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SCIENTIFIC OPINION

Scientific Opinion on genetic TSE resistance in goats in all European Union Member States[1]

EFSA Panel on Biological Hazards (BIOHAZ)[2, 3]

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ABSTRACT

This scientific opinion of the EFSA Panel on Biological Hazards (BIOHAZ) assesses the use in goats of breeding for genetic resistance to TSEs as a tool for the control of the different TSEs these ruminants can naturally host (i.e. Classical scrapie, Atypical scrapie and BSE) in all EU Member States (MSs), with the exception of Classical scrapie in Cyprus as this has been addressed in a previous scientific opinion. To carry out this task, currently available scientific knowledge on the subject is reviewed in this opinion, with particular emphasis on the candidate polymorphisms and the different TSE agents considered. Also, a preliminary evaluation of the availability in the EU MSs of those logistical and technical elements needed for a large-scale breeding program is presented based on the replies received to a questionnaire sent to the EFSA BSE-TSE Network. The BIOHAZ panel identified at least three polymorphisms of interest, enabling the conclusion that there are encouraging but as yet incomplete data to consider supporting a breeding programme in goats for genetic resistance to Classical scrapie. Moreover, the effects of these polymorphisms on resistance in goats to Atypical scrapie and BSE are insufficiently known. Furthermore, it seems that (at present) only a few EU MSs have in place the necessary elements to introduce this kind of breeding programme. Further detailed conclusions and recommendations are made, aiming at both addressing particular issues that have to be taken into account in a breeding programme for genetic TSE resistance in goats and on the particular data needs that would increase the confidence in the successful outcome of such breeding programme. Some of the key elements in this respect are the real protection (i.e. either to disease or to infection) provided by the candidate polymorphisms for the different TSEs, possible adverse effects and epidemiological considerations.

KEY WORDS

Goat, TSE, Classical scrapie, Atypical scrapie, BSE, genetic resistance, breeding programme, European Union.

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SUMMARY

Following a request from the European Commission, the Panel on Biological Hazards (BIOHAZ) was asked to deliver a scientific opinion on genetic resistance to Transmissible Spongiform Encephalopathies (TSE) in goats.

For a first part of that request, the BIOHAZ Panel adopted on 5th March 2009 a scientific opinion on the scientific validity of a study carried out by the Cypriot authorities under the auspices of the Community Reference Laboratory (CRL) for TSEs. That scientific opinion also indicated to what extent the information contained in the study could be used as relevant tools to control Classical scrapie in Cyprus.

In the current scientific opinion the BIOHAZ Panel addresses genetic resistance as a relevant tool for breeding for resistance to all TSEs of goats (including Atypical scrapie and BSE) in all the Member States (MSs) (except for Classical scrapie in Cyprus).

To carry out this task, available scientific knowledge on genetic TSE resistance in goats in the EU is reviewed, addressing those *PRNP* polymorphisms for which a capacity to provide resistance to TSEs in goats has been (or is being) investigated. Details tailored to the different TSEs found in this small ruminants (*i.e.* Classical scrapie, Atypical scrapie and BSE) are also considered and presented.

Further on, the feasibility of a large-scale breeding program in animal populations would need to be supported by a sound logistical and technical infrastructure in any given territory. In order to collect preliminary data that could help to evaluate the specific situation in the different EU MSs, a questionnaire was developed and circulated among the EFSA BSE-TSE Network. The results of the analysis of the replies received are also presented herewith.

The BIOHAZ Panel concluded that there are encouraging but as yet incomplete data to consider supporting a breeding programme for resistance in goats against Classical scrapie in all EU MSs, and ongoing studies are expected to provide a more robust scientific background in the coming years. On the other hand, at this moment there are not enough data available to consider supporting a breeding programme for resistance against Atypical scrapie and BSE in goats in all EU MSs. Experiments are ongoing on BSE in goats and results will be available in the next years. Furthermore, there are limited data suggesting that an allele (H154) might confer resistance to Classical scrapie but increase susceptibility to Atypical scrapie.

The frequency of the wild type allele, which is known to confer susceptibility to Classical scrapie, is high in all goat breeds considered. Thus, selection for putative resistance alleles will be slow, complicated and highly dependent on breeding structure.

It is acknowledged that any large scale breeding programme for TSE resistance in goats must take into consideration key elements related to the current dissemination of potentially TSE protective polymorphisms in the goat population of each EU MS and the characterisation of the real protection provided by those polymorphisms. At present, only a few EU MSs seem to have in place the necessary elements to introduce a breeding for resistance programme for Classical scrapie in goats.

The BIOHAZ Panel makes a series of recommendations on new investigations in order to assess the efficacy of breeding for the candidate *PRNP* alleles as a mean to control TSEs in goats. Furthermore, research on the possible adverse effects of the candidate *PRNP* polymorphisms on other production traits should be encouraged. In addition, it is recommended that a breeding for resistance programme for TSE in goats is first implemented in the seven EU MSs with the largest goat population as this would have the most impact.

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BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

It is scientifically recognised since several years 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). This association has led to the development at European Union (EU) level of breeding programmes based on the selection of animals known to be genetically resistant to TSE and to the implementation of eradication measures in TSE infected flocks based on a selective elimination of genetically susceptible animals. The appropriateness of these measures has been confirmed in the EFSA opinion on the breeding programme for TSE resistance in sheep[4].

In goats, the association of genetic variability of *PRNP* with resistance or susceptibility to TSE, and in particular to Classical scrapie, remains unknown. However, the Cypriot authorities have recently sent to DG SANCO the final results of a Commission funded pilot project study conducted in Cyprus and which has been just submitted for publication. These results indicate that polymorphisms of the *PRNP* gene at codons 146 and 154 could be associated with resistance/susceptibility to Classical scrapie in goats in Cyprus. If confirmed, these results could be very interesting in view of a possible future EU policy as regards scrapie control measures in goats.

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

Following consultation with the EU Commission Services, the original terms of reference were amended and split into two different parts, addressed by two different Scientific Opinions as follows:

- EFSA-Q-2008-744 (adopted on 5 March 2009), in which EFSA was requested to provide an opinion on the scientific validity of a study carried out by the Cypriot authorities under the auspices of the Community Reference laboratory (CRL) and to indicate to what extent the information contained in this study can be used as relevant tools to control Classical scrapie in Cyprus.

- The second and current part of the mandate asks EFSA to consider this information in the study and any other available information as basis for a relevant tool to control all TSE agents in goats in all EU Member States. This second EFSA opinion thus addresses genetic resistance as a relevant tool for breeding for resistance to all TSEs of goats (including Atypical scrapie and BSE) in all the EU Member States (MSs) (except for Classical scrapie in Cyprus).

[4] The EFSA Journal (2006) 382, 1-46

ASSESSMENT

1. Introduction

This EFSA Scientific Opinion addresses genetic resistance as a relevant tool for breeding for resistance to all TSEs of goats (including Atypical scrapie and BSE) in all the EU MSs (except for Classical scrapie in Cyprus). It follows the original request made by the European Commission to EFSA in December 2008, and the publication of a first Opinion that dealt with genetic resistance to Classical scrapie in goats in Cyprus (EFSA Panel on Biological Hazards, 2009).

In the current Scientific Opinion, available scientific knowledge on genetic TSE resistance in goats in the EU is reviewed, addressing those *PRNP* polymorphisms for which a capacity to provide resistance to TSEs in goats has been (or is being) investigated. Details tailored to the different TSEs found in this small ruminant (*i.e.* Classical scrapie, Atypical scrapie and BSE) are also considered and presented.

Further on, the feasibility of a large-scale breeding program in animal populations would need to be supported by a sound logistical and technical infrastructure in any given territory. In order to collect preliminary data that could help to evaluate the specific situation in the different EU MSs, a questionnaire was developed and circulated among the EFSA BSE-TSE Network. The results of the analysis of the replies received are also presented herewith.

2. Aspects needed to assess the feasibility for breeding for resistance against TSEs in goats in the EU

Several aspects would need to be considered when assessing the feasibility of breeding for resistance against TSEs in goats in the EU.

1. Demonstration and measurement of resistance levels associated with the identified goat *PRNP* polymorphism towards Classical scrapie, BSE and Atypical scrapie.

2. Assessment of the effect of the identified goat *PRNP* polymorphism on pathogenesis and TSE agent distribution in the organism.

3. Assessment of the epidemiological role of different genotypes in the maintenance of infection under natural conditions.

4. Evaluation of other aspects related to breeding for resistance:

    - Level of the identified polymorphism in the goat population in Member States.
    - Possible adverse effects on other production traits.
    - Structure of breeding systems for goats in Member States.

3. Historical knowledge of TSE in goats

Scrapie is a neurodegenerative prion disease affecting sheep and goats that invariably leads to the death of affected animals. Like other prion diseases, it is linked with accumulation of PrP^{Sc}, the pathological isoform of the cellular protein PrP^{C}, mainly in the central nervous system (Oesch et al., 1985; Prusiner, 1998).

The first observations of natural scrapie in sheep date back to 1732 in England and to 1759 in Germany (Laplanche et al., 1999). In the following centuries, scrapie endemically affected flocks in several countries. Goat scrapie was reported for the first time in a goat living in a sheep flock where the disease had prevailed for several years (Chelle, 1942). Since then, clinical scrapie cases in goats have been recorded throughout Europe and in other regions of the world. Although scrapie in goats is often found in mixed herds with sheep, it has also been observed to spread also from goat to goat (Hourrigan et al., 1979).

The first experimental challenges of goats with sheep scrapie showed 100% susceptibility and suggested that goats were highly susceptible to scrapie (Pattison et al., 1959, and Cuillé and Chelle, 1969). This contrasted with the results of similar experiments in sheep where survivors were regularly observed (Gordon et al., 1966).

Since 1942, when the first case of natural goat scrapie was reported, clinical scrapie cases in goats have been recorded throughout Europe and in other regions of the world (Hourrigan et al., 1969; Stemshorn et al, 1975; Toumazos et al., 1988; Wood et al., 1992; Leontides et al., 2000; Thuring et al., 2004; Seuberlich et al., 2007) albeit with a significantly lower incidence than seen with ovine scrapie. Thus, in the EU, very few cases of goat scrapie have been recorded in any member country prior to 2002. However, since 2002, when the EU's active TSE surveillance programme commenced, more than 1.14 million EU goats have been tested and over 3,000 scrapie cases have been detected in nine EU MSs (European Commission, 2009).

4. TSE Agents diversity in goats

There are overwhelming evidences that different TSE Agents circulate in small ruminant populations in Europe (Bruce et al., 2002; Beringue et al., 2008). The extent of such variability in sheep has been a matter of intense investigations in recent years but a conclusive picture is not yet available. The situation in goats is even less understood than in sheep.

The variability of the clinical phenotype of scrapie, possibly reflecting the involvement of different strains, was described for the first time in goats by Pattison and Millson in 1961. The transmission of a pool of scrapie infected sheep brains (SSBP/1) to goats resulted in two clinical disease phenotypes – either a 'scratching' or a 'nervous' syndrome – that were conserved on sub-passage.

Very few studies have been published dealing with the characterisation of the agents involved in naturally-occurring goat TSEs. Nevertheless, the same broad operational categories of TSE agents described in sheep have been observed in goats, namely: Classical scrapie and Atypical scrapie (European Food Safety Authority Panel on Biological Hazards, 2006). These encompass the vast majority of isolates reported in goats in Europe. However, two important cases of naturally-occurring BSE have also been detected in goats (Eloit, 2005; Jeffrey et al., 2006; CRL, 2008a).

An EU project 'goatBSE' (FOOD-CT-2006-36353[5]) dealing with goat TSEs aims to investigate – among other things – the variability of prion strains in goats and their geographical distribution in

[5] Further information available at: http://www.goatbse.eu/

Europe. That part of the project will use established strain typing methods in Classical mouse lines as well as (newly generated) transgenic mouse lines and biochemical assays.

Classical scrapie

Concerning typing studies by bioassay in rodent models, only limited information on the characteristics of goat isolates is available and that is dispersed in papers dealing with genetics and typing of sheep isolates.

In particular, the study of Vaccari et al., 2006, reports that all scrapie cases investigated showed the same profile of pK-resistant PrP^{Sc}, different from BSE and indistinguishable from that reported in a number of other Italian sheep scrapie cases (Nonno et al., 2003). The study also mentions unpublished results of strain typing in rodent models which confirmed the similarity of the scrapie isolate involved in that goat outbreak with isolates from other Italian sheep and goat outbreaks. The transmission characteristics of a scrapie isolate from an Italian goat to bank voles, C57Bl mice and hamsters were reported by Piening et al. in 2006, and appear to be very similar to those observed with an Italian sheep isolate (Agrimi et al, 2008). They both transmitted with short survival time (~ 190 days post infection) to bank voles but very inefficiently or not at all to wild type mice.

As far as studies in the natural host are concerned, Sofianidis et al. (2006) reported that histopathological and immunohistochemical examinations of several goats from two Greek scrapie-affected herds showed diverse lesion profiles and PrP^{Sc} distribution patterns. The authors suggest that this variability might be influenced by PrP genotype, age and clinical stage and that further studies are needed to investigate the possible role of the strain. Another study from Sofianidis et al. (2008) describes asymptomatic goats from scrapie-affected herds in which PrPSc deposition was mainly confined to cortex and rostral brain areas. PrP^{Sc} was found by ELISA at the level of the obex only in a limited number of animals and was never associated with immunoreactivity in the dorsal vagal nucleus. Unfortunately, no Western blot analysis is available and it cannot be concluded whether this pattern is just the result of the early clinical stage or is the mark of an unusual scrapie type.

Although a formal strain comparison between sheep and goat isolates has not been carried out, four categories of isolates are distinguishable in both sheep and goats through bioassay in tg338 transgenic mice expressing the VRQ sheep allele (Beringue et al., 2008). However, whether these categories are equivalent in sheep and goats is not yet known.

Finally, molecular analyses of goat isolates from France, UK and Cyprus suggest that the recently recognised ovine “CH1641-like” isolates (Baron et al., 2008), are also present in goats (CRL, 2008b).

Atypical scrapie

Following its recognition in sheep, Atypical scrapie was detected in goats, as well. Specifically, cases of Atypical caprine scrapie have been described in Switzerland (Nentwig et al., 2007), France (Le Dur et al., 2005 ; Arsac et al., 2007), Spain (European Commission, 2004) and Italy (Colussi et al., 2008).

Although the information on Atypical goat scrapie is limited, there is already clear evidence of similarities with the distinctive features observed in sheep. In particular, the molecular pattern of pK-resistant PrP^{Sc} obtained in most goat cases, as well as the very frequent involvement of animals carrying the AHQ allele, is very similar to that observed in Nor98 in sheep (Moum et al., 2005). In a single Atypical case reported in a goat in Switzerland, differences in the distribution of histopathological lesions and PrP^{Sc} deposition compared to Atypical sheep scrapie were described but the meaning of such differences is unknown (Seuberlich et al., 2007). As far as biological characterisation is concerned, a single Atypical scrapie isolate from a French goat transmitted to Tg338 mice with the same features as sheep Nor98 (Le Dur et al., 2005).

BSE

One confirmed case of natural goat BSE was discovered in France in 2002 through active surveillance (Eloit et al., 2005). A retrospective examination of early TSE cases in the UK using a more sensitive diagnostic test has revealed a second possible natural case of goat BSE that occurred in Scotland in 1990 (Jeffrey et al., 2006) (CRL, 2008a).

Regarding TSE Agents diversity in goats, it can be concluded that:

- In field cases Classical scrapie, Atypical scrapie and BSE have been identified in goats.
- Diversity of Classical scrapie in goats is currently under investigation through an EU funded project.
- The current ability to fully describe and identify TSE Agent diversity in goats, as in sheep and cattle, remains limited.

5. *PRNP* polymorphism and susceptibly to TSE infection in goats

5.1. Variability of the goat *PRNP* gene

The PrP^C gene (*PRNP)*, which encodes the PrP^C protein, is highly polymorphic in both sheep and goats.

To date, 27 polymorphic positions resulting in non-synonymous single-nucleotide polymorphisms (SNPs) of the caprine PRNP have been reported in different breeds (Goldmann et al., 1996 and 1998; Billins et al, 2002; Agrimi et al., 2003; Goldmann et al, 2004; Zhang et al., 2004; Kurosaki et al., 2005; Acutis et al., 2006 and 2008; Papasavva-Stylianou et al., 2007; Zhou et al., 2008, Serrano et al., 2009; Vaccari et al., 2009). In addition, in 1998 Goldmann et al. described a smaller version of the *PRNP*, found in Siberian goats that encodes a protein variant encompassing only 3 octapeptide repeats at the N-terminus.

PRNP variations in goats from European and non-European countries are reported below in Table 1.

Table 1: Reported variations of the goat *PRNP* gene (silent mutations in bold with italics).

Polymorphism*	EU countries reported from	Non-EU countries reported from	First reference
W18R	ES		Vaccari et al., 2009
V21A	GR		Billinis et al., 2002
L23P	GR		Billinis et al., 2002
G37V	IT	MO	Agrimi et al., 2003
P42P	UK, IT, FR, GR	US, CN, JP, PK	Goldmann et al., 1996
G49S	GR		Billinis et al., 2002
Q101R	UK	MO	Vaccari et al., 2009
Q101Q	NL		Vaccari et al., 2009
W102G	UK	CN,JP	Goldmann et al., 1998
K107K	GR		Billinis et al., 2002
T110P	IT		Agrimi et al., 2003
V125V		CN	Zhou et al., 2008
G127S	IT, UK, ES, FR	US, CN, JP, MO	Zhang et al., 2004
L133Q	IT		Acutis et al., 2006
M137I	IT	MO	Acutis et al., 2006
S138S	UK, IT, FR, CY, GR	US, CN, JP, PK	Goldmann et al., 1996
R139S		MO	Serrano et al., 2009
I142M	UK, FR, ES	US, JP, MA	Goldmann et al., 1996
I142T	IT		Acutis et al., 2008
I142I	NL		Vaccari et al., 2009
H143R	UK, IT, GR, NL	US, CN JP	Goldmann et al., 1996
G145D		MO	Serrano et al., 2009
N146D	CY		Papasavva-Stylianou et al., 2007
N146S	CY, UK	US, CN, JP	Zhang et al., 2004 Kurosaki et al., 2005
R151H	CY		Papasavva-Stylianou et al., 2007
R154H	GR, IT, ES, CY, FR	US, CN, MO	Billinis et al., 2002
P168Q	IT, GR, CY		Billinis et al., 2002
V179V	CY		Papasavva-Stylianou et al., 2007
D181D	CY		Papasavva-Stylianou et al., 2007
T194P	IT		Acutis et al., 2008
F201F	ES		Vaccari et al., 2009
T202T	IT		Acutis et al., 2006
K207K	GR		Billinis et al., 2002
R211Q	UK, FR, ES	US, CN, JP	Wopfner et al., 1999
R211G		CN	Zhou et al., 2008
I218L		CN	Zhang et al., 2004
T219I	ES	CN	Zhou et al., 2008
T219T	IT		Vaccari et al., 2006
Q220H	FR, CY, GR		Billinis et al., 2002
Q222K	IT, FR, UK, ES	US, CN, MO	Agrimi et al., 2003
Q222Q	NL		Vaccari et al., 2009
G232W	ES		Vaccari et al., 2009
G232G	IT		Vaccari et al., 2006
S240P	IT, FR, UK, GR, ES,	US,CN, JP, PK, MO	Goldmann et al., 1996

* *PRNP* polymorphisms are indicated using the format: the wild-type aminoacid, the codon umber and the variant observed.
CN=China; CY= Cyprus; FR= France; UK=United Kingdom; GR=Greece; JP=Japan; IT=Italy; MO=Morocco; NL=The Netherlands; PK=Pakistan; ES=Spain; US=United States of America.

The most common polymorphism is S240P, which leads to the presence in goat populations of two highly frequent haplotypes: one with P240 and one with S240, the latter being identical in amino acid sequence to the ovine *wild-type* (ARQ) *PRNP*. The other alleles seem to have arisen by mutations on the background of these two central haplotypes, being generally related to one of them by a single amino acid substitution. Some exceptions are given by mutations M142 and H154, which have been found in linkage with both S240 and P240 (Goldmann et al., 1998; White et al., 2008; Billinis et al., 2002; Acutis et al., 2006). Furthermore two haplotypes with a double mutation (V37K222 and H154K222) have been reported (Vaccari et al., 2006; Colussi et al., 2008). These last alleles might derive from recombination events.

5.2. Susceptibility/resistance to disease and infection

scrapie association studies in goats are far more limited than those in sheep. Data available in goats have mainly resulted from case control field studies carried out in field infected flocks. These usually involved only a limited number of animals (in particular positive animals), and flocks (limited range of TSE agents). A detailed review of both experimental and case control studies reporting on these is included in a table in Appendix A.

In sheep, a strong, demonstrated association of scrapie susceptibility with certain polymorphisms in the *PRNP* constitutes the basis for selective breeding strategies directed towards controlling the disease in ovine populations. At present, genetic associations between caprine PrP^{C} and scrapie are not as clear. However, several authors have studied the association of polymorphisms at the *PRNP* with Classical scrapie susceptibility in goats.

Case studies in Italy and France involving a variety of goat breeds have demonstrated a protective role for allele K222 (Acutis et al., 2006; Vaccari et al., 2006; Barillet et al., 2009) against Classical scrapie. Similarly, the substitution of glutamine for arginine at codon 211 has been associated with an increase in resistance to Classical scrapie in French Alpine and Saanen goats (Barillet et al., 2009). Allele H154 has been associated with limited resistance to naturally occurring Classical goat scrapie in case studies from Greece, France, Italy and Cyprus (Billinis et al., 2002). The presence of allele M142 has been linked with increased disease incubation time in goats experimentally challenged with Classical scrapie or BSE (Goldmann et al., 1996). In a French field study the haplotype M142 P240 seemed associated with an increased scrapie resistance (Barillet et al., 2009).

A pilot project study conducted in Cyprus investigated if polymorphisms of the *PRNP* gene at codons 146 and 154 could be associated with resistance/susceptibility to Classical scrapie in goats in Cyprus. This report was evaluated in a recent EFSA Scientific Opinion on genetic TSE resistance in goats in Cyprus (EFSA Panel on Biological Hazards, 2009). Among other findings, that EFSA Scientific Opinion concluded that the observed resistance to disease could not be extended to infer an association between H154, D146 and S146 and resistance to infection, nor could it be extrapolated to cover resistance to other types of goat TSEs such as BSE or Atypical scrapie. It has to be mentioned that H154 has been associated with some increased susceptibility to Atypical scrapie (Colussi et al., 2008).

Similar data for a genetic association to Atypical scrapie in goats is scarce. However, recent work analyzing *PRNP* genotypes associated with "Atypical" goat scrapie clearly suggests an influence of the AHQ allele in affected goats (Colussi et al., 2008).

With the exception of the studies from Vaccari et al. (2006) and from Barillet et al. (2009) in which diagnostic investigations in both nervous and lymphoid tissues were carried out, in most case control studies the investigated event of interest was the number of clinical scrapie cases or the presence of abnormal PrP^{Sc} in the obex of the studied animals at culling. Due to individual particularities in the TSE pathogenesis (*i.e.* long incubation period, late neuro-invasion), such information is not sufficient for assessing the infectious status of the investigated individuals. Consequently, rather than truly

measuring the susceptibility / resistance to TSE infection that is associated with a particular *PRNP* allele or genotype, those studies only provide a measurement of their protective effect on the clinical incidence of the disease within the context of the flock (limited life time of individuals in the flocks plus the consideration of variable infectious pressure).

Studies on the molecular level underlying host PrP^C conversion have shown that *in vitro* methods can be an invaluable tool in assessing some (if not all) of the *PRNP* variants association with TSE development. It has been demonstrated by conversion studies performed for sheep (Bossers et al., 1997 and 2000; Kirby et al., 2006; and Eiden et al., 2006) and deer (Raymond et al., 2000) that conversion efficiency differences of various ovine based *PRNP* variants correlated well with observed differences of these variants with susceptibility and transmissibility *in vivo*. These conversions tools, and variations thereof, are also currently used in the context of the EU 'goatBSE' project to assess the potential association of goat variants with differences in susceptibility to various goat scrapie and cattle BSE isolates. These studies aim to try to assess the potential association of various goat *PRNP* variants with scrapie and BSE susceptibility.

Despite the encouraging results, there is still insufficient evidence that these in vitro tools are able to unambiguously establish resistance or susceptibility to TSE infection.

In conclusion and within these methodological limitations, some caprine *PRNP* alleles have been reported to be associated with increased resistance to Classical scrapie.

5.3. Concluding remarks on TSE Agents diversity and resistance/susceptibility associated to the different *PRNP* alleles

TSE agents responsible for Classical scrapie in small ruminants represent a mosaic of infectious agents harbouring distinct biological properties as mentioned in a previous EFSA Scientific Opinion on certain aspects related to TSEs in small ruminants (EFSA, 2007). Amongst the biological properties of TSE Agents, the ability to develop and to transmit disease in individuals harbouring different *PRNP* polymorphisms has been established (EFSA Panel on Biological Hazards, 2006).

For instance, while in sheep the ARQ variant is considered to be fairly susceptible to TSE in general, some particular Classical scrapie isolates (like SSBP-1) seem unable to propagate in ARQ/ARQ animals (Goldmann et al., 1994). Conversely, the AHQ allele seems to provide a certain level of resistance towards some TSE agents but is associated with fair susceptibility to experimental BSE (Foster et al., 2001) and increased risk for the development of Atypical scrapie in both sheep (Moum et al., 2005) and goats (Colussi et al., 2008).

To date there is only very limited information on the potential diversity of TSE agents circulating in sheep and goat populations within EU MSs.

This paucity of information is a clear limitation for evaluating the interest of the different alleles in the control of TSEs in the EU MSs goat population.

In order to clarify this point, several types of investigations would be helpful:

- Rational characterisation (using biochemical tools and bioassay) of EU MSs TSE isolates;
- Experimental transmission of a panel of TSE isolates (including BSE and Atypical scrapie) to goats or another animal model (transgenic mouse model) harbouring the alleles of interest;
- In vitro conversion assays using a panel of TSE isolates.

In conclusion:

- There is a large diversity of alleles in the goat population.
- There are some alleles that have been identified as possible candidates for selection in a breeding for resistance programme to Classical scrapie in goats (S146, D146, Q211, R211, K222 and H154).
- For the case of BSE in goats there are no similar data available.
- For Atypical scrapie in goats, no resistance-associated polymorphisms have been identified but H154 may be a risk factor coding for susceptibility.

6. Pathogenesis linked to polymorphism in goats

Most of the data related to TSE in small ruminants were generated in ovine populations, and publications on TSE in goats remain sparse (Hadlow, 1961; Pattison & Millson, 1961a; Stemshorn, 1975, Wood and Done, 1992; Wood et al., 1992; Foster et al., 1993).

Different scrapie isolates, mink transmissible encephalopathy and BSE, were experimentally transmitted to goats. These experiments confirmed that goats are susceptible to BSE and scrapie Agents, but due to their experimental design those studies are non informative in term of pathogenesis.

Following the implementation of active surveillance measures in small ruminants, an increase of TSE cases was recorded in the EU goat population. As a consequence, several projects aiming at clarifying TSE pathogenesis in that particular species were undertaken both in naturally infected and in experimentally challenged animals.

6.1. Natural infection with Classical scrapie

In France, investigations were carried out in two highly affected herds (Project: UMR INRA1225 - AFSSA niort).

In a first herd 19 animals belonging to different age cohorts were selected on the basis of a positive PrP^{Sc} tonsil biopsy. These animals (and appropriate control) were culled and a large panel of tissues sampled for PrP^{Sc} detection by immunohistochemistry.

The results indicated that under the condition of natural exposure the contamination occurred through the Gut Associated Lymphoid Tissues (GALT) before PrP^{Sc} dissemination to the other secondary lymphoid organs. PrP^{Sc} entry into the nervous system occurs at gut level through the autonomic enteric system. Central nervous system invasion occurs at both the obex and thoracic spinal cord level through the autonomic nerves roots. Detailed results are presented in Appendix A. This general PrP^{Sc} dissemination scheme is identical to that described in sheep naturally infected with the Classical scrapie agent (Andreoletti et al., 2000; Van Keulen et al., 2002).

In a second herd, all the animals (442 animals) were submitted to clinical examination and sampled at culling for several lymphoid tissue (tonsil, spleen) and obex. Immunohistochemical and ELISA PrP^{Sc} detection were performed on all samples. In 81 goats, PrP^{Sc} was detected in at least one tissue and birth date was available. Detailed results are presented in Figure 1.

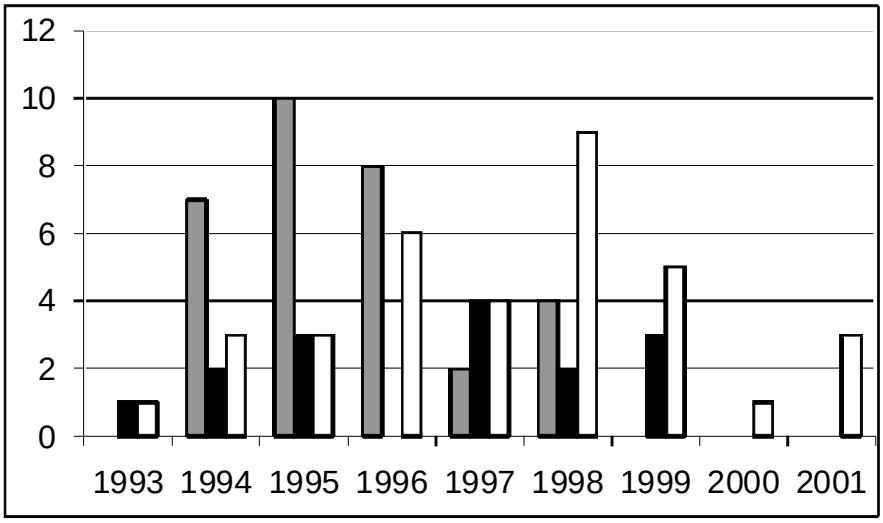

Figure 1: PrP^{Sc} distribution in tissues from 81 scrapie positive goats collected in a field affected herd (442 individuals). Animals are presented according to their year of birth (**grey**: preclinical animals with both CNS and LRS PrP^{Sc} positive; **white**: preclinical with CNS negative, LRS positive; **black**: clinical animal with both CNS and LRS positive. Animals were culled in March 2001.

In this herd, clinically affected animals were aged between 3 years and 7 years old. In all these animals PrP^{Sc} was detected in both CNS and lymphoid organs. In a large number of animals PrP^{Sc} was only evidenced in lymphoid tissue, which is compatible with an earlier stage of the incubation period. Some of these animals were younger than 12 months which is consistent with a contamination at early age. However a fraction of the animals harbouring similar feature were older than 7 years. This result indicates that infection probably occurred in adult animals, as previously described in sheep (Hourrigan et al., 1979).

In a UK study (DEFRA Project code SE 1956[6]; Gonzalez et al., 2009), two UK scrapie –affected goat herds were culled in 2008, and brain and lymphoid tissues examined for the prion protein by immuno-histochemistry (PrP^{d} IHC), and PrP^{Sc} ELISA and western blot.

In that UK study, if any tissue of an animal was found positive for PrP^{Sc} , the animal was classified as infected; the prevalences of infection were : Herd A : 4.2% (7/166), Herd B : 36 % (72/200). Table 2 illustrates the inter-relationship between age, PRNP genotype, TSE ELISA and WB screening data and clinical status of animals in Herd B that were positive in at least one tissue by PrP^{Sc} IHC.

[6] Further details at: http://randd.defra.gov.uk/Document.aspx?Document=SE1956_7847_FRP.doc

Table 2: Summary of laboratory results, genotype and age distribution, and clinical status of the 72 scrapie-infected goats according to their PrP^{Sc} distribution in brain and LRS tissues (Taken from Gonzalez et al., 2009).

	PrP^{Sc} IHC positive in		
	Brain only (n=4)	LRS only (n=34)	Brain and LRS (n=34)
Other results			
ELISA brain	(0) 0 (0)	(0) 0 (0)	(56) 19 (100)
WB brain	(0) 0 (0)	(0) 0 (0)	(56) 19 (100)
Vacuolation	(0) 0 (0)	(0) 0 (0)	(38) 13 (100)
Genotype at 142			
II	(0) 0 (0)	(76) 26 (65)	(41) 14 (35)
IM	(50) 2 (7)	(21) 7 (25)	(56) 9 (68)
MM	(50) 2 (50)	(3) 1 (25)	(3) 1 (25)
Age (months)			
< 60	(75) 3 (9)	(38) 13 (41)	(47) 16 (50)
> 60	(25) 1 (4)	(62) 21 (61)	(53) 18 (35)
Clinical signs			
Normal	(75) 3 (7)	(68) 23 (50)	(59) 20 (43)
Other	(0) 0 (0)	(9) 3 (50)	(9) 3 (50)
Inconclusive	(25) 1 (8)	(18) 6 (46)	(18) 6 (46)
Definite	(0) 0 (0)	(5) 2 (29)	(14) 5 (71)

Results are indicated as number of animals falling in each group. Percentage in brackets to the left of the number refers to the total of goats in each IHC category (brain only, LRS only or brain and LRS). Percentage in brackets to the right of the number refers to the total of goats in each set of other results, genotype, age and clinical condition (rows).

Open reading frame sequencing of the caprine prion protein gene of infected and non-infected herd mates in A and B showed a clear association of infection with the I142I homozygotes, although I142M and M142M infected animals were also found in the herd with the higher prevalence of infection (Herd B); younger M142M animals in this herd were more likely to be infected than older M142M animals which is consistent with the idea that the pressure of infection was increasing in this herd over time and that M142 carriers require a higher exposure to infection (pressure of infection) than I142 animals at the herd level to become infected.

In Italy, a study was carried out on a goats herd exposed to the subcutaneous administration of a vaccine against 'contagious agalactia' possibly contaminated with the scrapie Agent (Vaccari et al. 2006). Due to the nature of the infectious source, the study of such outbreaks could provide information in a similar manner to that which can be achieved through experimental-transmission studies by peripheral routes.

Clinical examination and laboratory diagnosis on the obex and LRS (tonsil, lymph nodes, spleen and third eyelid) were carried out on all the animals (n=100). They revealed a high prevalence of the infection with 39 out of 100 animals infected. Out of them, 11 goats showed clinical symptoms and harboured PrP^{Sc} in both the obex and the LRS, 9 did not show clinical symptoms but were positive at both the obex and the LRS and 19 were negative at the obex but positive at LRS. In the goats under investigation, the high number of animals that showed PrP^{Sc} accumulation at the LRS but not at the CNS level, along with the constant involvement of lymphoid tissues in goats that had PrP^{Sc} at the CNS level, suggest that the pathogenesis model proposing the presence of a lymphotrophic phase preceding neuroinvasion is likely to be valid in sheep scrapie as well as in goats.

Statistical analysis revealed that there were at least two main *PRNP* polymorphisms that could influence goat susceptibility, namely those at codons 154 and 222. Within these polymorphisms, that at codon 154 did not appear to confer scrapie resistance. However, considering the existence of a time

zero of infection, the data obtained in that study suggested that the polymorphism at codon 154 plays a role in the elongation of scrapie incubation time in goats.

6.2. Experimental oral infection with Classical scrapie

In France (Project UMR INRA1225 - AFSSA niort) goat kids (n=54) were orally challenged with Classical scrapie around birth (1.5g brain homogenate from naturally affected goats administered through natural suckling).

Groups of wild type genotype challenged (n=3 or 4) and control animals (n=1) were sequentially killed at 21 days, 1 month, 4 months, 12 months and 21 months post challenge. A last group (n=4) was observed till the occurrence of clinical signs (41 months post challenge).

No PrP^{Sc} was detected in any tissue of animal at 21 days and one month post-challenge.

In wild type PrP^{C} genotype goat kids:

- PrP^{Sc} was first detected in GALT (ileal peyer patches) of 4 months old kids.
- At 12 months post challenge all secondary lymphoid formations were PrP^{Sc} positive. CNS and peripheral nervous system were found PrP^{Sc} positive in 21 months post-challenge animals. At this age, low PrP^{Sc} amount were also observed in skeletal muscular tissue.

Some goat kids that were included in these experiments were harbouring I142M, R211Q, Q222K polymorphisms (All heterozygotes). All the animals (n=6) bearing the I142M *PRNP* polymorphism killed over 12 months old were found PrP^{Sc} positive in at least one tissue. The 3 I142M allele carriers goats were included in the clinical monitoring group developed clinical scrapie with an incubation period of 48 months. This data indicate that under the used experimental challenge condition, I142M polymorphism impact on the length of the incubation period but do not prevent infection.

Conversely, in the R211Q (n=7) and Q222K (n=5) heterozygote animals that were investigated during the kinetics experiment, no PrP^{Sc} was detected in any tissues. The R211Q (n=2) and Q222K (n=2) heterozygote animals that were belonging to the clinical monitoring group are still alive and healthy (84 months post challenge). These observations seem to indicate that the heterozygosis for the R211Q or Q222K polymorphisms could provide a certain level of resistance towards Classical scrapie oral infection.

However, because of the limited number of animals, further confirmation are needed before drawing definitive conclusion.

In the framework of the French INRA/AFSSA funded project (UMR INRA1225 - AFSSA niort), an experiment aiming at determining the impact of I142M, Q211R and K222Q heterozygosis on the susceptibility to oral Classical scrapie infection is ongoing. In that experiment a different scrapie isolate than the one previously used was selected.

The first available results (12 months post challenge) confirm that:

- I142M polymorphism is not associated with strong resistance to the oral infection.
- R211Q and Q222K heterozygosis seems to be associated with a certain level of resistance against oral infection to the used scrapie agent.

6.3. Experimental oral infection with Classical BSE

There are on-going experimental studies on goat genetic susceptibility to BSE. In the framework of the EU project 'goatBSE' (FOOD-CT-2006-36353), an experiment aiming at determining the dynamic and kinetics of BSE and BSE adapted to goat with dissemination in organs of animals bearing various *PRPN* polymorphisms (heterozygotes for I142M, R211Q and Q222K is in progress. This will provide indispensable data on genetic susceptibility in the most commonly used goat breeds. Partial but already informative results should be made available in 2011-2012.

6.4. Atypical scrapie in goats

To date Atypical scrapie cases in goats were reported in several EU countries. Like in sheep, there is no available information related to the pathogenesis of this TSE form in goats. While in sheep several projects related to Atypical scrapie are ongoing, in goats no specific on-going project has been identified.

6.5. Concluding remarks on the effect of different *PRNP* alleles on the pathogenesis of the different goat TSEs

From this section, it can be concluded that:

- TSE pathogenesis in goats is similar to what is known in sheep.
- Considering the available data, the impact of most of the described *PRNP* allelic variants on TSE susceptibility/resistance is unclear. Some of the polymorphisms like M142 seems to influence the incubation period without providing substantial protection towards contamination while other could be associated with strong resistance to infection.
- In Classical scrapie oral infection, available results seem to indicate some protective effects of K222 and Q211 allelic variants.
- There are no available data on a potential effect of K222, Q211 and other goat *PRNP* polymorphism on BSE and Atypical scrapie susceptibility. H154 seems to be associated with some susceptibility towards Atypical scrapie.
- Experiments to clarify the impact of different polymorphisms that could be associated to resistance to TSE in goats are currently on-going for Classical scrapie and BSE. To date, no experiment related to Atypical scrapie is on-going.

7. Epidemiology and breeding for resistance

7.1. Theoretical basis for breeding for resistance

The reproduction Ratio (R_0) (number of secondary cases generated by an infected individual) is a key factor determining the expansion or the fading of a disease in a population. R_0 is the results of dynamic interactions between the host/ the infectious agent and the environment. This is an ideal tool to calculate the allele distributions at which the infection cannot persist in the population (i.e. $R_0 < 1$). In TSEs in small ruminants, similarly to other infections, resistance in a major part of the population can protect the more susceptible animals in the population in the form of a herd immunity (Diekmann and Heesterbeek, 2001).

To quantify the transmission of TSEs in goats, data that give information about exposure should be evaluated in combination with data about new infections in that same period. This usually requires

data on infections/disease over a period of time preferably in combination with a good overview of the herds in which the data concerning genotype and age are collected. Typically, case-control data are not suitable for this question, since the background data on the full herd are lacking, thus the level of exposure which led to the case can not be estimated. Such data partially allow determining the relative susceptibility of the animals, but does not allow analysis of the effect of the relative infectiousness of these animals in the field cannot be derived from the data. This can partially be compensated with pathogenesis studies. However, these still do not give definite answers on infectiousness in the field, only indications thereof.

To calculate the required allele frequency for scrapie control for a candidate allele, transmission parameters of animals with this allele need to be quantified. This requires data from field herds with scrapie and a minimal allele frequency of preferably 20-30% over several years. Estimates from other data sources can be used, but are less reliable than field data.

7.2. Experience from breeding for resistance in sheep.

In the Netherlands, a breeding programme for ARR scrapie resistance was introduced very early on. In 1998, the top breeders already started their ARR selection, to produce ARR homozygote rams, which could be used to distribute in the national herd. In the years following, a few infected flocks which started early on with the breeding programme (voluntary) were monitored intensively. Each adult animal was sampled by tonsil biopsy annually, and a blood sample for genotyping was taken too. These herds were monitored for 3 to 5 years. A clear increase of resistance combined with a decline of the scrapie prevalence was the result in these herds.

To reduce the R_0 of scrapie below one, the frequency of ARR allele animal carrier below 100% would suffice. Some work is in progress on the basis of data collected in the Netherlands, despite the required ARR allele frequency can not be accurately estimated at the moment, current opinions in this field would indicate that the 60-80% frequency could be sufficient. At present the ARR allele frequency in the Netherlands is estimated at 55% and the scrapie prevalence as detected by active surveillance is declining fast (Melchior et al., 2009a), More data will become available in the near future.

Within the framework of a large simulation model of the British sheep population and its breeding and trading structure Truscott and Ferguson (2008a, 2008b) used various data obtained by passive and active surveillance of sheep, and by genotype profiling of different breeds in the UK national flock, to model the impact of various parts of the UK breeding for resistance plan on:

- The number of confirmed cases;
- The frequency of *PRNP* alleles;
- R_0 for Classical scrapie.

Truscott and Ferguson build a non-intervention baseline scenario that predicted the persistence of scrapie within the national sheep flock for the next 300-400 years. A more optimistic view considers that current measures should be effective in 20 to 30 years (Hope, 2009).

In conclusion, to foresee and assess the potential for breeding for resistance to TSE in goats existing modelling tools to analyse the breeding for resistance in sheep can be applied. Even if done with the current limited data available, modelling would aid the identification of important data gaps on experimental or surveillance data.

8. Other aspects related to breeding for resistance

8.1. Geographical distribution and frequency of polymorphism in the goat population

Estimating the frequency of candidate alleles in a population is a pre-requisite in evaluating the feasibility of a genetic selection programme. Studies on *PRNP* allele frequencies in goats in Italy, France, USA, Japan, China and Morocco (Zhang et al., 2004; Kurosaki et al., 2005; Acutis et al., 2008; White et al., 2008; Barillet et al., 2009; Serrano et al., 2009) have been published so far. Table 3 shows the minimum and maximum frequencies reported for the polymorphic variants associated with potential resistance to scrapie in a given population.

Table 3: Summary of minimum and maximum frequencies for the polymorphic variants associated with some resistance to Classical scrapie reported in literature (Data taken from: Zhang *et al*., 2004; Kurosaki *et al*., 2005; Acutis *et al*., 2008; White *et al*., 2008; Barillet *et al*., 2009).

Polymorphic variant	***Country (number of goat breeds)***					
	Italy (8)	**France (2)**	**USA (10)**	**Japan (1)**	**China (5)**	**Morocco (2)**
M142	0.0-28.2* (5/8)* *	3.9-8.7 (2/2)	0.0-43.2 (4/10)	4.6	0.0	0.6-0.8 (2/2)
R143	0.0-5.4 (4/8)	0.0	0.0-10.9 (4/10)	3.0	14.3-57.1 (5/5)	0.0
S/D146	0.0	0.0	0.0-35.2 (7/10)	1.7	0.0-57.4 (1/5)	0.0
H154	0.0-11.3 (6/8)	0.5-5.4 (2/2)	0.0-1.8 (1/10)	0.0	7.4-6.6 (5/5)	21.9-25.4 (2/2)
Q211	0.0-13.7 (4/8)	7.1-18.5 (2/2)	0.0-9.7 (5/10)	12.7	0.0 (0/5)	0.0
K222	1.3-17.2 (8/8)	4.9-7.5 (2/2)	0.0-5.4 (2/10)	0.0	0.0-21.7 (1/5)	1.25-1.75 (2/2)

*Minimum and maximum percentage frequencies of the polymorphic variant relative to all haplotypes observed in the respective study.
**number of breeds in which the polymorphism has been found out of the total breeds included in the study

What is clear is an uneven distribution of alleles in the world goat population with the absence of some polymorphisms in some countries (e.g. N146S and N146D in Italy) or in some breeds (e.g. I142M found only in dairy breeds in USA).

In Tables 4 and 5 the results of the Italian and French studies are presented in more details, showing the frequencies (%) of the polymorphisms of interest within breed, with 95% confidence intervals, and the number of animals analysed per breed.

Table 4: Detailed results of Italian studies referred in Table 3.

Polymorphism	*Breed abbreviation* (number goats)*							
	CA (n=84)	**SA (n=69)**	**RO (n=70)**	**VA (n=77)**	**GA (n=58)**	**MA (n=25)**	**IO (n=27)**	**RM (n=28)**
I142M	8.9** (5.1-14.3)***	7.2 (3.5-13.0)	5.7 (2.5-11.0)	28.2 (21.6-36.4)	2.6 (0.5-7.4)	0.0 (0.0-7.1)	0.0 (0.0-6.6)	0.0 (0.0-6.4)
H143R	0.0 (0.0-2.2)	0.0 (0.0-2.6)	0.0 (0.0-2.6)	0.0 (0.0-2.4)	1.7 (0.2-6.1)	4.0 (0.5-13.7)	3.7 (0.4-12.7)	5.4 (1.1-14.9)
N146S/D	0.0 (0.0-2.2)	0.0 (0.0-2.6)	0.0 (0.0-2.6)	0.0 (0.0-2.4)	0.0 (0.0-3.1)	0.0 (0.0-7.1)	0.0 (0.0-6.6)	0.0 (0.0-6.4)
R154H	11.3 (6.9-17.1)	0.0 (0.0-2.6)	3.6 (1.2-8.1)	0.0 (0.0-2.4)	11.2 (6.1-18.4)	6.0 (1.2-16.5)	7.4 (2.0-17.9)	5.3 (1.1-14.9
R211Q	13.7 (8.9-19.9)	10.2 (5.7-16.4)	13.6 (8.4-20.4)	9.6 (5.5-15.5)	0.0 0.2-6.1)	0.0 (0.0-7.1)	0.0 (0.0-6.6)	0.0 (0.0-6.4)
Q222K	2.4 (0.6-6.0)	3.0 (0.8-7.2)	4.3 (1.6-9.1)	1.3 (0.2-4.6)	17.2 (10.9-25.4)	12.0 (4.5-24.3)	7.4 (2.0-17.9)	5.4 (1.1-14.9

*CA= Camosciata delle Alpi; SA= Saanen; RO= Roccaverano; VA= Valdostana; GA= Garganica; MA= Maltese; IO= Ionica; RM= Red Mediterranean

** Mean value; *** Confidence limits (with 95% confidence interval)

Table 5: Detailed results of French studies referred in Table 3.

Polymorphism	*Breed (number goat bucks)*	
	Alpine (n=184)	**Saanen (n=220)**
I142M	3.9* (2.1-6.4)**	8.7 (6.2-11.6)
H143R	0.0 (0.0-1.0)	0.0 (0.0-0.8)
N146S/D	0.0 (0.0-1.0)	0.0 (0.0-0.8)
R154H	5.4 (3.3-8.3)	0.5 (0.0-1.6)
R211Q	7.1 (4.7-10.2)	18.5 (15.1-22.6)
Q222K	7.5 (5.1-10.8)	4.9 (3.2-7.5)

* Mean value; ** Confidence limits (with 95% confidence interval)

In the Italian study nearly all the major breeds reared in the country have been considered, but the numbers of animals analysed are higher for the Northern breeds (first four breeds in Table 3) than for the southern ones. This study highlighted differences in allele distribution according to both breeds and geography. Significant differences in the frequencies of some alleles were found between the two geographical groups: in Southern goats, M142 was nearly absent and the mutations H154 and K222 were significantly more frequent than in the North. A genetic distance analysis showed that breeds belonging to the same geographical location clustered tightly. Caňòn *et al*. (2006) have previously pointed out the fact that the phylo-geographical structure of goat populations is more obvious than in other domestic species. In fact, gene flow among breeds has been restricted by spatial isolation, whereas the use of herd books is rarely practiced, so that geographical clones are maintained that predate breeds formation.

In the French study two main breeds, which comprise about 90% of the national goat population, have been examined. The sampled animals were all bucks born between 1998 and 2005, representing 82% of all the artificial insemination buck progeny tested in France between 1999 and 2006. In this study as well, significant differences in some allele frequencies have been found in the two breeds: H154 was more frequent in Alpine bucks and Q211 had a significantly higher frequency in Saanen bucks.

An overview of both studies shows that in general the polymorphisms are present at low frequencies. Furthermore, the codon 146 polymorphisms, associated with resistance in Cypriot goats, are completely absent. Only the K222 mutation is present in all breeds while the mutations at codons 142 and 211 could be of interest only for French and North-Italian breeds and H154 only for Southern-Italian goats.

Comparison of the polymorphism frequencies (%) of Saanen breed in Italy, France and the USA is shown in Table 6.

Table 6: Comparison of the polymorphism frequencies (%) of Saanen breed in Italy, France and USA.

Polymorphism	***Country (number goats)***			
	Italy (n=138)	**France (n=368)**	**USA (n=60)**	**p-value**
I142M	7.2	8.7	0.0	Not significant
H143R	0.0	0.0	0.0	Not significant
N146S/D	0.0	0.0	1.8	Not significant
R154H	0.0	0.0	1.8	Not significant
R211Q	10.2	18.5	3.6	0.002
Q222K	3.0	4.9	0.0	Not significant

The Q211 polymorphisms has a significantly greater presence in French Saanen than in Italian and American Saanen, showing that it is important to carry out breed surveys in each country, and not only in autochthonous but also in cosmopolitan breeds. This action is ongoing at the European level in the frame of the Specific Targeted Research Project "Goat BSE: Proposal for improvement of goat TSE discriminative diagnosis and susceptibility based assessment of BSE infectivity in goat milk and meat"[7], from which the Italian and French studies have also been partly funded. Some details on surveys in other EU MSs, include:

- In The Netherlands 1,000 goat samples from routine slaughter and about 500 from fallen stock have been collected to get a "representative" overview of polymorphisms present in the populations. These samples come from a variety of flocks and from the major breeds going into slaughter. An estimate of the distribution of the collected breeds and their geographical mapping, as well as an analysis of the sequencing results is still underway.

- In the United Kingdom more than 1,000 samples, coming from Saanen, Alpine, Anglo-Nubian, Toggenburg and Boer breeds, have been collected and will be analysed.

- In Spain goats from Saanen, Alpine, Moncaina, Pirenaica, Retinta breeds have been genotyped.

[7] Details available at www.goatBSE.eu

- In Greece the analysis of 500 samples from local Greek goats is ongoing.

The results will be available in the near future, providing important elements to assess the feasibility of future breeding programmes.

The frequency of the wild type allele, which is known to be susceptible to scrapie, is high in all goat breeds considered. This would represent a drawback for a breeding programme aimed at selecting putative resistant alleles in the general goat population.

8.2. *PRNP* allele selection and possible adverse effect on production or health traits

Adverse effect on health and production traits associated with breeding for particular trait is a well recognised risk in genetics. Such potential risk was largely debated when considering the breeding for resistance policy in sheep (European Food Safety Authority Panel on Biological Hazards, 2006), but no adverse effect has been observed in these species at this time. This has been more recently confirmed by Sweeney and Hanrahan in 2008, who published an extensively reviewed research on this topic. They concluded that "there is no evidence for a negative association between PrP^C genotype and reproduction traits, lamb performance traits or milk production". However, they did not exclude that genes linked to *PRNP* locus may influence resistance / susceptibility to other diseases and recommended further studies on this particular point.

Contradictory to the general tendency, Sawalha et al., 2008, found in Scottish Black face animals differences between ARQ carriers and non carriers in term of ewe body weight and seasonal mobilization of body reserve, but excluded negative effect of a selection against VRQ. Similarly, Gubbins at al., 2009, concluded that despite the evidence of limited *PRNP* effect on different traits, "a selective breeding programme based on *PRNP* genotype will not have a detrimental effect on lamb survival".

While a large number of studies and datasets were available for discussion of such risk in sheep, in goats data remain sparse. However, the sheep and goat genomes are very close as indicated for instance by Maddox and Cockett (2007) and it can be expected that observations made in the sheep are good indicators of the goat situation.

The probability that an adverse effect would be associated with a particular *PRNP* allele selection in the goat species is generally considered to be low. However, the risk of a "founder effect" should be considered.

8.3. A survey on the structure of production and breeding systems in goats in the different EU Member States

The organisation of goat breeding and genetic selection systems in the EU MSs has an impact on the feasibility and potential duration of any *PRNP* selection scheme for introducing or increasing the frequency of particular alleles of the caprine *PRNP* into their national goat herds.

Linked with experience on breeding for resistance in sheep, the following could be considered as the minimum requirements for starting up a breeding programme:

- Presence of a nationwide goat-disease monitoring programme;
- An on-going large scale goat breeding scheme;
- Existence of a logistical and technical infrastructure for *PRNP* genotyping.

As EU MSs specific information on the fulfilment of those requirements is not readily available, a questionnaire aimed at collecting data on goat farming and breeding practices in the individual EU MSs was developed and circulated among the EFSA BSE-TSE Network[8]. Full details of this survey, including the questionnaire and the results can be found in Appendix C.

Some limitations have to be considered when interpreting the results obtained:

- Some replies highlighted difficulties when gathering the requested data in the given timeframe from different sources (*e.g.* Ministries, breeding organisations). This influenced the level of detail reported compared to that required;

- Even though some questions could benefit from quantitative replies, in most occasions replies received were of qualitative nature.

Taking those limitations into account, some preliminary conclusions could still be made:

- In general, there was variability in the amount and quality of data returned by EU MSs in the questionnaire. This may reflect the different levels of centralisation of data among the reporting countries – thus, MSs with a less centralised data system for goat herds may not be able to provide the requested information with a great level of detail in the given timeframe – or it could represent real differences in the level of data control concerning goat herds in different Mss.

- There is a high level of diversity on breeding programmes and management systems for goats in the different MSs.

- Goat-specific disease programs are present in most of the reporting MSs. A breeding strategy in goats, mostly for production traits, is present in the majority of the MSs and in all but one of those with reported populations of over 100,000 head.

- Based on the replies received, only a few EU MSs seem to have in place the necessary elements to introduce a breeding for resistance programme for goats.

- In 2008, 7 EU MSs accounted for nearly 90% of the total goat population in the EU[9]. The application of a breeding for resistance programme for goat scrapie in MSs with the larger goat populations would have the most impact within the EU, and these MSs are therefore the candidates to pioneer this initiative.

It is recommended that before starting up such a breeding programme in any particular EU MSs the capacity to fulfil these elements should be thoroughly evaluated in this respect.

[8] This network is currently composed by representatives of 26 EU MSs who act as contact points on BSE-TSE risk assessment related matters, plus 12 representatives from further countries and international organisations who act as observer members.

[9] Based on EUROSTAT data: Total EU: 13,314,300 head; Sum of Bulgaria, France, Greece, Italy, Spain, Portugal and Romania: 11,988,700 head.

CONCLUSIONS AND RECOMMENDATIONS

CONCLUSIONS

General Conclusions

- Field cases of Classical scrapie, Atypical scrapie and BSE have been identified in goats.
- Diversity of Classical scrapie in goats is currently under investigation through national projects and an EU funded project. However, the current ability to fully describe and identify TSE Agent diversity in goats, as in sheep and cattle, remains limited.
- Data currently available are not sufficient to definitively determine the resistance/susceptibility level towards the different TSE agents associated with different *PRNP* polymorphisms. Candidate polymorphisms of interest for breeding resistance to Classical scrapie are SD146, H154, Q211 and K222. However, H154 seems to be associated with susceptibility to Atypical scrapie. The effects of SD146, Q211 and K222 on susceptibility to Atypical scrapie and BSE in goats are still unknown.
- It remains unclear whether or not the reported *PRNP* allelic variants in goats are associated with resistance to infection or only prolong incubation time.
- With regard to pathogenesis and tissue distribution of PrP^{Sc} and/or infectivity, experiments to clarify the impact of different *PRNP* polymorphisms that could be associated with resistance to TSE in goats are currently on-going for Classical scrapie and BSE. No such experiments related to Atypical scrapie in goats seem to be on-going.
- No information is available on the possible adverse effects of the candidate *PRNP* polymorphisms on other goat production traits.
- The frequency of the wild type allele which is known to confer susceptibility to Classical scrapie is high in all goat breeds considered. This would represent a drawback for a breeding programme aimed at selecting putative resistant alleles in the general goat population.
- To foresee and assess the potential for breeding for resistance to TSEs in goats existing modelling tools to analyse the breeding for resistance in sheep can be applied. Even if done with the current limited data available, modelling would aid the identification of important data gaps in experimental or surveillance data.
- Based on the replies received to a questionnaire circulated among the EFSA BSE-TSE Network, there is a high level of diversity in breeding programmes and management systems for goats in the responding MSs. Quantitatively, seven MSs account for over 90% of the total EU goat population. Based on the replies received, only a few MSs seem to have in place the necessary elements to introduce a breeding for resistance programme for goats.

Answer to the Terms of Reference

- There are encouraging but as yet incomplete data to consider supporting a breeding programme for resistance in goats against Classical scrapie in all MS, and ongoing studies will provide a more robust scientific background in the coming years.
- At this moment there are not enough data available to consider supporting a breeding programme for resistance against Atypical scrapie and BSE in goats in all MS. Experiments are ongoing on BSE in goats and results will be available in the coming years.
- There are limited data suggesting that an allele (H145) might confer resistance to Classical scrapie but increase susceptibility to Atypical scrapie.
- The frequency of the wild type allele, which is known to confer susceptibility to Classical scrapie, is high in all goat breeds considered. Thus selection for putative resistance alleles will be slow, complicated and highly dependent on breeding structure.
- Any large scale breeding programme for TSE resistance in goats must take into consideration key elements related to the current dissemination of potentially TSE protective polymorphisms in the goat population of each MS and the characterisation of the real protection provided by those polymorphisms. However, these elements seem to be quite breed-diverse within and between MSs.
- At present and based on the replies received to a questionnaire circulated among the EFSA BSE-TSE Network, only a few MSs seem to have in place the necessary elements to introduce a breeding for resistance programme for goats.

RECOMMENDATIONS

- Before starting any breeding programme for genetic TSE resistance in goats, completion of on-going studies aimed at measuring the resistance provided by the candidate *PRNP* polymorphisms (SD146, H154, Q211 and K222) towards Classical scrapie and BSE is recommended.
- It is recommended that when all results of these experiments are available, a breeding for resistance programme for TSE in goats is first implemented in the seven MSs with the largest goat population as this would have the most impact.
- A programme for characterising EU TSE isolates in goats using biochemical and bioassay methods should be supported and extended to all MSs.
- Experimental transmission of a panel of different isolates of Atypical scrapie and BSE in goats harbouring the PRNP alleles of interest (SD146, H154, Q211 and K222) should be supported.
- Experimental transmission of a panel of goat TSE isolates in other animal models (transgenic mouse model) harbouring the alleles of interest and in vitro conversion assays using a panel of TSE isolates would be useful.
- The continuous observation of the disease dynamics in herds of affected goats over a period of time, where TSE isolate would also be characterised (using biochemical tools and bioassay), would be useful.
- Research on the possible adverse effects of the candidate PRNP polymorphisms on other production traits should be encouraged.

DOCUMENTATION PROVIDED TO EFSA

1. Letter from the European Commission (Ref. SANCO.E.2/MP/khk/520865) on a request for a scientific opinion on the analysis of a Cypriot study on genetic TSE resistance in goats in Cyprus.

2. Letter from the European Commission (Ref. SANCO.E.2/MP/bo – D(2009)520019) on the revision of the request made under bullet point 1 above.

REFERENCES

Acutis PL, Bossers A, Priem J, Riina MV, Peletto S, Mazza M, Casalone C, Forloni G, Ru G and Caramelli M, 2006. Identification of prion protein gene polymorphisms in goats from Italian scrapie outbreaks. Journal of general virology, 87, 1029-1033.

Acutis PL, Colussi S, Santagada G, Laurenza C, Maniaci MG, Riina MV, Peletto S, Goldmann W, Bossers A, Caramelli M, Cristoferi I, Maione S, Sacchi P and Rasero R, 2008. Genetic variability of the PRNP gene in goat breeds from Northern and Southern Italy. Journal of applied microbiology, 104, 1782-1789.

Agrimi U, Nonno R, Dell'Omo G, Di Bari MA, Conte M, Chiappini B, Esposito E, Di Guardo G, Windl O, Vaccari G and Lipp HP, 2008. Prion protein amino acid determinants of differential susceptibility and molecular feature of prion strains in mice and voles. PLoS Pathogens 4(7): e1000113 doi:10.1371/journal.ppat.1000113.

Andreoletti O, Berthon P, Marc D, Sarradin P, Grosclaude J, van Keulen L, Schelcher F, Elsen JM and Lantier F, 2000. Early accumulation of PrPres in gut-associated lymphoid and nervous tissues of susceptible sheep from a Romanov flock with natural scrapie. Journal of General Virology 81, 3115-3126.

Arsac JN, Andreoletti O, Bilheude JM, Lacroux C, Benestad SL and Baron T, 2007. Similar Biochemical Signatures and Prion Protein Genotypes in Atypical scrapie and Nor98 Cases, France and Norway. Emerging Infectious Diseases 13, 58-65.

Barillet F, Marita D, Amigues Y, Foucras R, Caillat H, Moazami-Goudarzi K, Rupp R, Babilliot JM, Lacroux C, Lugan S, Schelcher F, Charter C, Corbière F, Andreoletti O, 2009. Identification of seven haplotypes of the caprine PrP gene at codons 127, 142, 154, 211, 222 and 240 in French Alpine and Saanen breeds and their association with Classical scrapie. Journal of General Virology. Ahead of publication: doi:10.1099/vir1090.006114-006110

Baron T, Bencsik A, Vulin J, Biacabe A-G, Morignat E, Verchere J and Betemps D, 2008. A C-Terminal Protease-Resistant Prion Fragment Distinguishes Ovine 'CH1641-Like' scrapie from Bovine Classical and L-Type BSE in Ovine Transgenic Mice. PLoS Pathog 4(8), e1000137. doi:10.1371/journal.ppat.1000137.

Beringue V, Vilotte JL and Laude H, 2008. Prion agent diversity and species barrier. Veterinary Research, 39(4), 1-47. DOI: 10.1051/vetres:2008024.

Billinis C, Panagiotidis CH, Psychas V, Argyroudis S, Nicolaou A, Leontides S, Papadopoulos O, and Sklaviadis T, 2002. Prion protein gene polymorphisms in natural goat scrapie. The Journal of general virology, 83, 713-721.

Bossers A, Belt P, Raymond GJ, Caughey B, de Vries R and Smits MA, 1997. scrapie susceptibility-linked polymorphisms modulate the in vitro conversion of sheep prion protein to protease-resistant forms. Proceedings of the Nationall Academt of Science of the United States of America, 94, 4931-4936.

Bossers A, de Vries R and Smits MA, 2000. Susceptibility of sheep for scrapie as assessed by in vitro conversion of nine naturally occurring variants of PrP. Journal of Virology 74, 1407-1414.

Bruce ME, Boyle A, Cousens S, McConnell I, Foster J, Goldmann W and Fraser H, 2002. Strain characterisation of natural sheep scrapie and comparison with BSE. Journal of General Virology, 83(3), 695-704.

Chelle PL, 1942. Un cas de tremblante chez la chèvre. Bulletin de l'Académie Vétérinaire de France, 15, 294-295.

Colussi S, Vaccari G, Maurella C, Bona C, Lorenzetti R, Troiano P, Casalinuovo F, Di Sarno A, Maniaci MG, Zuccon F, Nonno R, Casalone C, Mazza M, Ru G, Caramelli M, Agrimi U and Acutis PL, 2008. Histidine at codon 154 of the prion protein gene is a risk factor for Nor98 scrapie in goats. Journal of General Virology, 89, 3173-3176.

CRL (Community Reference Laboratory for TSEs), 2008a. Summary report of the TSE Community Refernce Laboratory Expert Group on Strains of 30 April 2008. Accessed : 9/10/2009. Available at : http://www.defra.gov.uk/vla/science/docs/sci_tse_rl_steg.pdf

CRL (Community Reference Laboratory for TSEs), 2008b. The TSE community reference laboratory strain typing expert group (STEG) Summary of the STEG opinion on two caprine isolates (08-357 from France; G08-1469 from the UK) referred to the group following the identification of unusual molecular profiles on discriminatory Western blot (as required in EU Regulation 36/2005). Accessed : 9/10/2009. Available at : http://www.defra.gov.uk/vla/science/docs/sci_tse_rl_steg1008.pdf

Cuillé J, Chelle PL, 1969. Transmission expèrimentale de la tremblante à la chèvre, Comptes Rendus Hebdomadaires des Seances de l'Academie des Sciences, 208, 1058-1060.

Diekmann O and Heesterbeek JAP, 2000. Mathematical Epidemiology of Infectious Diseases: model building, analysis and interpretation. John Wiley & Sons, Chichester, 303.

EC (European Commission), online. Cumulative TSE testing in goats since 2002. Accessed: 12/10/2009. http://ec.europa.eu/food/food/biosafety/bse/mthly_gt_cml_reps_tse2002_en.pdf

EC (European Commission), 2004. Report on the monitoring and testing of ruminants for the presence of transmissible spongiform encephalopathy (TSE) in the EU in 2004. European Commission, Luxembourg: Office for Official Publications of the European Communities, 2005, ISBN 92-894-7431-9.

EFSA Panel on Biological Hazards (BIOHAZ), 2007. Certain aspects related to the risk of Transmissible Spongiform Encephalopathies (TSEs) in ovine and caprine animals. The EFSA Journal, 466, 1-10

EFSA Panel on Biological Hazards (BIOHAZ), 2006. The breeding programme for TSE resistance in sheep. The EFSA Journal, 382, 1-46.

EFSA Panel on Biological Hazards (BIOHAZ), 2009. Genetic TSE resistance in goats. The EFSA Journal, 995, 1-25.

Eiden M, Palm GJ, Hinrichs W, Matthey U, Zahn R and Groschup MH, 2006. Synergistic and strain-specific effects of bovine spongiform encephalopathy and scrapie prions in the cell-free conversion of recombinant prion protein. Journal of General Virology 87, 3753-3761.

Eloit M, Adjou K, Coulpier M, Fontaine JJ, Hamel R, Lilin T, Messiaen S, Andreoletti O, Baron T, Bencsik A, Biacabe AG, Beringue V, Laude H, Le Dur A, Vilotte JL, Comoy E, Deslys JP, Grassi J, Simon S, Lantier F AND Saradin P, 2005. BSE agent signatures in a goat. Veterinary Record (Letters) 156, 523–524. And erratum in: Veterinary Record, 156(19), 620.

Foster JD, Hope J and Fraser H, 1993. Transmission of bovine spongiform encephalopathy to sheep and goats. Veterinary Reccord, 133(14), 339-41.

Foster JD, Parnham DW, Hunter N and Bruce M, 2001. Distribution of the prion protein in sheep terminally affected with BSE following experimental oral transmission. Journal of General Virology, 82, 2319–2326.

Goldmann W, Hunter N, Smith G, Foster J and Hope J, 1994. PrP genotype and agents effects in scrapie: change in allelic interaction with different isolates of agent in sheep, a natural host of scrapie. Journal of General Virology, 75, 5: 989-995.

Goldmann W, Martin T, Foster J, Hughes S, Smith G, Hughes K, Dawson M and Hunter N, 1996. Novel polymorphisms in the caprine PrP gene: a codon 142 mutation associated with scrapie incubation period. The Journal of general virology, 77(11), 2885-2891.

Goldmann W, Chong A, Foster J, Hope J and Hunter N, 1998. The shortest known prion protein gene allele occurs in goats, has only three octapeptide repeats and is non-pathogenic. The Journal of general virology, 79(12), 3173-3176.

Goldmann W, Perucchini M, Smith A and Hunter N, 2004. Genetic variability of the PrP gene in a goat herd in the UK. Veterinary Record, 155(6), 177-178.

Gonzalez L, Martin S, Siso S, Konold T, Ortiz-Pelaez A, Phelan L, Goldmann W, Stewart P, Saunders G, Windl O, Jeffrey M, Hawkins SA, Dawson M, Hope J, 2009. High prevalence of scrapie in a dairy goat herd: tissue distribution of disease-associated PrP and effect of PRNP genotype and age. Veterinary Research, 40(6), 1-65. DOI: 10.1051/vetres/2009048.

Gordon W, 1966. Variation in Susceptibility of Sheep to scrapie and Genetic Implications. Report of scrapie Seminar, ARS 91-53, US Department of Agriculture, 53–67.

Gubbins S, Cook CJ, Hyder K, Boulton K, Davis C, Thomas E, Haresign W, Bishop SC, Villanuea B and Eglin RD, 2009. Associations between lamb survival and prion protein genotype: analysis of data for ten sheep breeds in Great Britain, BMC Veterinary Research, 5(3). DOI:10.1186/1746-6148-5-3.

Hadlow WJ, 1961. The pathology of experimental scrapie in the dairy goat. Research in Veterinary Science 2, 289-314.

Hourrigan JL, Klingsporn AL, McDaniel HA and Riemenschneider MN, 1969. Natural scrapie in a goat. Journal of the American Veterinary Medical Association 154, 538-539.

Hope J, 2009. Eradicating scrapie. Epidemiology and Infection, 137(6), 792-793.

Hourrigan JL, Klingsporn A, Clark WW and De Camp M, 1979. Epidemiology of scrapie in the United States. In Slow Transmissible Diseases of the Nervous System volume 1. Eds Prusiner SB and Hadlow WJ. Academic Press, New York, 331-356.

Jeffrey M, Martin S, Gonzalez L, Ryder SJ, Bellworthy SJ and Jackman R, 2001. Differential diagnosis of infections with the Bovine Spongiform Encephalopathy (BSE) and scrapie Agents in Sheep. Journal of Comparative Pathology, 125, 271-284.

Kirby L, Goldmann W, Houston F, Gill AC and Manson JC, 2006. A novel, resistance-linked ovine PrP variant and its equivalent mouse variant modulate the in vitro cell-free conversion of rPrP to PrP(res). Journal of General Virology, 87, 3747-3751.

Kurosaki Y, Ishiguro N, Horiuchi M and Shinagawa M, 2005. Polymorphisms of caprine PrP gene detected in Japan. The Journal of veterinary medical science / the Japanese Society of Veterinary Science, 67, 321-323.

Laplanche JL, Hunter N, Shinagawa S, Williams E, 1999. scrapie, Chronic Wasting Disease, and Transmissible Mink Encephalopathy. In: Prion biology and diseases. Ed Prusiner S. Cold Spring Harbor, New York, 393-429.

Le Dur A, Béringue V, Andréoletti O, Reine F, Lan Laï T, Baron T, Bratberg B, Vilotte JL, Sarradin P, Benestad SL and Laude H, 2005. A newly identified type of scrapie agent can naturally infect sheep with resistant PrP genotypes. PNAS 102 :16031–16036.

Leontides S, Psychas V, Argyroudis S, Giannati-Stefanou A, Paschaleri-Papadopoulou E, Manousis T and Sklaviadis T, 2000. A survey of more than 11 years of neurologic diseases of ruminants with special reference to transmissible spongiform encephalopathies (TSEs) in Greece. Journal of Veterinary Medicine Series B, 47, 303-309.

Maddox JF and Cockett NE, 2007. An update on sheep and goat linkage maps and other genomic resources. Small Ruminant Research, 7, 4-20.

Melchior M, Hagenaars T, Bossers A, Davidse A, Zijderveld F, 2009. scrapie surveillance in the Netherlands; the effect of selection for scrapie resistance in sheep. Proceedings of the Prion 2009 conference, Porto Carras, Ghaldiki, Greece, 23, 24 and 25 September 2009, 170.

Moum T, Olsaker I, Hopp P, Moldal T, Valheim M, Moum T and Benestad SL, 2005. Polymorphisms at codons 141 and 154 in the ovine prion protein gene are associated with scrapie Nor98 cases. Journal of General Virology, 86, 231–235.

Nentwig A, Oevermann A, Heim D, Botteron C, Zellweger K, Drögemüller C, Zurbriggen A and Seuberlich T, 2007. Diversity in Neuroanatomical Distribution of Abnormal Prion Protein in Atypical scrapie. PLoS Pathog 3(6): e82. doi:10.1371/journal.ppat.0030082.

Nonno R, Esposito E, Vaccari G, Conte M, Marcon S, Di Bari M, Ligios C, Di Guardo G and Agrimi U, 2003. Molecular analysis of cases of Italian sheep scrapie and comparison with cases of bovine spongiform encephalopathy (BSE) and experimental BSE in sheep. Journal of Clinical Microbiology, 41(9), 4127-4133.

Oesch B, Westaway D, Wälchli M, McKinley MP, Kent SB, Aebersold R, Barry RA, Tempst P, Teplow DB, Hood LE, Prusiner SB and Weissmann C, 1985. A cellular gene encodes scrapie PrP 27-30 protein. Cell, 40(4), 735–746

Papasavva-Stylianou P, Kleanthous M, Toumazos P, Mavrikiou P, Loucaides P, 2007. Novel polymorphisms at codons 146 and 151 in the prion protein gene of Cyprus goats, and their association with natural scrapie. Veterinary Journal, 173, 459-462.

Pattison IH, Gordon WS, Millson GC, 1959. Experimental production of scrapie in goats. Journal of Comparative Pathology, 69, 300-312.

Pattison IH and Millson GC, 1961. scrapie produced experimentally in goats with special reference to the clinical syndrome. Journal of Comparative Pathology, 71, 101-9.

Piening N, Nonno R, Di Bari M, Walter S, Windl O, Agrimi U, Kretzschmar HA and Bertsch U 2006. Conversion efficiency of bank vole prion protein in vitro is determined by residues 155 and 170, but does not correlate with the high susceptibility of bank voles to sheep scrapie in vivo. Journal of Biological Chemistry 281, 9373-9384.

Prusiner SB, 1998. Prions. Proceedings of the Nationall Acaddemy of Science of the United States of America, 95(23), 13363-83.

Raymond GJ, Bossers A, Raymond LD, O'Rourke KI, McHolland LE, Bryant IP, Miller MW, Williams ES, Smits M and Caughey B, 2000. Evidence of a molecular barrier limiting susceptibility of humans, cattle and sheep to chronic wasting disease. The EMBO Journal 19, 4425-4430.

Sawalha RM, Brotherstone S, Lambe NR and Villanueva B, 2008. Association of the prion protein gene with individual tissue weights in Scottish Blackface sheep. Journal of Animal Science, 86(8), 1737-1746.

Serrano C, Hammouchi M, Benomar A, Lyahyai J, Ranera B, Acín C, el Hamidi M, Monzón M, Badiola JJ, Tligui N, Zaragoza P and Martín-Burriel I, 2009. PRNP haplotype distribution in Moroccan goats. Animal Genetics. 40(4), 565-568.

Seuberlich T, Botteron C, Benestad SL, Brünisholz H, Wyss R, Kihm U, Schwermer H, Friess M, Nicolier A, Heim D and Zurbriggen A, 2007. Atypical scrapie in a Swiss goat and implications for transmissible spongiform encephalopathy surveillance. Journal of Veterinary Diagnostic Investigation, 19(1), 2-8.

Sofianidis G, Psychas V, Billinis C, Spyrou V, Argyroudis S and Vlemmas I, 2006. Histopathological and immunohistochemical features of natural goat scrapie. Journal of Comparative Pathology, 135(2-3), 116-129.

Sofianidis G, Psychas V, Billinis C, Spyrou V, Argyroudis S and Vlemmas I, 2008. Atypical PrPSc Distribution in Goats Naturally Affected with scrapie. Journal of Comparative Pathology, 138(2-3), 90-101.

Stemshorn, BW, 1975. Case of scrapie in a goat. Canadian Veterinary Journal, 16(3), 84-86.

Sweeney T and Hanrahan JP, 2008. The evidence of associations between prion protein genotype and production, reproduction, and health traits in sheep. Veterinary Research, 39(4), 1-28. DOI: 10.1051/vetres:2008004 v08089 [pii]

Thuring CM, Erkens JH, Jacobs JG, Bossers A, Van Keulen LJ, Garssen GJ, Van Zijderveld FG, Ryder SJ, Groschup MH, Sweeney T and Langeveld JP, 2004. Discrimination between scrapie and bovine spongiform encephalopathy in sheep by molecular size, immunoreactivity, and glycoprofile of prion protein. Journal of Clinical Microbiology, 42(3), 972-980.

Toumazos, P 1988. First report of ovine scrapie in Cyprus. British Veterinary Journal, 144(1), 98-100.

Truscott JE and Ferguson NM, 2009a. Control of scrapie in the UK sheep population. Epidemiology and Infection, 137 (6), 775-86.

Truscott JE and Ferguson NM, 2009b. Transmission dynamics and mechanisms of endemicity of scrapie in the UK sheep population. Epidemiology and Infection, 137 (6), 762-764.

Vaccari G, Di Bari MA, Morelli L, Nonno R, Chiappini B, Antonucci G, Marcon S, Esposito E, Fazzi P, Palazzini N, Troiano P, Petrella A, Di Guardo G and Agrimi U, 2006. Identification of an allelic variant of the goat PrP gene associated with resistance to scrapie. Journal of General Virology, 87, 1395-1402.

Vaccari G, Panagiotidis CH, Acin C, Peletto S, Barillet F, Acutis P, Bossers A, Langeveld J, Van Keulen L, Sklaviadis T, Badiola JJ, Andreéoletti O, Groschup MH, Agrimi U, Foster J and Goldmann W, 2009. State-of-the-art review of goat TSE in the European Union, with special emphasis on PRNP genetics and epidemiology. Veterinary Research, 40(5), 1-18. DOI: 10.1051/vetres/2009031.

Van Keulen LJM, Vromans MEW and Van Zijderveld FG, 2002. Early and late pathogenesis of natural scrapie infection in sheep. Acta pathologica, Microbiologica et Immunologica 110(1), 23-32

White S, Herrmann-Hoesing L, O'Rourke K, Waldron D, Rowe J and Alverson J, 2008. Prion gene (PRNP) haplotype variation in United States goat breeds. Genetics Selection Evolution, 40, 553-561.

Wood JN, Done SH, Pritchard GC and Wooldridge MJ, 1992. Natural scrapie in goats: case histories and clinical signs. Veterinary Record 131, 66-8.

Wood JN and Done SH, 1992. Natural scrapie in goats: neuropathology. Veterinary Record 131, 93-96.

Zhang L, Li N, Fan B, Fang M and Xu W, 2004. PRNP polymorphisms in Chinese ovine, caprine and bovine breeds. Animal Genetics, 35, 457-461.

Zhou RY, Li XL, Li LH, Wang HY, Lu JG, 2008. Polymorphism of the PRNP gene in the main breeds of indigenous Chinese goats. Archives of Virology, 153, 979-982.

APPENDICES

A. EXPERIMENTAL AND CASE CONTROL STUDIES REPORTING ON *PRNP* ALLELES ASSOCIATED WITH RESISTANCE TO SCRAPIE

Type of study	*PRNP* variation	TSE agent	Exposure route	N of goats	Testing tissue targeted	Effect claimed by author(s)	Limitations	Reference
Experimental studies	142M	Sheep scrapie CH1641	Intra-cerebral (i.c.)	6	Brain tissue	Extended incubation period	- No infectivity study performed - No Lymphoreticular system tested	Goldmann et al., 1996
			Subcutaneous	4				
		Sheep-passaged ME7 scrapie	i.c.	12				
		BSE agent	i.c.	9				
			Subcutaneous	5				
	102G	Sheep-passaged SSBP/1	i.c.	5	Brain tissue	Extended incubation period	- No infectivity study performed - No Lymphoreticular system tested	Goldmann et al., 1998
Case control studies	143R 154H	Classical scrapie	n/a[10]	51	Cerebrum, brain stem, cerebellum	Resistant to clinical disease	- No infectivity study performed. - No Lymphoreticular system tested	Billinis et al., 2002
	222K	Classical scrapie	n/a - Linked in some of the studied outbreaks to subcutaneous administration of a vaccine against contagious agalactia (*Mycoplasma agalactiae*))	177	Obex	Protective against scrapie	- No infectivity study performed - No Lymphoreticular system tested	Acutis et al., 2006

[10] n/a = Non applicable. These are scrapie cases naturally acquired. When further information is presented in the study on potential route, this is included in also included here.

Type of study	*PRNP* variation	TSE agent	Exposure route	N of goats	Testing tissue targeted	Effect claimed by author(s)	Limitations	Reference
Case control studies	143R 154R/H (heterozygous)	Classical scrapie	n/a – Subcutaneous, linked to administration of a vaccine against contagious agalactia (*Mycoplasma agalactiae*))	100	Obex, Lymphoreticular system (tonsil, lymph nodes spleen, third eyelid)	Extended incubation period	- No infectivity study performed	Vaccari et al., 2006
	222K	Classical scrapie	n/a (subcutaneous?) Linked to administration of a vaccine against contagious agalactia (*Mycoplasma agalactiae*))	100	Obex, Lymphoreticular system (tonsil, lymph nodes spleen, third eyelid)	scrapie resistance	- No infectivity study performed	Vaccari et al., 2006
	146S, D 154H	Classical scrapie	n/a	250	Brain tissue.	Protection against scrapie	- No infectivity study performed - No Lymphoreticular system tested	Papasavva-Stylianou et al., 2007
	154H	Nor98	n/a	254	Obex.	Risk factor for Nor98 occurrence	- No infectivity study performed - No Lymphoreticular system tested	Colussi et al., 2008
	222K	Classical scrapie	n/a	254	Blood, spleen, ileum, mesenteric lymph nodes, tonsil and obex.	Protection against natural scrapie infection	- Positive when PrP^{Sc} present in at least one tissue, but not specified.	Barillet et al., 2009
	154H	Classical scrapie	n/a	254	Blood, spleen, ileum, mesenteric lymph nodes, tonsil and obex.	Protection against natural scrapie infection	- Positive when PrP^{Sc} present in at least one tissue, but not specified.	Barillet et al., 2009
	211Q	Classical scrapie	n/a	254	Blood, spleen, ileum, mesenteric lymph nodes, tonsil and obex.	Protection against natural scrapie infection	- Positive when PrP^{Sc} present in at least one tissue, but not specified.	Barillet et al., 2009

B. IMMUNE HISTO CHEMISTRY DETECTION OF PrP^{SC} IN LYMPHOID AND NERVOUS TISSUE OF GOATS WITH NATURAL SCRAPIE

Table 7: IHC PrPSc detection in lymphoid tissues and digestive tract of 19 goats naturally infected with scrapie. Animals were selected in a field herd on the basis of a PrPSc positive tonsil biopsy. Intensity of the IHC PrPSc deposits are semi quantitatively scaled : negative (-), minimal (+), light (++), moderate (+++) severe (++++).

Organ[1]	2289 cont	2244	6109	6053	9036	2229	2246	2275	2144	2279	9027	9034	9002	2298	2268	9009	9013	6076	2285	2260
Tonsil	-	+++	++++	++++	+++	++++	+++	++++	++++	+++	+++	++++	+++	++++	++++	++++	++++	++++	++++	++++
Oesophagus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Reticulum	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	++
Rumen	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-
Omasum	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Abomasum	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
ENS duo	-	-	-	-	-	-	-	-	-	0/+	0/+	0/+	-	+	+	+	+++	++	+	+
PP duo	-	+	ND	ND	ND	+++	ND	ND	++++	++++	++++	+++	ND	ND	+++	ND	ND	ND	ND	ND
MLN 25%	-	+++	-	+++-	++	+++	+++	++++	++++	++++	++++	+++	+++	+++	++++	++++	++++	+++	+++	+++
ENS jeju-25	-	-	-	-	-	-	-	0/+	-	0/+	0/+	0/+	0/+	+	+	+	+++	++	++	++
PP jeju-25%	-	++	ND	ND	ND	+++	ND	++++	++++	++++	++++	ND	ND	ND	++++	ND	ND	ND	ND	+++
MLN 25%	-	++	-	+++	+++	+++	+++	++++	++++	++++	++++	+++	+++	+++	++++	++++	++++	+++	+++	+++
ENS jeju-50	-	-	-	-	-	-	-	0/+-	-	0/+-	0/+-	+-	++	+	++	++	+++	++	+++	++
PP jeju 50%	-	++	++	ND	ND	+++	+++	+++	+++	+++	+++	ND	+++	ND	++++	ND	ND	ND	++++	++++
MLN 50%	-	+	+	++	++	+++	+++	++++	++++	++++	++++	+++	++++	++++	++++	++++	++++	++	++	++
ENS jeju-75	-	-	-	-	-	-	-	-	+	+	+	+	++	++	++	++	+++	++	++	++
PP jeju 75%	-	++	++	+++	+++	+++	+++	+++	+++	+++	++++	ND	ND	++++	++++	ND	ND	ND	++++	++++
MLN 75%	-	++	-	-	-	+++	+++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++
ENS ileon	-	-	-	-	+	-	-	++	+	+	++	++	++	++	+++	+++	+++	+++	+++	+++
PP ileon	-	++	++	++	++	+++	+++	+++	+++	+++	++++	ND	++++	++++	++++	++++	++++	++++	++++	++++
MLN ileon	-	++	-	+++	+++	+++	+++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++	++	++
ENS cæcum	-	-	-	-	-	-	-	++	+	++	++	++	++	++	+++	+++	+++	+++	+++	+++
PP cæcum	-	-	+++	ND	ND	+++	ND	ND	+++	ND	++++	ND+	ND	++++	++++	++++	ND	ND	++++	ND
Colic LN	-	-	-	++++	+++	+++	+++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++
Hepatic LN	-	-	0/+	+	0/+	+++	+++	++	++++	++++	+++	0/+	+++	++++	++++	++	++++	++	+++	++
Mediastinal LN	-	-	+	0/+	0/+	-	++	++	++	++	+++	++	++	++++	++++	++++	++++	++++	++	++
Prescapular LN	-	-	0/+	++	-	+/++	+++	++	+++	++	++	+	++	+++	+++	++++	++++	++++	+++	++++
Precrural LN	-	-	0/+	0/+	0/+	++	++	++	+	+++	+	-	++	++	+++	+++	+++	+++	+++	+++
Spleen	-	-	+	+	++	++	-	++	++	++	++	0/+	++	+	++	++	++	+	++	+
3 rd Eye Lid	-	-	-	++	+/++	-	++	+++	+++	+++	0/+	0/+	++++	++	+++	++++	++++	+++	++++	+++

Table 8: IHC PrPSc detection in obex and spinal cord of 19 goats naturally infected with scrapie. Animals were selected in a field herd on the basis of a PrPSc positive tonsil biopsy. Intensity of the IHC PrPSc deposits are semi quantitatively scaled : negative (-), minimal (+), light (++), moderate (+++) severe (++++).

Organ *	2289 cont	2244	6109	6053	9036	2229	2246	2275	2144	2279	9027	9034	9002	2298	2268	9009	9013	6076	2285	2260
Obex	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+
C1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+/++	++	+++	+++
C2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+/++	++	+++	+++
C3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+/++	++	+++	+++
C4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+/++	+/++	+++	+++
C5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+/++	+/++	+++	+++
C6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	++	++	+++	+++
C7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	++	+++	+++	+++
T1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0/+	+/++	+++	+++	++++	++++
T2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+++	+++	++++	++++
T3	-	-	-	-	-	-	-	-	-	-	-	0/+	0/+	+	+/++	+	+++	+++	++++	++++
T4	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+/++	+++	+++	++++	++++
T5	-	-	-	-	-	-	-	-	-	-	-	0/+	+	+	+/++	+/++	+++	+++	++++	++++
T6	-	-	-	-	-	-	-	-	-	-	-	0/+	+	+	+/++	++	+++	++++	++++	++++
T7	-	-	-	-	-	-	-	-	-	-	-	+	+	+	0/+	++	+++	++++	++++	++++
T8	-	-	-	-	-	-	-	-	-	-	-	0/+	0/+	+/++	+	++	+++	++++	++++	++++
T9	-	-	-	-	-	-	-	-	-	-	-	0/+	0/+	+/++	+	++	+++	+++	++++	++++
T10	-	-	-	-	-	-	-	-	-	-	-	0/+	0/+	-	+	++	+++	+++	++++	++++
T11	-	-	-	-	-	-	-	-	-	-	-	0/+	-	-	0/+	++	+++	+++	++++	++++
T12	-	-	-	-	-	-	-	-	-	-	-	0/+	-	-	0/+	+/++	+++	+++	++++	++++
T13	-	-	-	-	-	-	-	-	-	-	-	-	-	0/+	0/+	+/++	++	++	+++	+++
L1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+/++	++	++	++++	++++
L2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	++	++	++++	++++
L3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	++	++	++++	++++
L4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	++	+++	+++
L5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0/+	+	++	+++	+++
S1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0/+	+	+

[1] duo, duodenum ; jeju, jejunum ; ENS, enteric nervous system ; PP, Peyer's patches ; MLN, mesenteric lymph node. ND, not determined.
[2] C, cervical, T, thoracique, L, lombaire et S, sacré. ND, non determiné.

C. QUESTIONNAIRE FOR DATA COLLECTION ON GOAT FARMING AND BREEDING PRACTICES IN THE EU MEMBER STATES

Introduction

The organisation of goat breeding and genetic selection systems in the EU Member States has an impact on the feasibility and potential duration of any *PRNP* selection scheme for introducing alleles of the caprine prion protein gene into their national goat herds. This information is not readily available and so a questionnaire aimed at collecting data on goat farming and breeding practices in the different EU Member States (MSs) was developed and circulated among the EFSA BSE-TSE Network[11].

Questions were categorised in two different groups:

- Regarding the *goat population*:
    - Total numbers of goats in the country of interest[12];
    - breeds and their proportion in the total goat population;
    - are goat herd registers kept;
    - age structure of the goat population[12];
    - types of herds;
    - number of goats slaughtered yearly for human consumption[12];
    - age at slaughter[12].
- Regarding management, breeding and disease surveillance programmes:
    - Intensive versus extensive production systems;
    - are there any animal disease control programmes for goats (e.g. for CAE/ retroviruses) running?;
    - is there a national programme for breeding for TSE resistance in sheep in the country?;
    - is there any *PRNP* genotyping done in goats? If yes, how many are typed, which *PRNP* polymorphisms are looked at, and are the results per breed available? (2007 – 2008);
    - are records kept for *PRNP* genotyping in rams and could you give results for these in 2007 and 2008?;
    - is there a breeding strategy including handling of males, Artificial Insemination (AI);
    - is there a breeder association(s) controlling this ;

[11] This network is currently composed by representatives of 26 EU MSs who act as contact points on BSE-TSE risk assessment related matters, plus 12 representatives from further countries and international organisations who act as observers members.

[12] This was requested for the years 2006, 2007 and 2008.

- are there any genetic programs for improving production traits? For meat or milk type of goats?;
- how many herds are included in these breeding programmes and this compared to all herds?;
- Is there an organised milk industry? Industrialised, number of plants, etc?

Questions 10a and 10b of the questionnaire were cancelled, as there was misinterpretation between countries on the meaning of "rams" in the context of the questionnaire (some referred to adult breeding males sheep, others to adult breeding male goats or "billy-goats").

The full questionnaire can be found at the end of this Appendix.

Summary of the questionnaire replies

a) Overall response and breeds

Seventeen countries of the EU27 and Switzerland replied to the questionnaire. The size of their goat populations ranged from 10,000 to over 5,000,000 animals and, for those 12 countries providing the information, specific national breeds or cross-breeds formed over 50% of the total population.

Goat registers are kept, mostly in a centralised database, in the majority of those countries which responded to the questionnaire. In three countries, national registers were supplemented by breed society databases.

b) Age structure

There was variable reporting of the age structure of the live goat population in EU MSs and this data was not available at all from 4 countries, while one advised that this could not be obtained in the timeframe of the request. Only 4 countries were able to provide figures for each of the age ranges requested (*i.e.* less than 3 months, 3 to 6 months, 12 to 24 months and over 24 months) while the majority presented data for two age ranges, a) less than 12 months old and b) 12 or more months old. When detailed age-stratified data were reported, a higher proportion of goats were reported to be over 24 months old apart for one case where most animals fell in the age range 12 to 24 months. **The EFSA WG found inconsistencies in the returns from some member states which lowered their confidence on the usefulness of these data.**

c) Production systems

Countries were asked to indicate, and if possible quantify, both the type of goat herds regarding production (milk, meat, mixed) and species composition (goats only, sheep only or mixed herds). The majority of the responding countries reported higher percentages of either milk production herds (6 countries, 2 of them 100% milk herds) or mixed production herds (6 countries, 2 of them 100% mixed herds).

Thirteen countries reported they had an organised milk industry and the number of goat dairy plants varied from 2 to 16. Two countries provided extra detail on their primary production capability and goat milk collection companies.

Fifteen of the reporting countries keep goat herds in extensive or mixed husbandry systems, while two countries have mainly intensive husbandry systems. One country reported higher prevalence of "semi-intensive" production systems.

d) Slaughter statistics

Reported figures on slaughtered animals were quite constant between years, with variations of less than 10% in most of the cases. Regarding age at slaughter, only one country was able to provide details for the age ranges requested (same as per live goat data). Overall, stratification of the data on slaughter age was presented by most of the countries in the same fashion as per the live goat population (*i.e.* less than 12 months or 12 and over. Contrary to what it was reported for the live animal population, the majority of the goats slaughtered for human consumption were less than 12 months old. One country reported data for home slaughter separated from abattoir slaughter, were the former was eight fold higher than the number of goats slaughtered in licensed meat premises. Some inconsistencies in the returns from some member states were found.

e) Breeding for TSE resistance in Sheep, and *PRNP* typing of goats

Out of all the reporting EU MSs that reported positive TSE cases in sheep during 2007 (European Commission, 2009), four of them do not run a program for breeding for TSE resistance in sheep.

Eight of the reporting countries carry out same type of *PRNP* genotyping in goats. Some countries reported they did this on positive cases only, while a few (4) run *ad hoc* projects aiming at gathering data on *PRNP* genotype of their goat populations.

f) Goat health control programmes

Goat-specific disease control programs (beyond TSE monitoring) are carried out in 14 of the reporting countries. Common goat diseases under surveillance and control include: Brucellosis, Paratuberculosis, Pseudotuberculosis, Caprine arthritis encephalitis, Contagious agalaxy, Maedi-Visna and Blue tongue.

g) Use of artificial insemination (AI)

A breeding strategy for goats was reported by 12 countries, with 9 of these countries routinely used artificial insemination. Improving production traits was reported as part of a genetic program by 10 countries (mostly for milk producing goats), and this correlated with the same country keeping a breeding strategy. However, 2 reporting countries with a breeding strategy do not specify that this is for improving production traits. When asked about the number of herds included in the genetic program, 4 out of the 12 countries were able to provide further details.

Conclusions

- In general, there was variability in the amount and quality of data returned by EU MSs in the questionnaire. This may reflect the different levels of centralisation of data among the reporting countries – thus, countries with less centralised data system for goat herds may not be able to provide the requested information with great level of detail in the given timeframe – or it could represent real differences in the level of data control about goat herds in different countries.

- There is a high level of diversity on breeding programmes and management systems for goats in the different MS.

- Goat specific disease programs are present in most of the reporting countries. A breeding strategy in goats, mostly for production traits, is present in the majority of the countries and in all but one of those with reported populations of over 100,000 head.

- Based on the replies received, only a few EU MSs seem to have in place the necessary elements to introduce a breeding for resistance programme for goats.

- In 2007, when more detailed population data was available, 5 MSs accounted for over 85% of the total goat population of the 18 reporting countries.

Blank questionnaire

1.	Yes	No	System
Are any goat herd registers kept?			

2.	2006	2007	2008
Total number of goats			
What are the main breeds of goats (as proportion of the total goat population)?			
1			
2			
3			
4			
5			

3.	2006	2007	2008
Age structure of the goat population?			
o **Under 3 months**			
o **Between 3 and 6 months**			
o **Between 6 -12 months**			
o **Between 12 – 24 months**			
o **Over 24 months**			

4a	Milk	Meat	Mixed
Types of goat flocks (percentage of total)?			

4b	Goats only	Goats/sheep	Sheep only
Types of goat flocks (percentage of total)?			

5.	2006	2007	2008
Number of goats slaughtered per year?			
o **Under 3 months**			
o **Between 3 and 6 months**			
o **Between 6 -12 months**			
o **Between 12 – 24 months**			
o **Over 24 months**			

Comments

Please note: question 6 relates to sheep!

6.	YES	NO	Instance dealing with it (name, contact and address)
Is there a national programmes for breeding for TSE resistance in sheep in your country?			

7.	Intensive	extensive	MIXED
What is the main goat husbandry system?			

8.	YES	NO	Instance dealing with it (name, contact and address)
Are there any animal disease control programmes for goats on-going? **If yes, please provide details on each programmes (e.g. for CAE/retroviruses)?**			
1.			
2.			
3.			

Comments

9a	Yes	No
Is there any *PRNP* genotyping done in goats? If yes, consider also 9b and 9c		

9b	2007	2008
***PRNP* genotyping in goats: If yes, which *PRNP* polymorphisms are looked at?**		
1		
2		
3		

9c	2007	2008
***PRNP* genotyping in goats? If yes: results per breed available? (2007 – 2008)?**		
1		
2		
3		

Comments

10a *(cancelled)*	Yes	No	2007	2008
Are records kept for *PRNP* genotyping in rams? If yes could you give results per breed available (2007 and 2008)?				
1				
2				
3				

10b *(cancelled)*	2007	2008
If *PRNP* genotyping in rams? Indicate the *PRNP* polymorphisms that are looked at, and give the results per breed available (2007 and 2008)?		
1		
2		
3		

Comments

11.	YES	NO	Instance dealing with it (name, contact and address)
Is there a breeding strategy for goats including handling of males?			
Is Artificial Insemination used in goats breeding programmes?			
Is there a goat breeder association(s) supervising this?			

12.	YES	NO	Instance dealing with it (name, contact and address)
Are there any genetic programmes in goats for improving production traits?			
For meat production?			
For milk production?			

13.	Herds included	As per total herds
If a genetic programme in goats for improving production traits exists, how many flocks are included as compared to the total flocks?		
For meat production?		
For milk production?		

14.	YES	NO	Number of plants involved?	Instance dealing with it (name, contact and address)
Is there an organised goat milk industry? If yes how many plants does this include?				

Comments