extra selection rate of 0.28 in HGMF and, either a full replacement or half of the replacement in CF from the HGMF. Even with all these constraints, the evolution of the

ARR/ARR frequency in the CF is noticeable, but still far from establishing the R allele in this population.

## The Netherlands

### *The population*

The sheep population comprises 1 334 000 ewes, 5 % of them belonging to HGMF. The most numerous breed is the Texel, but many other breeds (67) are present.

### *The breeding programme in HGMF*

Selection for CS resistance started in 1998 on a voluntary basis, became compulsory in 2004, and continued up to 2007 when it moved to being voluntary. The general idea is that rams must be ARR/ARR, with some variability in the way this is applied from breed to breed.

Information on several questions provided in the questionnaire was lacking, and therefore it was difficult to obtain a clear picture on certain aspects of the situation in the country.

The allelic and genotypic frequencies were given for the whole population, not separating HGMF and CF tiers. An additional adjustment of the data was therefore made to estimate values for HGMF and CF. Given the global allelic frequency in 2005 (0.375) and the relative sizes of the two tiers (5 % for HGMF and 95 % for CF), the frequencies of the ARR allele in the two tiers in 2005 ( $f_{ARR}(HGMF)$  and  $f_{ARR}(CF)$ ) were chosen to obtain a model output fitting the observation in 2013, as follows:

- $0.05 \cdot f_{ARR/ARR}(HGMF) + 0.95 \cdot f_{ARR/ARR}(CF) = 0.524$ , given that:
- $0.05 \cdot f_{ARR}(HGMF) + 0.95 \cdot f_{ARR}(CF) = 0.375$  in 2005.

On average, 26 900 genotypes were obtained per year. There is no information about their repartition between HGMF and CF, or between males and females. To run the model, it was assumed that all rams produced were genotyped, meaning 42 % of genotyping for males.

The number of genotyped males allows a very strong selection of replacement rams.

The allelic and genotypic frequencies are roughly consistent with the HWE.

The model was run assuming no selection on the female path but a full selection for the male path.

### *Dissemination to the whole population*

The relative size of the two tiers only allows a full dissemination of genes from HGMF to the CF when there is no selection of rams moving to the lower tier. The model was run with this hypothesis, assuming a dissemination of ARR/ARR rams only. As the number of ARR/ARR rams in the HGMF is not sufficient to provide full replacement of rams in the CF, only a part of the replacement comes for HGMF during the first years.

## Slovenia

### *The population*

The sheep population is small (86 000 ewes), with 5 pure breeds, the most abundant being Bela Krajina Pramenka and Istrian Pramenka, and a number of crossed animals.

### *The breeding programme in HGMF*

The BP-CS started in 2005. It is mostly based on HGMF, and the CF make profit of the progresses through the dissemination of good rams. Breeders' participation is voluntary, with expenses covered

by the State. The main principle is the elimination of NSP4 and NSP5 rams, and a limited use of NSP3 rams. An auditing system controlled by official authorities exists. The rules are followed by 70-80 % of HGMF breeders and 30-40 % of CF breeders.

The number of genotyped rams is above the replacement needs, allowing a selection on *PRNP*. However, only 15 % of the genotyping effort is targeted to males, lowering its effectiveness.

The initial frequencies are not consistent with HWE. The limited sample sizes may be an explanation. The model was run with the allele frequency value, and assuming that the genotype frequencies followed HWE.

Comparing the results of the model and the data provided in the questionnaire, the selection of PrP genotypes is much lower than expected. An extra selection on other traits with a 0.50 rate was assumed for the rams.

#### *Dissemination to the whole population*

The relative size of the two tiers allowed a complete replacement of CF rams by HGMF animals. However, the dissemination rate was limited to 20 %, as indicated in the questionnaire 'Breeding programmes'. As a consequence, independently of the extra selection rate considered, the evolution in the commercial flocks is not as good as possible, reaching only 20 % ARR/ARR in 2013.

### **United Kingdom (Great Britain)**

#### *The population*

The British sheep population is very large (15.2 million animals). It includes about 100 breeds, 13 of which have a significant population size and represent 50 % of the total, and crossbred animals. The HGMF population is about 50 % of the entire population.

#### *The breeding programme in HGMF*

The BP-CS started in 2001 and stopped in 2009. It was mostly based on HGMF, i.e. pure breeds, and dissemination of males. The specificity of the British ovine industry is the large proportion of crossbred animals. As a result, the British HGMF population is extremely large (more than 7.5 million ewes). The BP-CS consisted of the *PRNP* genotyping of all males in the population, with an elimination of VRQ carriers. 80 % of the genotyping was performed on ewes, with a limited impact on the genetic progress.

Rams were classified with the NSP system and a selection favouring the most resistant classes was given as a rule.

These rules were compulsory, with an auditing system monitored by the NSP administration center.

The number of rams genotyped were above the replacement needs, allowing some selection on *PRNP*. Despite the very amount of genotyping, because only a small fraction is performed on the males the selection pressure is limited, with about half of the genotyped rams kept for replacement.

The initial frequencies are consistent with HWE. The final ARR allele and ARR/ARR genotype frequencies are consistent.

The results of the model fit well with the observed and do not suggest that selection was based on other factors.

### *The dissemination to the whole population*

The size of the HGMF population is huge compared to the CF. This is due to the definition of HGMF in the British context. No rules were in place for the dissemination (TypeDI4). This dissemination system, coupled with the hypothesis that 70 % of the CF rams came from HGMF, had a negative effect during the first years of the BP-CS, with a decrease of resistance in the CF. This is due to the fact that most of the resistant animals were selected for the HGMF replacement, leaving mostly susceptible rams for the dissemination process. The initial ARR/ARR frequency will recover only in 2016 and real progresses should be observed only after this date. It is interesting to note that, despite the cancellation of the BP-CS in 2009, the CF is still progressing in terms of favorable allele frequency.

## **B. Countries with a trend in the prevalence of Classical scrapie not statistically different from a flat one**

### **Belgium**

#### *The population*

The sheep population in Belgium consists of about 185 000 sheep (about 26 000 flocks).

#### *The breeding programme in HGMF*

The BP-CS started in 2005. It is still running and concerns only HGMF. On average, 733 genotypes were obtained per year, including both males and females. Ewes were genotyped for qualification of flocks and mating purposes. Rules for the selection and use of ARR/ARR rams exist, but on a voluntary basis, and are the basis for flock status classification. An auditing system exists, monitored by the breeders organisations and the authorities.

The genotyping programme allows some selection of the replacement males on their PrP genotype, but nearly no selection of females (VRQ elimination is marginally feasible).

The initial allelic and genotypic frequencies are inconsistent with HWE ( $0.87 \cdot 0.87 = 0.76$ , which is inconsistent with the reported value of 0.42). Previous information collected by the European Commission in 2010 indicated an earlier starting year for the BP-CS (2004), which suggests that some selection may have occurred before 2005.

The model was run assuming no selection on the female path but a full selection for the male path (selection rate 64 %, no deviation from the voluntary rules). After eight years of selection, the modelling of *PRNP* evolution reaches an end point of 100 % ARR for the young males whatever the starting point (0.42 or 0.76). An extra limited selection (rate = 0.655) adjusts the simulation to the observation.

However, the situation is polymorphic. About 60 % of the genotyping concerned the Texel breed. It may be that in some minor breeds the selection rate and rules were different, giving another final state.

#### *The dissemination to the whole population*

The size of HGMF was sufficient for a full dissemination of genes from HGMF to the CF. From the questionnaire 'Breeding programmes', in HGMF following the voluntary plan, all rams sold are ARR/ARR, and this is followed by all breeders in the major breeds. It must be concluded that only a maximum of 12.1 % of the males needed are provided to the commercial tier by the HGMF, all being ARR/ARR. The model was run assuming that only ARR/ARR rams were sold to the commercial tier, with no selection on the female path. After seven years of BP-CS, the model does not predict any change in the ARR/ARR frequency in the CF.



## Czech Republic

### *The population*

There are 25 different breeds in Czech Republic, the most important being the Suffolk (26 % of the total population), Romney Marsh (16 %) and Suvama sheep (11 %).

### *The breeding programme in HGMF*

The rules of the BP-CS are applied more strictly for the major breeds than for the minor ones.

The BP-CS started in 2003, is still running, and concerns only HGMF. The principle of the plan is to increase ARR with a selection of rams belonging to risk groups I and II, elimination of those from groups IV and V. The situation for Risk group III depends on the breed.

On average, 4 297 genotypes were obtained per year, from both males and females. Ewes were genotyped for mating purposes.

The genotyping programme allows a very strong selection of the replacement males based on their *PRNP* genotype, and a weak selection of females (which is not included in the model).

The initial allelic and genotypic frequencies are consistent with HWE. This is not the case for the final frequencies.

The model was run assuming no selection on the female path but a full selection for the male path (selection rate 13 %, no deviation from the voluntary rules). After 11 years of selection, the modeling of PrP evolution reaches an end point of nearly 100 % ARR and 84 % ARR/ARR. This is very optimistic as compared to the sampling result, suggesting that selection on traits is independent from selection on *PRNP* occurring after genotyping. The observed evolution only fits the modelled one when an extra strong selection rate of 0.184 is applied to the rams.

### *The dissemination to the whole population*

The size of HGMF was sufficient for a full dissemination of genes from HGMF to the CF. From the replies to the questionnaire 'Breeding programmes', in HGMF following the compulsory plan, rams sold should belong to Risk groups I and II with exception for group III depending on the breed. This suggests that the required number of rams could come from HGMF to the CF. The model was run with this hypothesis. The number of rams available for dissemination to the commercial tier being very large, the evolution in this subpopulation is not far from the evolution in the HGMF.

## Italy

### *The population*

The sheep population in Italy comprises 7.3 million ewes, in about 95 000 flocks, with about 10 different breeds participating in the BP-CS. The HGMF population is about 7 % of the entire population.

### *The breeding programme in HGMF*

The BP-CS started in 2005. It is not based on HGMF, and all flocks may be involved in the scheme. The BP-CS for the Sarda breed in Sardinia is an exception (see Appendix E). Rams and flocks are classified in categories: classes I (highest genetic resistance) to class IV (lowest genetic resistance) for rams, and level I (highest genetic resistance) to level IV (lowest genetic resistance) for flocks.

The BP-CS is based on rules defining the type of rams to be used, depending on the initial ARR frequency of the breed.

A maximum of a third of males used for replacement were genotyped (assuming no selection of these males). When selection occurs, a case expected in the BP-CS, this proportion decreases. Considering this point and the description provided, the BP-CS was classified as TypeBP2.

The Initial ARR frequency was given as  $f_{ARR} = 0.41$  at the global level in the first questionnaire, with some variability between breed (from 0.13 in Biellese to 0.56 in Merinizatta). No information was provided about the final frequencies.

The model slightly overestimated the effect of nine years of selection, an extra selection rate of 90 % for other traits giving the correct adjustment.

The expected improvement in the frequency of resistant alleles after eight years of selection is still very limited.

#### *The dissemination to the whole population*

The relative size of the two tiers did not allow a complete replacement of CF rams by HGMF animals, and a very small fraction of replacement rams are genotyped. From the qualitative analysis it seems that poor dissemination would make the BP-CS effective only in the longer term, and no positive results would be expected to be visible at present. However, the impact of genotyping in CF was not modelled.

### **Slovakia**

#### *The population*

The sheep population (417 000 ewes) comprises 34 breeds, 4 of them (Zoslachtena valaska, Cigaja, Lacaun and Merino) representing more than 80 % of the total. The HGMF population is about 6.5 % of the entire population.

#### *The breeding programme in HGMF*

The BP-CS started in 2004, and is based on HGMF. Compulsory rules, with an official auditing system, define the structure of the BP-CS. Genotypes are classified in groups based on the resistance to CS, and range from Group I (highest resistance) to Group V (lowest resistance). The BP-CS includes detailed rules for the selection and use of rams. The number of genotyped rams is far above the replacement needs, allowing a strong selection based on *PRNP*.

The initial frequencies are consistent with HWE; however the final ARR allele and ARR/ARR genotype frequencies are not consistent with HWE.

The results of the model indicates a strong selection for other factors. An extra selection rate of 0.18 gives a good fit for the observed evolution of resistance.

#### *The dissemination to the whole population*

The relative size of the two tiers did not allow a complete replacement of CF rams by HGMF animals. The dissemination type was considered to be TypeDI2. Applying these hypotheses, the modelled ARR/ARR frequency reached about 32 % in 2013.

### **Spain**

#### *The population*

The Spanish sheep population is very large (16.6 million animals in 112 000 flocks), with 50 different breeds.

#### *The breeding programme in HGMF*

The BP-CS started in 2003. Initially compulsory, it has become voluntary since 2013. It is mostly based on HGMF. A specificity of the programme is that a very high proportion of the genotyping effort is applied to females (91.25 %). Nevertheless, the BP-CS includes rules for the selection and use of rams, with a classification into three groups, depending on the genotypes, and ranging from Group I (highest resistance) to Group III (lowest resistance).

These rules are compulsory rules, but their application is left to breeding associations, without any official auditing system.

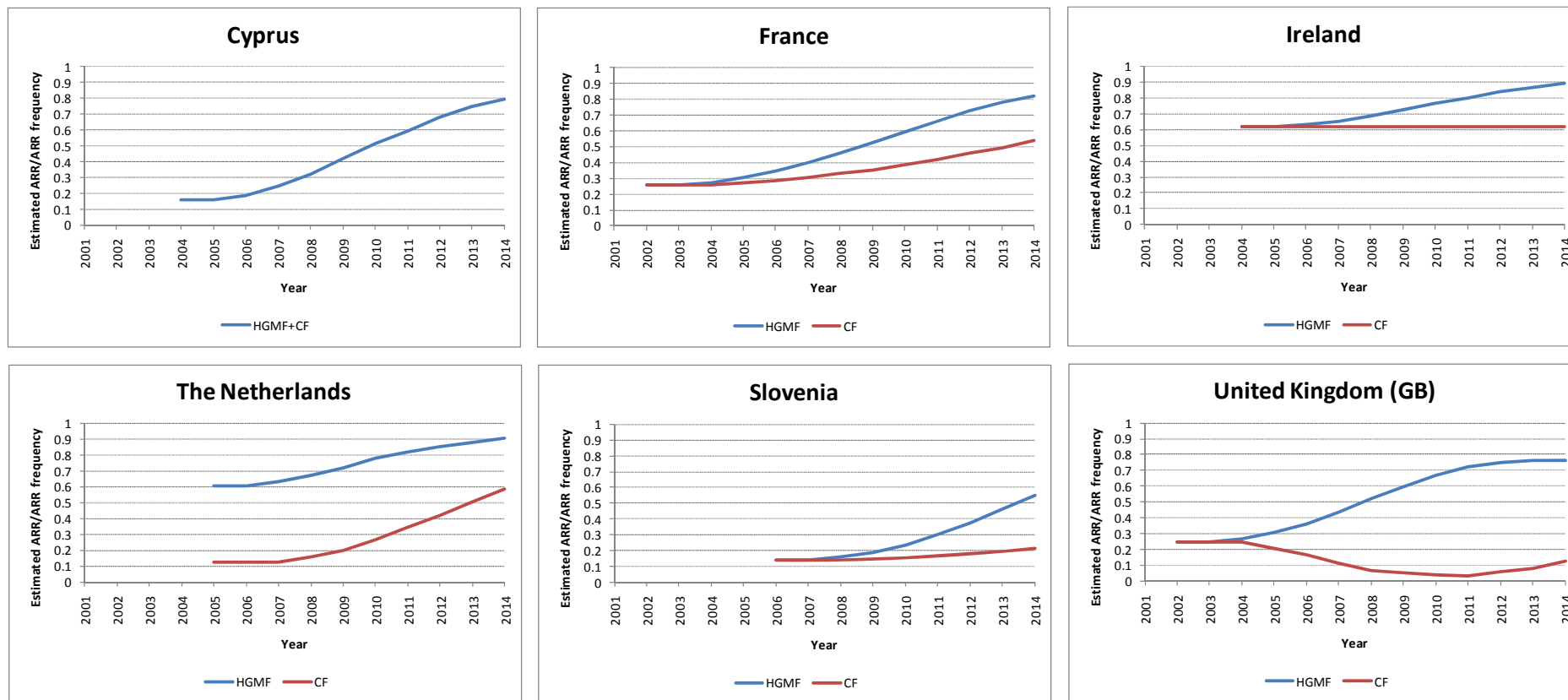
The number of rams genotyped is above the replacement needs allowing some selection on *PRNP*. Despite the very extensive genotyping programme, the selection pressure is rather low (0.636) because only a small fraction is performed on the males.

The initial frequencies are consistent with HWE. The final ARR allele and ARR/ARR genotype frequencies are also consistent.

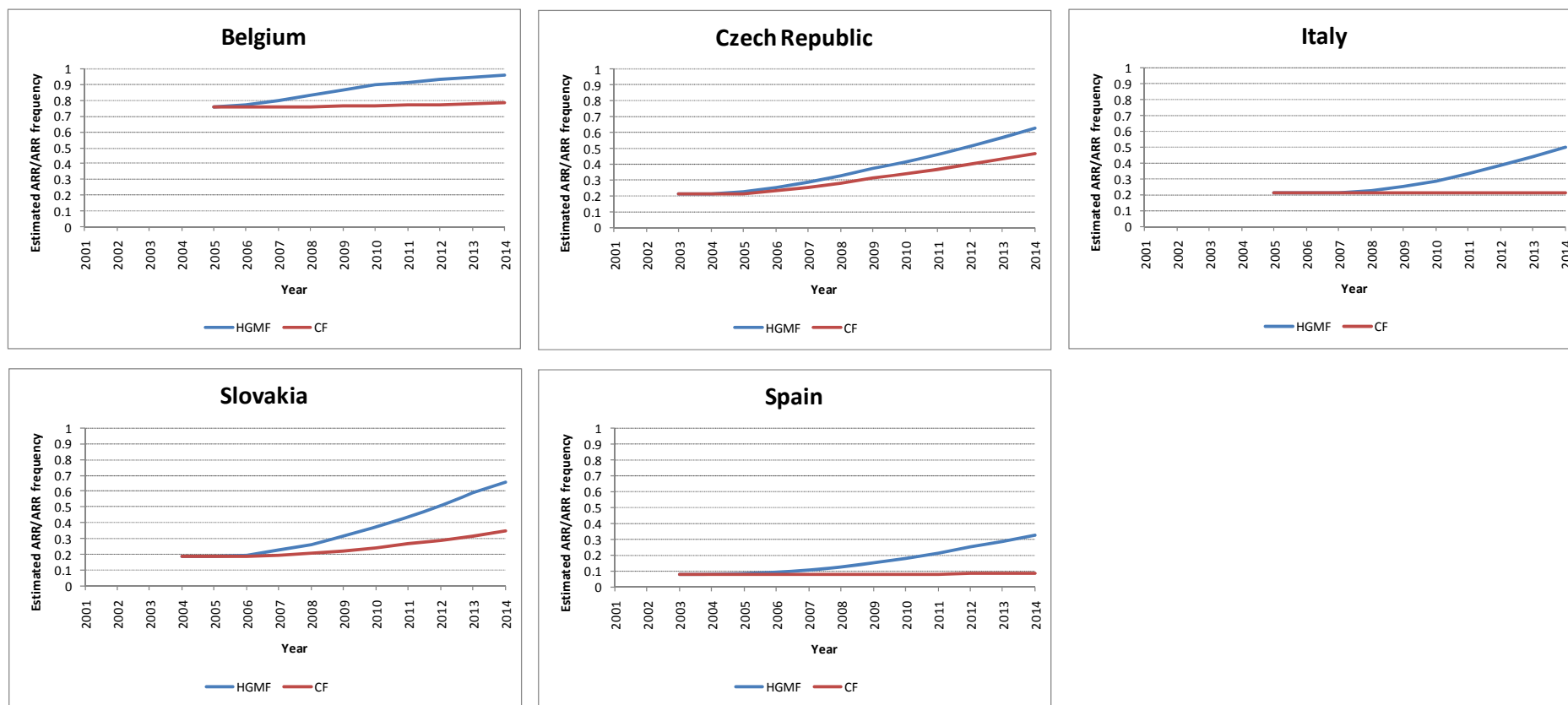
The results of the model fit well with the observed data (extra selection rate of 0.9 on production traits), showing that the effects of the BP-CS are only now starting to be visible.

#### *The dissemination to the whole population*

The relative size of the two tiers allowed a complete replacement of CF rams by HGMF animals. However, only a very small fraction are genotyped, and, considering the rules of dissemination, this TypeDI2 dissemination system is not efficient in terms of *PRNP* genotype evolution.



**Figure 18:** Evolution of the ARR/ARR frequency in HGMF and CF as estimated by the deterministic model (countries with a statistically significant decreasing trend of CS). For The Netherlands, the starting frequency for HGMF and CF is different, since an ad hoc adjustment of the model was needed to estimate those values from the available frequency value, related to the whole population (see text for details).



**Figure 19:** Evolution of the ARR/ARR frequency in HGMF and CF as estimated by the deterministic model (countries with a trend of CS not statistically different from a flat one).

## ABBREVIATIONS

### Member States of the European Union

AT	Austria
BE	Belgium
BG	Bulgaria
CY	Cyprus
CZ	Czech Republic
DE	Germany
DK	Denmark
EE	Estonia
EL	Greece
ES	Spain
FI	Finland
FR	France
HR	Croatia
HU	Hungary
IE	Ireland
IT	Italy
LT	Lithuania
LU	Luxembourg
LV	Latvia
MT	Malta
NL	Netherlands
PL	Poland
PT	Portugal
RO	Romania
SE	Sweden
SI	Slovenia
SK	Slovakia
UK	United Kingdom