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SCIENTIFIC OPINION

Statement on a protocol for additional data collection based on the EFSA recommendations about resistance to scrapie in goats in Cyprus[1]

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

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ABSTRACT

Following a request from the European Commission (EC), the Panel on Biological Hazards (BIOHAZ) was asked to deliver a technical advice on a research protocol prepared by the TSE Community Reference Laboratory (CRL) in the UK, aiming at supplying, in a 6-12 months time frame, additional data supporting the possibility for breeding for resistance to scrapie in goats in Cyprus. This protocol considers six research areas on resistance to scrapie in goats and follows an earlier EFSA scientific opinion, which evaluated a pilot project carried out in Cyprus. The BIOHAZ Panel was requested by the EC to evaluate this second CRL protocol. The BIOHAZ panel concluded that the protocol is an extension and an improvement of the earlier pilot project. The BIOHAZ panel made a series of detailed conclusions and recommendations for further development of the CRL protocol. Some of the studies will generate results in the time frame proposed by the EC, while other experiments may not do so. However, it is also recommended to start these as soon as possible, as valuable intermediate and final results are to be expected supporting the decision on implementing a breeding for resistance against scrapie in goats in Cyprus in particular and in all EU MS in general.

KEY WORDS

TSE, Scrapie, Goat, genetic resistance, breeding program, protocol evaluation, Cyprus.

SUMMARY

Following a request from the European Commission (EC), the Panel on Biological Hazards was asked to deliver a technical advice on a protocol for additional data collection supporting a possible breeding for resistance in goats in Cyprus based on the EFSA recommendations about resistance to

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1 On request of the European Commission, Question No EFSA-Q-2009-00631, adopted on 09 July 2009.

2 Panel members: Olivier Andreoletti, Herbert Budka, Sava Buncic, John D Collins, John Griffin, Tine Hald, Arie Hendric Havelaar, James Hope, Günter Klein, James McLauchlin, Winy Messens, Christine Müller-Graf, Christophe Nguyen-The, Birgit Noerrung, Luisa Peixe, Miguel Prieto Maradona, Antonia Ricci, John Sofos, John Threlfall, Ivar Vågsholm, Emmanuel Vanopdenbosch. One member of the Panel did not participate in the discussion on the subject referred to above because of potential conflicts of interest identified in accordance with the EFSA policy on declarations of interests.

3 This statement is based on major contributions from: Olivier Andreoletti, Pier Luigi Acutis, Herbert Budka, Jean Michel Elsen, Wilfred Goldmann and Emmanuel Vanopdenbosch.

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scrapie in goats in a previous EFSA Scientific Opinion on genetic TSE resistance in goats (EFSA, 2009).

Earlier in 2008, the Cypriot authorities sent to the Directorate-General for Health and Consumers (DG SANCO) of the EC the final results of an EC funded pilot project study conducted in Cyprus. This pilot project intended to investigate the association of variability of the PrP^{C} protein gene (*PRNP*) with resistance or susceptibility to TSE in goats, and in particular to Classical scrapie, which remains up to date unknown. The report of this pilot study presented a case control study in Cypriot goat herds aiming at the identification of the effect of *PRNP* polymorphisms on TSE susceptibility. The results in this report, if were to be confirmed, could be very interesting in view of a possible future EU policy as regards scrapie control measures in goats. EFSA was requested by the EC to provide an opinion on the scientific validity of this Cypriot study and to indicate to what extent the information contained in this study can be used as relevant tools to control Classical scrapie in goats in Cyprus. The EFSA Scientific Opinion (EFSA, 2009) concluded that, while the results are encouraging, it was too early to recommend breeding for the 'resistant' *PRNP* polymorphisms in goats in Cyprus and further research needed to be done. In support of the conclusions, six areas of further research were formulated in the opinion. Following this publication the European Commission tasked the Community Reference Laboratory (CRL) for TSEs to draft a new protocol for additional studies in order to adequately supplement the initial findings of the Cypriot pilot study and following the six areas of research as given in the EFSA Scientific Opinion (EFSA, 2009).

EFSA was tasked by the EC to evaluate this protocol of the TSE CRL. The protocol addresses the 6 areas of research that were suggested in the previous EFSA Scientific Opinion and comments on the feasibility of the suggested experiments. The EFSA Panel on Biological Hazards concluded that the protocol is mainly an extension and an improvement of the case control study that was presented in the first Cypriot pilot project. However, the low frequencies of the *PRNP* alleles of interest in the population compromise the statistical power of the present proposal. Since the potential diversity of TSE Agents in Cypriot goats cannot be foreseen, the systematic screening of all samples available by high throughput biochemical test would be advisable rather than the proposed reduced panel of isolates. It was further concluded that in vitro conversion assays are available and can be used to document potential resistance associated to the D146 and S146 *PRNP* alleles. Standardised bioassays of TSE isolates of interest, as used by the Strain Typing Expert Group (STEG), are necessary to document the biodiversity of TSE strains in goats in Cyprus. Considering the limited frequencies of the *PRNP* alleles of interest and the assumed prevalence of Classical scrapie in infected herds, it was concluded that the proposed experiments are unlikely to document the distribution of PrP^{Sc} in homozygous goats but could document, within some limits, the distribution of PrP^{Sc} in heterozygous goats.

Finally it was concluded that the modelling approach on feasibility and duration of selection for resistant alleles is subject to the availability of input data. This protocol cannot substitute the experiments advised in the earlier EFSA Scientific Opinion on cohort follow up and experimental inoculation. These experiments remain crucial for definitive assessment of D146 and S146 *PRNP* alleles' resistance.

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BACKGROUND

It is scientifically recognised since several years that some *PRNP* polymorphisms 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 EU level of breeding programs 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 program for TSE resistance in sheep[4].

In goats, the association of *PRNP* variations with resistance or susceptibility to TSE, and in particular to Classical scrapie, remains unknown. Earlier in 2008, the Cypriot authorities sent to DG SANCO the final results of an EC funded pilot project study conducted in Cyprus (also submitted for publication to "The Veterinary Journal"). The report of this pilot study presented a case control study in Cypriot goat herds aiming at the identification of the effect of *PRNP* polymorphisms on TSE susceptibility. The results in this pilot project indicated that polymorphisms of the PrP gene at codons 146 and 154 could be associated with resistance/susceptibility to classical scrapie in goats in Cyprus. As these results, if confirmed could be very interesting in view of a possible future EU policy as regards scrapie control measures in goats, EFSA was requested by the EC (DG SANCO) to provide an opinion on the scientific validity of this Cypriot study and to indicate to what extent the information contained in this study can be used as relevant tools to control classical scrapie in goats in Cyprus.

Following this request, EFSA published a Scientific Opinion (EFSA, 2009) on genetic TSE resistance in goats in consideration of this pilot project study carried out in Cyprus. The BIOHAZ Panel was requested to assess the scientific validity of the study and to indicate to what extent and based on this study genetic breeding can be used as a control program for Classical scrapie in goats in Cyprus.

The BIOHAZ Panel concluded that the study conducted in Cyprus brings additional proof of a potential lower susceptibility to Classical scrapie in goats in H154, D146 and S146 *PRNP* allele carriers and can be considered as encouraging information on the path for identifying *PRNP* polymorphisms that could be used as part of a genetic strategy to control and eradicate TSE agents in goats. However, the BIOHAZ Panel concluded at the same time that the study on its own was an insufficient basis to evaluate accurately and reliably the efficacy and the potential adverse consequence of the large-scale breeding for H154, D146 and S146 *PRNP* alleles as a tool to control and eradicate Classical scrapie in Cyprus and recommended new investigations in order to assess the efficacy of breeding for the PRNP alleles in goat populations in Cyprus. In addition, the operational possibility to conduct these alleles selection in the Cypriot goat population and the potential adverse effect of such selection on genetic variability in the Cypriot goat breeds should be evaluated. In addition, a set of recommendations for further research were formulated in the opinion.

Following this publication a video conference was organised by DG SANCO on 21/04/2009 between EC, EFSA and the Community Reference Laboratory (CRL) for TSE to discuss the EFSA opinion and its conclusions and recommendations. In view of the importance of the initial findings and the potential for their use within the framework of the TSE eradication in the goat population, the Commission tasked the CRL for TSEs to draft, based on the EFSA conclusions and recommendations, a protocol for additional studies in order to supplement the initial findings of the Cypriot pilot study.

The CRL for TSEs elaborated a draft protocol for additional experiments and data collection based on the EFSA recommendations and with the aim to progress knowledge about resistance to scrapie in goats in Cyprus. This protocol should focus on experiments that can be undertaken in a relatively

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

short time frame, *i.e.* within 6-12 months. It comprises of suggested work in the areas of the selection of herds, genotyping of animals, selection of cases for a screening cull, screening of tissues and some modeling exercises.

This protocol was forwarded to DG SANCO of the EC, and in turn DG SANCO asked EFSA on 4th June 2009 to provide, by end of July 2009, a technical advice on this report and to indicate if the protocol suggested by the TSE CRL is appropriate to adequately supplement the initial findings of the Cypriot study and address the recommendations of the EFSA opinion published on 24th March 2009. The protocol addresses the 6 areas of research as suggested in the EFSA Scientific Opinion (EFSA, 2009) and comments on the feasibility of the suggested experiments. The EFSA technical advice given in this statement elaborates further on the feasibility of the experiments both in terms of timing within the requested time frame (6-12 months) and technical their technical aspects.

ACKNOWLEDGEMENTS

The European Food Safety Authority wishes to thank the members of the Working Group on the Technical Advice on a protocol for additional data collection based on the EFSA recommendations about resistance to scrapie in goats in Cyprus for the preparation of this opinion: Olivier Andreoletti (Rapporteur), Pier Luigi Acutis, Herbert Budka, Jean Michel Elsen, Wilfred Goldmann and Emmanuel Vanopdenbosch (Chair). At the same time, assistance to the working group by the following Hearing Experts is gratefully acknowledged: James Hope, Penelope Papasavva-Stylianou and Otto Windl.

ASSESSMENT

1. Introduction

The aim of the document prepared by the TSE CRL and presented by the European Commission to EFSA is to provide a protocol for additional data collection based on the EFSA opinion on 'Genetic TSE Resistance in Goats' (EFSA, 2009) to progress knowledge about resistance to scrapie in goats in Cyprus. The protocol of the TSE CRL is included in the EFSA web as a separate document to this EFSA Statement.

2. Background of the request for Technical Advice

Scrapie is a problem for the sheep and goat industry in Cyprus. Control of the disease in sheep through breeding for resistance is well underway in Cyprus, but breeding for TSE resistance in goats is not established as there is limited knowledge about the genetic resistance of goats to scrapie in Cyprus and worldwide. A pilot study was carried out between 2006 and 2007 in goats in Cyprus, which addressed the role that specific *PRNP* alleles have in contributing to the absence of clinical scrapie in an animal/herd. This study was taken into consideration by a European Food Safety Authority (EFSA) working group and led to an EFSA Scientific Opinion, published in March 2009 (EFSA, 2009).

The EFSA Scientific Opinion concluded that, while the results are encouraging, it was too early to recommend breeding for the 'resistant' *PRNP* alleles in goats in Cyprus and further research needs to be done. Six areas of further research were identified and experimental approaches were stipulated (see below).

The EC DG-SANCO convened a consultation meeting with EFSA and the TSE CRL, and asked the TSE CRL to draft a protocol for short term research over the next 6 to 12 months in order to supplement the initial findings of the Cypriot study. This protocol is the subject of the current Statement.

3. Evaluation

3.1 Overview of research suggested by the EFSA Scientific Opinion

The published opinion of the EFSA working group (EFSA, 2009) identified six areas for further research (chapter 4 of the published opinion).

The experiments in these six areas are outlined in the Scientific Opinion and it is clear that the collection of relevant data would need a research programme with a duration of several years. Furthermore, neither the experiments in this protocol nor the more elaborated experiments outlined in the Scientific Opinion would eliminate all uncertainty of the outcome of a policy that could be based on these findings, *i.e.* a selective cull of susceptible animals and the breeding for resistant animals. Research findings may only reduce the uncertainty of the outcome of such policies.

In practical terms, the protocol below focuses on the experiments that can be undertaken in a relatively short time frame, *i.e.* the selection of herds, genotyping of animals, selection of cases for a screening cull, screening of tissues and some modelling exercises. It will not be possible to get data from challenge studies or via herd monitoring protocols, as this would take several years.

The six areas of recommended further research are:

1. Effect of H154, D146 and S146 *PRNP* alleles on individual susceptibility to classical scrapie infection.

2. TSE agent diversity and resistance/susceptibility associated to H154, D146 and S146 *PRNP* alleles.

3. Effect of H154, D146 and S146 *PRNP* alleles on classical scrapie pathogenesis.

4. Effect of H154, D146 and S146 PRNP alleles on the dynamics of TSE agent transmission in affected herds.

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

6. Capacity for selection and diffusion of *PRNP* allele in Cypriot goat population.

The following general comments have to be considered before going into detail in evaluating the individual sections addressed in the protocol:

- The BIOHAZ Panel and the Working Group (WG) understands that this protocol is intended as a first step that cannot replace a complete research programme on genetic resistance in goats in Cyprus. More exhaustive research protocols will have to be developed based on the conclusions and recommendations made here.

- It is very important to indicate that even in case the effect of one of the identified *PRNP* alleles towards resistance to Classical scrapie would be substantiated in the next 18 months, the selection for such *PRNP* alleles in the general goat population or even in Classical scrapie affected goats (considering their number in Cyprus) will require several years (like occurred with the performances of the breeding for TSE resistance policy in sheep). The main constraints in the system are the initial frequencies of the allele of potential interest and the breeding system organisation.

- The use of resources, external to the TSE CRL, which are available in EU for particular experiments, could substantially improve several aspects of the proposed protocol.

- The most relevant experiments that are necessary to assess and measure the level of resistance to Classical scrapie infection in goats carrying particular *PRNP* alleles will clearly exceed the allocated time frame of 6-12 months given by the EC. However, this should not prevent their initiation. The earlier those critical experiments will start the sooner their results will become available.

  Moreover, even if those experiments cannot be completed in the time frame allocated by the EC, they are likely to provide intermediate results that will be extremely valuable for reconsidering the interest of the selection of the proposed *PRNP* alleles.

- Although atypical scrapie is not the subject of this mandate, it should be noted that THE H154 *PRNP* allele seems associated with a higher risk of developing Atypical scrapie in goats (Colussi *et al*., 2008). Thus, the selection of animals bearing such *PRNP* allele in the Cyprus goat population could have adverse effect.

In the assessment hereunder reference is made to each of the six areas for research addressed in the EFSA Scientific Opinion. *Relevant extracts from the TSE CRL protocol are quoted in italics,* and comments made immediately below address the opinion of the BIOHAZ Panel and its WG of experts

or highlight issues that have to be considered. Individual conclusions are included in each section. Final conclusions and recommendations are summarised at the end.

3.2. Research Area 1: Effect of H154, D146 and S146 *PRNP* alleles on individual susceptibility to Classical scrapie infection.

Two types of experiments were suggested in the EFSA Scientific Opinion (EFSA, 2009):

a) oral and intra-cerebral challenge of goats homozygous and heterozygous for the alleles of interest;

b) production of animals harbouring the genotypes of interest in a number of affected herds and follow up evaluation of these goats.

Regarding the suggested experiment (a), the TSE CRL protocol indicates that:

"The inoculation experiments would take several years to complete and cannot be done in the timeframe of this protocol."

The BIOHAZ Panel and its WG of experts would like to point out that:

The fact that the inoculation experiment will probably exceed the allocated time frame of 6-12 months should not be a reason not to initiate them. These experiments are of high relevance to assess and measure the resistance towards Classical scrapie infection in animals bearing the particular *PRNP* alleles. The earlier the experiments will start, the sooner their results will be available.

Moreover, if some experiment may not been completed in the time frame allocated by the EC, they will provide intermediate results (*e.g.* pre-determined kill point with examination of peripheral tissue in orally and intra-cerebrally challenged animals) that will be extremely valuable for reconsidering the interest of the selection of the proposed alleles.

Furthermore, the TSE CRL protocol indicates that:

"A plan for such an experiment could be devised in the framework of the protocol. However, such a plan has to address, amongst other considerations, the important points of the selection of scrapie free animals (breed/genotype/country of origin/ biosecurity of facility in which experiment would be conducted) and the selection of appropriate inocula (there is currently little information on the diversity of natural isolates in Cyprus, or their potential host genotype tropisms)."

The BIOHAZ Panel and its WG of experts would like to point out that:

Animals carrying the H154, D146 or S146 *PRNP* alleles (including both homozygous and heterozygous goats) are currently available in the two 'Government-owned' goats herds, described here in section 3.6. bellow. These two herds are considered by the Cypriot authorities to be Classical scrapie free. These animals and their progeny could be used to rapidly start the oral and intra-cerebral inoculation experiments.

The realisation of the experiments within a Classical scrapie affected herd would only require basic precaution.

Furthermore, the TSE CRL protocol indicates that:

"It would only be possible to cover a few of these variables at a practical level, and it should be anticipated that the group sizes would not be considered statistically robust. The experiment itself, if thought to be necessary, would have to happen in parallel and contemporaneously with the practical measures to control scrapie in Cyprus."

The BIOHAZ Panel and its WG of experts would like to point out that:

If goats bearing a certain *PRNP* genotype are strongly resistant to infection, reducing the number of individuals will be sufficient to substantiate it (groups of 5 animals)

Regarding the suggested experiment (b), the TSE CRL protocol indicates that:

"The production of animals harbouring the genotypes of interest is in all likelihood unnecessary as it is our understanding that in most affected herds there are already a considerable number of animals present with these genotypes."

The BIOHAZ Panel and its WG of experts would like to point out that:

The initial pilot protocol was suggested in order to introduce or create cohorts of animals harbouring the *PRNP* alleles of interest in the same environment. This would mean that all the studied goats are submitted to a similar infection pressure and herd management practise. Investigating a group of animals from different birth cohorts in a herd at a determined time point does not allow to take into account such differences (in particular in term of infection pressure) and reduces the power of the performed analysis.

Furthermore, the TSE CRL protocol indicates that:

"The genotypes (codons 146 and 154) of all goats in Cyprus is expected to be determined by the end of 2009 provided that the tender will be awarded without any appeals from other tenderers, with the potential partial funding of the European Commission. The genotypes of selected herds will be available by the end of July 2009. While following up animals with resistant genotypes over several years will not be possible within this protocol, some estimate of true resistance (i.e. absence of PrPSc as opposed to absence of clinical disease) to scrapie could be obtained from the screening of selected animals.

These will be adult animals with homozygous and heterozygous resistant alleles, at least 6 years old, culled for management purposes from herds with confirmed cases of scrapie for at least the lifetime of the selected animals for testing, i.e., 6 years. This will ensure that culled animals have been exposed to scrapie throughout their lifetime."

The BIOHAZ Panel and its WG of experts would like to point out that:

To fulfil the objectives described in the EFSA Scientific Opinion, such experiment would require that each animal belonging to the same cohort as the investigated animals (carrying the *PRNP* allele of interest) will have been genotyped and subjected to adequate Classical scrapie detection test when leaving the herd. Otherwise it will not be possible to determine if:

- the studied animals were exposed to an efficient infectious pressure during their lifespan;
- some of the animals bearing the *PRNP* alleles of interest did not die of Classical scrapie at earlier age.

Furthermore, the TSE CRL protocol indicates that:

"It is anticipated that 2000 genotyped animals will be available and the average genotype distribution will be for codon 146 (assuming the distribution is similar to the frequencies in the pilot study, see Annex 1): ~ 75% NN, >10% ND, >10% NS, <1% DD, <1% SS, <1%DS. It is expected that around 20 animals will therefore have each of the rare genotypes DD, SS, and DS out of the 2000 genotyped animals. All animals with DD, SS and DS genotypes, as well as all animals with the

genotypes NS and ND (over 200 each) make a minimum total of 460 resistant or semi-resistant animals to be examined, plus an equal number of susceptible NN animals matched by age and herd.

For the codon 154 (R or H), it is expected that approximately 10% of animals are heterozygous RH and a small amount (0.5 – 1%) will be homozygous HH (see Annex 1).”

The BIOHAZ Panel and its WG of experts would like to point out that:

Considering the number of investigated animals according to proposed *PRNP* allele frequencies, the number of potentially infected animals (with the hypothesis that all the *PRNP* genotypes would be equally susceptible to Classical scrapie and that investigated herds would be affected under a 5% or 10 % prevalence) are presented in Table 1.

Table 1. Expected maximum number of Scrapie cases considering a 5% or 10% prevalence in the different allele combination subgroups. Calculated from data presented in Annex 1 of the TSE CRL protocol, included in this statement in Appendix A.

	Allele combinations							
	NN (non H carrier)	**HH**	**H/R**	**ND**	**NS**	**DD**	**SS**	**DS**
Frequency	0.64	0.01	0.1	0.1	0.1	0.01	0.01	0.01
Maximum Number of animals	1,280	20	200	200	200	20	20	20
Maximum number of cases (5%-10% prevalence)	64-128	1-2	10-20	10-20	10-20	1-2	1-2	1-2

Under the hypothesis of a similar capacity of detection of cases in the different *PRNP* genotypes and in the absence of bias due to age and herd, the minimal number of HH, DD, SS and DS animals to be investigated for demonstrating a difference of susceptibility by comparison with wild-type, would have to be multiplied by at least 3.5 or 2 (respectively for a target prevalence of 5% or 10 %). In homozygous animals, the proposed experiments (2,000 animals investigated) would have too low power to demonstrate statistical difference. At least 7,000 animals would have to be investigated in order to have a statistically significant result at $p < 0.05$ (after exclusion of potential bias in the population).

Furthermore, the CRL protocol indicates that:

“Therefore, assuming the presence among the 460 NN controls of approximately 46 RH and 4 HH animals, the rest of the homozygous HH (10) and heterozygous RH (108) in the remaining NN cull population (1080) will be sampled and tested. Thus the total number of animals to be sampled and tested to cover the resistant and semi-resistant alleles of codons 146 and 154 independently of each other will be 1,038, distributed as follows (assuming the presence of all possible combination of alleles):

Codon 154: 154 NN/RH, 14 NN/HH and 410 NN/RR

Codon 146: 200 NS/RH-HH-RR, 200 ND/RH-HH-RR, 20 SS/RH-HH-RR, 20 DS/RH-HH-RR and 20 DD/RH-HH-RR (RH-HH-RR referring to codon 154 genotypes).

The number of controls for codon 154 within the 1038 tested animals will be approximately 820 (animals with the genotype RR at codon 154).

It should be emphasised again that the number of animals tested in each of the available genotypes may vary if the allele frequency in the selected herds for testing is different from the one reported by the pilot study in Annex 1.

As H154 does occur, unlike S146 and D146, on P240 and S240, the codon 240 would need to be determined in each animal that harbours H154. In addition, the coupling of H at codon 154 with P or S at codon 240 would need to be determined in all animals that are heterozygous for both codons. This more extensive genetic analysis cannot be done within the time frame of the protocol, but it could be done in subsequent years. Care has to be taken that genomic DNA or fresh-frozen tissue is kept from all animals.

The analysis of obex and tonsil will be done by immunohistochemistry."

The BIOHAZ Panel and its WG of experts would like to point out that:

- For this initial screening, the use of biochemical rapid tests will be quicker and of lower (labour) cost than by immunohistochemistry (IHC). However, IHC would allow validating the presence of lymphoid tissue in case of improper sampling.

In summary for Research Area 1:

- This protocol is an extension of the case control study that was presented in the first pilot project. In this extension, the scrapie status of the newly investigated goats will be properly assessed (PrP^{Sc} presence in tonsil and obex) which is a clear improvement compared to the pilot project study. Hence, this protocol could start immediately and could be completed within the proposed time frame. However, considering the frequencies of the alleles of interest in the population, the capacity of this protocol to bring additional data on resistance will be insufficient to demonstrate statistically significant differences of susceptibility in homozygous D146 and S146 and H154 goats if only 2,000 animals will be tested.

- This protocol cannot substitute the advised experiments on cohort follow up and experimental inoculation, which remain crucial for definitive assessment of D146 and S146 *PRNP* allele resistance. Consequently, it would be highly advisable that both the cohort follow up study and the experimental inoculation start immediately, however, these studies are unable to produce results within the time frame given by the EC (6-12 months).

3.3. Research Area 2: TSE agent diversity and resistance/susceptibility associated to H154, D146 and S146 *PRNP* alleles.

The following experiments were suggested in the EFSA Scientific Opinion (EFSA, 2009):

a) Rational characterisation (using biochemistry and bioassay) of a panel of Cypriot TSE isolates

b) Experimental transmission of a panel of TSE isolates (including BSE and Atypical scrapie) in goats or transgenic mice harbouring the resistant alleles;

c) In vitro conversion assays using a panel of TSE isolates.

Regarding point (a), the TSE CRL protocol indicates that:

"Around 400 samples of scrapie-affected animals were examined between 2005 and 2009 by discriminatory Western blot and a BSE-specific pattern was excluded for all of them. These cases were sourced from 305 herds and mixed flocks covering all geographical areas in Cyprus where

scrapie cases had been confirmed and represented approximately half of all infected herds (e.g. 62.6% of all infected goat herds in 2007 and 42.5% of the all infected herds in 2009).”

The BIOHAZ Panel and its WG of experts would like to point out that:

- These samples represent an ideal panel of samples (number of involved herds and geographical distribution of cases) to characterise the diversity of TSE Agents circulating in the Cyprus goat population. The sample set is available and ready for investigation. The screening of all these samples will maximise the possibility to identify potential biochemical diversity in Cypriot goats TSE cases. Hence, this can start immediately and will generate results within the time frame of 6-12 months.

Furthermore, the TSE CRL protocol indicates that:

“Given the lack of information on the number of field strains present in the Cypriot goat population, the prevalence of strains in scrapie-affected herds and the presence of co-infection with multiple strains, it is not possible to estimate the sample size required to detect strain diversity if present at all. However the number of cases and affected herds widely geographically distributed will maximize the chances to detect strain diversity.

While a BSE-specific pattern was excluded for all of them, one unusual case was however identified and is currently being examined by the Strain-typing Expert Group (STEG) of the CRL. A further case may be unusual as well, but this is currently unverified. The remaining results will be scrutinised and some of the Western blots will be repeated, if necessary. Due to the bad quality of the frozen tissue particularly form the early years of submission, it will be necessary to repeat the analysis of approximately 100 samples. It will be possible to replace them with samples from the same or neighbouring herds from the Cypriot archive. Similarly, fixed tissue of all 400 samples can be retrieved from the Cypriot archive, and analysed by IHC methods. It will be necessary to determine the genotype (determine codons 146 and 154) of all cases from the brain material.

Further analysis of some samples might be necessary, should the primary tests (Western blot and IHC) be unusual. The biochemical evaluation of these samples will depend to some extent on the available amount of tissue. We would anticipate that at most 10% of these samples would be subject to further analysis, such as PK-titration, guanidine melting, conformation-dependent immunoassay (CDI), and the detection of a C-terminal protease resistant prion protein fragment that distinguishes ‘CH1641-like’ scrapie from BSE (Baron et al., 2008, PLoS Pathog 4(8)).”

The BIOHAZ Panel and its WG of experts would like to point out that:

- The methodology applied only allows identifying samples that would share some phenotypic features evocative for BSE. It does not provide elements on the possible diversity in isolates not harbouring the features of the BSE Agent.

- The biochemical approach for investigating TSE Agents’ diversity in goats is currently under evaluation in a European Union (EU) funded project, so called ‘EU goatBSE project’ and with reference FOOD-CT-2006-36353[5]. It would certainly be beneficial to use a similar approach as the one described in the ‘EU goatBSE project’ for studying Cypriot scrapie isolates (comparison between results obtained / up to date approach).

- In particular and beyond the capacity to identify BSE in small ruminants, the carcinoembryonic (CEA) ELISA typing method (Simon *et* al., 2008) was able to demonstrate

[5] Details of the the ‘EU goatBSE project’ reference FOOD-CT-2006-36353 can be found at www.goatbse.eu/site/index.php?option=com_content&view=article&id=8&Itemid=4

a certain biochemical diversity in TSE isolates. This methodology also showed evidence of biochemical diversity in EU goat TSE isolates ('EU goatBSE project', reference FOOD-CT-2006-36353). Because it is an ELISA method it appears more adapted than other approaches for treating a large number of samples in a reduced time frame.

Furthermore, the TSE CRL protocol indicates that:

"Unusual cases will be further analysed alongside established Strain-Type Expert Group (STEG) procedures.

Three samples will be inoculated within the time frame of the protocol (the two that have shown some different characteristics, see above, and one 'ordinary' case for comparison)."

The BIOHAZ Panel and its WG of experts would like to point out that:

- Determining the number of samples (very low number) at this stage is probably not the best approach. The number of apparently different TSE agents in Cyprus could be greater than 3.

Furthermore, the TSE CRL protocol indicates that:

"It is proposed to use the transgenic lines tg338 and TgshpXI for this purpose. These lines are sensitive models for classical scrapie and some preliminary data already exists for UK goat isolates."

The BIOHAZ Panel and its WG of experts would like to point out that:

The use of a similar approach as the one used by the STEG would be a beneficial comparison. It would also allow comparing the obtained results with those already available for sheep scrapie isolates across the EU (documentation of the variability of TSE agents between goat and sheep). In that perspective, the additional use of other PrP transgenic (Tg) mice model would complete the mouse panel.

Furthermore, the TSE CRL protocol indicates that:

"The completion time of such bioassays is entirely dependent on the incubation period of the disease, and would probably not be available until some time in 2010. Ultimately, a representative panel of up to 10 cases could be selected for bioassays."

The BIOHAZ Panel and its WG of experts would like to point out that:

The incubation period for scrapie in goats is indeed unpredictable. However, for most of the sheep Classical scrapie isolates tested so far in Tg 338 and Tg XI and Tg bov gave an incubation period of less than 300 days in at least one model. Even when incomplete, bioassay results can help to establish the biological difference between TSE agents.

Furthermore, the CRL protocol indicates that:

"The bioassays, if thought to be necessary, would have to be undertaken in parallel to the practical measures to control scrapie in Cyprus."

The BIOHAZ Panel and its WG of experts would like to point out that:

Bioassays are the gold standard to establish the identity/difference between TSE Agents. Consequently, they will be necessary in the context of this protocol.

Regarding the point (b), the TSE CRL protocol indicates that:

"See 1a regarding goat transmissions. It should be added that transgenic mouse models carrying the various goat alleles are not available and would need to be established. The experiments, if thought to be necessary, may happen in parallel to the practical measures to control scrapie in Cyprus."

The BIOHAZ Panel and its WG of experts would like to point out that:

- Tg goat mice are currently being produced in Germany and Spain in the framework of the 'EU goatBSE project' and other national funded projects. It would be wise to contact laboratories involved in producing those tools.

Regarding the suggested experiment (c), the TSE CRL protocol indicates that:

"These assays need to be established and cannot be done in the framework of this protocol. The experiments, if thought to be necessary, may happen in parallel to the practical measures to control scrapie in Cyprus."

The BIOHAZ Panel and its WG of experts would like to point out that:

- This is maybe true for the TSE CRL, however the Friedrich Loeffler Institute (FLI, Germany) has developed in vitro assays for that purpose with already available data for codons 154 and 146('EU goatBSE project', reference FOOD CT- 2006-36353). The use of this technique on a panel of properly selected goat TSE isolates (including BSE) would provide very valuable elements.

In summary, for research area 2:

- With regards to the rational characterisation (using biochemistry and bioassay) of Cypriot TSE isolates, the biochemical screening could be started immediately and is likely to produce results within the 6-12 months time frame given by the EC.

  Since the potential diversity of TSE agents in Cyprus goats can not be foreseen, the systematic screening of samples available by high throughput biochemical test would certainly be a more appropriate approach than the pre-selection of a limited number of samples. The use of ELISA PK resistant assay (the TSE CRL, 2009) has shown to identify some variability into EU Scrapie goat isolates ('EU goatBSE project', reference FOOD-CT-2006-36353). Such tests could be used for screening Cypriot goat tissue samples.
  The Panel and the WG of experts considers bioassay still necessary to confirm TSE Agents diversity (Groschup *et al*., 2008). The bioassay experiments could start after the biochemical analysis but are unlikely to produce results within the time frame of 6-12 months given by the EC

- With regards to the experimental transmission of a panel of TSE isolates (including BSE and Atypical scrapie) in goats or tg mice harbouring the resistant *PRNP* alleles, it has to be pointed out that relevant mice models are currently under production, but still remain uncharacterised. Bioassay could be started after the completion of the biochemical screening but will probably not produce results within the time frame of 6-12 months given by the EC

- With regards to the in vitro conversion assays using a panel of TSE isolates, the Panel and the WG of experts considers in vitro conversion as a useful tool to approach potential resistance associated to the 146 *PRNP* allele (Priem *et al.,* 2008). The in vitro conversion experiments could start after the biochemical screening completion and could produce relevant results within the time frame of 6-12 months given by the EC.

3.4. Research Area 3: Effect of H154, D146 and S146 *PRNP* alleles on classical scrapie pathogenesis

The following were suggested in the EFSA Scientific Opinion (EFSA, 2009):

a) A systematic assessment of PrP^{Sc} presence in peripheral tissue of clinically healthy and scrapie affected animals from infected herds to look at distribution of TSE agent in resistant animals.

b) A kinetic study of TSE Agent dissemination in organs of orally challenged goats, *i.e*. sequential kill and analysis as above.

Regarding the point (a), the TSE CRL protocol indicates that:

"Following discussions with the Veterinary Services in Cyprus the following two strategies for the sampling of animals to examine the presence of PrPSc in peripheral tissue are practical within the time frame of the protocol (although there are some logistical dependencies relating to the seasonality of the culls, and the availability of appropriate staff at the right time for sampling).

(i) a cull of all animals in several highly infected herds.

(ii) the management cull of older animals in a number of highly infected herds (this part of the experiment is already described in 1. b)).

(i) According to our information from the Veterinary Services in Cyprus some flock owners may be willing to submit their whole herd for culling, which would enable screening of CNS and peripheral tissue involvement throughout the age and genotype range. The selection criteria for the whole-herd cull will be:

- *herds whose owners are willing to submit the herd for culling,*
- *herds with the largest prevalence of scrapie cases in the last two years,*
- *herds with minimum herd size of 200.*

Once the sampling frame is extracted from the database, a list will be produced by ranking the herds according to the selection criteria. The Cypriot veterinary services will approach the herd owners in the same order to propose the whole cull. The number of herds will depend on how many herds are needed to complete the target of 1000 animals over 6 months of age. It is expected though that the number of herds culled will range between 3 and 7. The convenient sample of 1000 animals will ensure that enough animals with the rare homozygous genotypes in codons 146 and 154 are available. A clinical assessment will be conducted by official veterinarians in order to ascertain the health status of the animal prior to the cull."

The BIOHAZ Panel and its WG of experts would like to point out that:

According to the frequencies of the different *PRNP* alleles that were presented into the upper sections, 1,000 animals will only provide few animals bearing the genotype of interest. Additionally, it could not be assumed that all the animals will have been exposed to similar (if any) infectious pressure. Together, these elements make the proposed approach inadequate for documenting the impact of alleles of interest on the pathogenesis of classical scrapie.

The oral inoculation of groups of goats (homozygote and /or heterozygote for the *PRNP* alleles of interest) with a subsequent time point killing strategy remains the most efficient and rational way to obtain interpretable and clear results.

Furthermore, the TSE CRL protocol indicates that:

"The cull will take place in the one operating culling centre already available, which will serve all 5 districts, with sufficient trained staff and disposal capacity. The sampling rate and incineration of carcases in these centres is 40 animals per centre and day. Thus the cull and sampling of all animals could be carried out in approximately six weeks.

After the cull a systematic assessment of PrPSc distribution will be carried out using IHC and ELISA. Given the scale of such a cull and the time-limits of the protocol we would propose a restricted sample panel consisting of:

- *whole brain,*
- *thoracic spinal cord (to identify the earliest site of neuroinvasion, and inform on pathogenesis),*
- *distal ileum to indicate possible oral route of infection and involvement of GALT and/or enteric nervous system (again for the purpose of informing on pathogenesis),*
- *tonsil (or lateral retropharyngeal lymph node – whichever is the most straightforward to sample) – to represent the lymphoreticular system.*

We recognise that this is not an exhaustive list of tissues, but it represents a pragmatic approach to the major systems known to be involved in TSE pathogenesis in small ruminants, given the large number of animals we would wish to sample and the limited time available. Thus selection is also based on a personal communication from Martin Groschup regarding the full PrPSc distribution in Cypriot goat scrapie examined within an EU research project (full data not yet available). We propose to multiblock obex and LN together for IHC. Any animal which screened negative would be considered negative, and no further investigation will be done. Any positive animal would be fully tested. Once the genotypes are known (from the national genotyping study) full screening of animals homozygous or heterozygous for one of the resistant alleles (S146, D146 or H154) will also be performed.

The data collected from the whole-herd cull including genotype, age and disease status will be used to estimate the relative risk of infection/disease by genotype and the incubation periods for the different genotypes present in goats using the back-calculation model (Arnold and Wilesmith, 2003, 2004)."

The BIOHAZ Panel and its WG of experts would like to point out that:

According to the results already available on the pathogenesis of scrapie in goats, spinal cord does not appear to be an earlier site of neuro-invasion than the obex ('EU goatBSE project', reference FOOD CT- 2006-36353). Consequently, the collection and analysis of this samples could be avoided.

With regards to point (b), the TSE CRL protocol addresses the same points as under point (a) of the area 1 (see section 3.2. of this report).

In summary for research area 3.

- With regards to the systematic assessment of PrP^{Sc} presence in peripheral tissue of clinically healthy and scrapie affected goats from infected herds to look at distribution of TSE agent in resistant animals, the protocol proposed by the TSE CRL could be started immediately.

  However, considering the limited frequencies of the alleles of interest and the prevalence of classical scrapie in infected herds (see Table 1, section 3.2) the expected results:

- Are unlikely to document the distribution of PrP^{Sc} in homozygous goats
- Could document, within some limits, the distribution of PrP^{Sc} in heterozygous goats.

- A kinetic study of TSE Agent dissemination in organs of orally challenged goats remains the most appropriate approach to document the impact of the allele of interest on the pathogenesis and TSE Agent distribution in exposed animals. This experiment could start rapidly but is likely not to produce results within the time frame of 6-12 months given by the EC.

3.5. Area 4: Effect of H154, D146 and S146 *PRNP* alleles on the dynamics of TSE agent transmission in affected herds.

The following experiments were suggested in the EFSA Scientific Opinion (EFSA, 2009):

a) Genetic distribution of alleles in full herds (at least 10).

b) Screening of genetic distribution over few years.

c) Include TSE evaluation by testing on more tissues or using ante mortem test.

Regarding point (a), the TSE CRL protocol indicates that the genotypes of all goats will be determined by the end of December 2009.

Regarding points (b) and (c), the TSE CRL protocol indicates that:

"The screening of genetic distribution over few years would require the follow-up of infected herds with known genotype distribution, so as to detect significant changes over time due to the theoretical impact of the disease on it. In discussions with the Veterinary Services in Cyprus, it was considered impractical to set up such study given the ongoing genotype programme as in point a) and the uncertain scientific management for a study of this kind over many years:

(i) Since farmers will have access to full details of the genotypes of their animals in the next six months, it is expected that they will intervene soon after to cull animals of susceptible genotypes interfering with the potential natural changes of genotype distribution of the affected herds.

(ii) The TSE evaluation of these herds would require the identification of all diseased and infected animals from the selected herds in the study in order to relate the transmission dynamics within the herds and the changes in genotype distributions. With the current surveillance of scrapie-affected herds in Cyprus that covers the testing of a small proportion of clinical suspects, it would be insufficient to evaluate the TSE status of these herds over time.

(iii) The follow up time necessary to detect significant changes in genotype distribution is beyond the timeframe of this protocol."

In summary for research area 4:

- In agreement with the TSE CRL protocol, it is acknowledged that assessing the effect of H154, D146 and S146 *PRNP* alleles on the dynamics of TSE Agent transmission in affected herds will remain (if at all feasible) a difficult task requiring a long time and a complicated protocol.

3.6. Research Area 5: *PRNP* allele selection and adverse effect on production or health traits.

The EFSA Scientific Opinion (EFSA, 2009) suggests the consideration of the risk of "founder effect" due to the low frequency of potentially resistant alleles.

The TSE CRL protocol indicates that:

"In the discussion with the Veterinary Services of Cyprus, it became clear that data from government herds could be used to approach the question about the association between genotype and production and health traits."

The BIOHAZ Panel and its WG of experts would like to point out that:

- The approach proposed is inadequate to fulfil the objective. A herd cannot be considered as a model for a whole population. What is important at this stage, if breeding for some *PRNP* allele would be decided, is to ensure that there will be no "founder effect" (management of the genetic variability in the population). Nothing of what is described here allows judging that aspect.

Furthermore, the CRL protocol indicates that:

"Production data from two government herds, the Athalassa farm of the Agricultural Research Institute and the Achelia farm of the Department of Agriculture have been collected for more than 20 years and will be made available for analysis. Apart from individual data like ID, sex, date of birth, mating dates, type of mating etc, production parameters recorded include monthly milk yield, dam, sire, fertility and abortion rates, birth weight, weaning weights (49 days), weight at 120 days. However, the genotypes are only known for two breeding seasons in the Athalassa farm with approximately data from 170 genotyped goats and four breeding seasons in the Achelia farm with approximately 350 genotyped goats. These two farms are currently implementing a breeding programme to increase the prevalence of resistant alleles in the female population and produce homozygous resistant billy goats. Although no accurate information could be obtained from the farm managers at short notice, it is expected that the proportion of animals with susceptible genotypes included in the analysis will be sufficient to detect significant differences if they were present.

Raw data will be extracted and collated by the farm management. Further manipulation of the data might be necessary to produce a final dataset for analysis. Appropriate statistical analyses will be conducted and to test differences by genotypes in production parameters of goats born and raised in the two governmental herds

Irrespective of future breeding programmes, care should be taken to keep a subpopulation with at least one susceptible allele (such as N146). The goats in Cyprus are genetically fairly unique and it might not be possible to restock from somewhere else in case of a genetic problem linked with the resistant alleles.

The Cypriot authorities have indicated that an embryo and semen cryobank is to be established for which additional funding is being sought. The target set is to extract and store 1,500 embryos, 300 from each of the five selected groups in a two-year period: local Machaeras goats, local Akama goats, local fat-tailed sheep, and goats and sheep with susceptible genotypes."

The BIOHAZ Panel and its WG of experts would like to point out that:

- It is not possible to judge if this approach will allow detecting significant differences in the production parameters and health traits, as the groups are relatively small and it is unclear at

this moment what the distribution of the susceptible *PRNP* genotypes within the five groups will be.

In summary for research area 5:

- The proposed approach will provide very limited data on the health traits associated with the different *PRNP* genotypes.

3.7. Research Area 6: Capacity for selection and diffusion of *PRNP* allele in Cypriot goat population.

The EFSA Scientific Opinion (EFSA, 2009) suggests the following experiments:

a) determine the genetic structure of Cypriot goat population;

b) to get data on feasibility and duration of selection for resistant alleles;

c) to determine capacity of breeding system to support diffusion of resistant alleles by modelling.

Regarding the point (a), the TSE CRL protocol indicates that this will be done separately as a national genotyping programme to be completed by the end of 2009.

Regarding the points (b) and (c), the TSE CRL protocol indicates that:

"A metapopulation model of the British sheep industry, based on that used to predict the progress of the GB NSP's Ram Genotyping Scheme (Arnold et al., 2002), will be adapted to the structure of the Cypriot goat population using the genotype data as in point a) and additional input parameters collected during the genotyping of all animals (breed, age and sex), available in the literature and/or elicited through expert opinion. Different breeding strategies will be discussed and agreed with the Cypriot veterinary authorities, including different rates of introduction of resistant males and mating regimes. The output of the model would be the allele prevalence in the total population per year and the changes in the frequency of alleles in the total population over a 10-year period."

The BIOHAZ Panel and its WG of experts would like to point out that:

- In order to evaluate the possibility for modelling the diffusion of alleles in a population, important parameters are needed (e.g. population structure, organization of breeders and diffusion of genetic progress in the population). It is not possible from what is presented here to judge if those data exist, considering that in all cases:
    - the main identified alleles of interest frequencies are low and so that bucks carrying these alleles will also be low;
    - the homozygote bucks will be needed for efficient diffusion if a male diffusion scheme is chosen;
    - at least four generations are necessary to renew a dairy producing herd without impairing its capacities.
- It must be clearly indicated that in the best possible conditions, the diffusion of one of the identified *PRNP* alleles in the general population or just in affected herds (considering their number) will probably require several years to be properly achieved.

Furthermore, the TSE CRL protocol indicates that:

"While the model can be adapted to the Cypriot goat population in advance, the simulations cannot be carried out until genotype data is available (i.e. earliest Jan 2010). The subsequent interpretation of the preliminary results, further refinement of the model to produce the final results and sensitivity analysis is predicted to require at least 4 months."

In summary for research area 6:

- With regards to the intention to get data on feasibility and duration of selection for resistant *PRNP* alleles, and to determine capacity of breeding system to support diffusion of resistant alleles by modelling, the following has to be considered:
    - The modelling could be started after completing the genotyping of the Cypriot goat population and is likely to produce results within the time frame given by the EC (6-12 months). However, availability of data to feed the model would need to be confirmed.
    - Considering the low frequencies of the alleles of interest, a breeding strategy for particular PRNP alleles should take particular care of preservation and management of the genetic diversity in the different breeds. These parameters must also be taken into account in the models proposed by the protocol.

CONCLUSIONS

The EFSA Panel on Biological Hazards concludes that:

- The protocol as submitted by the Community Reference Laboratory (CRL) for TSEs is mainly an extension and an improvement of the case control study that was presented in the first Cypriot pilot project (EFSA, 2009). However, the low frequencies of the *PRNP* alleles of interest in the population compromise the statistical power of the present proposal.
- Since the potential diversity of TSE Agents in Cypriot goats cannot be foreseen, the systematic screening of all samples available by high throughput biochemical test would be advisable rather than the proposed reduced panel of isolates.
- In vitro conversion assays are available and can be used to document potential resistance associated to the D146 and S146 *PRNP* alleles.
- Standardised bioassays of TSE isolates of interest, as used by the Strain Typing Expert Group (STEG), are necessary to document the biodiversity of TSE strains in goats in Cyprus.
- Considering the limited frequencies of the *PRNP* alleles of interest and the assumed prevalence of Classical scrapie in infected herds, the proposed experiments are unlikely to document the distribution of PrP^{Sc} in homozygous goats but could document, within some limits, the distribution of PrP^{Sc} in heterozygous goats.
- The modelling approach on feasibility and duration of selection for resistant alleles is subject to the availability of input data.
- This protocol cannot substitute the experiments advised in the earlier EFSA opinion (EFSA, 2009) on cohort follow up and experimental inoculation. These experiments remain crucial for definitive assessment of D146 and S146 *PRNP* allele resistance.

DOCUMENTATION PROVIDED TO EFSA

1. TSE resistance in goats in Cyprus – Protocol for additional data collection. *Draft protocol version 8, 22/05/2009*. The EU Centre Reference Laboratory for Transmissible Spongiform Encephalopathies. Published in the EFSA web as a separate document.

REFERENCES

Colussi, S., Vaccari, G., Maurella, C., Bona, C., Lorenzetti, R., Troiano, P., Casalinuovo, F., Di Sarno, A., Maniaci, M.G., Zuccon, F., Nonno, R., Casalone, C., Mazza, M., Ru, G., Caramelli, M. and Agrimi, U., 1989. Histidine at codon 154 of the prion protein gene is a risk factor for Nor98 scrapie in goats. J. Gen. Vir., 89(12), 3173-3176.

EFSA (European Food Safety Authority), 2009. Scientific Opinion of the Panel on Biological Hazards on a request from the European Commission on genetic TSE resistance in goats. The EFSA Journal, 995, 1-25.

Groschup, M. and Buschmann, 2008. Rodent models for prion disease. *Veterinary Research*, 39(32), DOI: 10.1051/vetres:2008008. www.vetres.org/index.php?option=toc&url=/articles/vetres/abs/2008/04/contents/contents.html

Priem, J., Langeveld, J., Van Keulen, L., Van Zijderveld, F., Bossers, A., 2008. Protein Misfolding Cyclic Amplification (PMCA) using ovine and bovine species. Prion 2008 Book of Abstracts, P8.25, p. 137. Madrid, 8th-10th October 2008. www.neuroprion.org/resources/pdf_docs/conferences/prion2008/abstract-book-prion2008.pdf

Simon, S., Nugier, J., Morel, N., Boutal, H., Créminon, C., Benestad, S.L., Andréoletti, O., Lantier, F., Bilheude, J.M., Feyssaguet, M., Biacabe, A.G., Baron, T., Grassi, J., 2008. Rapid typing of transmissible spongiform encephalopathy strains with differential ELISA. Emerg. Infect. Dis., 14(4), 608-16.

TSE CRL (Transmissible Spongiform Encephalopathies Community Reference Laboratory), 2009. TSE strain characterisation in small ruminants: A technical handbook for national reference laboratories in the EU. Version 3, March 2009. www.defra.gov.uk/vla/science/docs/sci_tse_rl_handbookv3mar09.pdf

APPENDICES

APPENDIX A: COPY OF ANNEX 1 OF THE TSE CRL PROTOCOL USED FOR THE CALCULATIONS MADE IN TABLE 1 OF THIS STATEMENT

Current knowledge about allele and genotype distribution in the Cypriot goat population (from the report on the pilot project: 'Polymorphims of caprine PrP gene and their association with resistance or susceptibility to natural scrapie' by Papasavva-Stylianou, 2006/07, addressed in the EFSA Opinion)

The frequency of the different alleles for the 146 codon was determined for (i) scrapie-free herds (n=9615), (ii) healthy controls in affected flocks (n=219) and (iii) scrapie-negative controls in affected flocks (n=280) in the pilot study during 2006/07. The allele frequencies were 6.9% (i), 5.7% (ii) and 5.9% (iii) for D146 and 7.1% (i), 7.3% (ii) and 6.1% (iii) for S146. The frequency for H154 was only determined in (ii) and (iii) and was 5.9% and 5.1%, respectively.

The genotype frequencies were the following for codon 146:

(i) 74.1% NN, 11.7% ND, 12%NS, 0.7% DD, 0.6%SS and 0.9% SD

(ii) 76.2% NN, 9.6% ND, 11.9% NS, 0.5% DD, 0.9% SS and 0.9% SD

(iii) 78.2% NN, 9.3% ND, 10.4% NS, 1% DD, 0.7% SS and 0.4% SD

The genotype frequencies were the following for codon 154:

(ii) 88.5% RR, 11.5% RH and 0.5% HH

(iii) 90.7% RR, 8.2% RH and 1.1% HH

While D146 and S146 occur most probably only on the wild type allele that carries P at codon 240 (P240), the H154 occurs on P240 and the variant S240 in about similar frequency (regarding this parameter the pilot study provides only limited information).