PrP allele frequencies in non-infected Valle del Belice and infected cross-bred flocks

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INTRODUCTION – Transmissible Spongiform Encephalopathies (TSE) are fatal transmissible neurodegenerative diseases occurring in a number of mammalian species such as Creutzfeldt-Jakob disease affecting humans, BSE affecting bovines, FSE affecting felines (cats) and Scrapie affecting moufflons, sheep and goats. TSE are characterised by the accumulation of an abnormal form of a host-encoded prion protein (PrP) in the central nervous system and lymphoid tissues of the affected individuals, eventually leading to death. Susceptibility to TSE diseases is partly genetically controlled and mainly depending on the inherited alleles of the PrP gene encoding the PrP. If it would be found that BSE could be transmitted from cattle to sheep then the effects would be huge due to the known effect of BSE causing Creutzfeldt-Jakob disease in humans. To avoid human health risks the EU is implementing policies for selection against certain alleles of the PrP gene, which make sheep susceptible to Scrapie. An initial step is the investigation of the allele frequencies in various breeds. At least 15 allelic variants of the PrP gene are known in sheep. But generally (e.g. Goldmann et al., 1990; Belt et al., 1995), the PrP genotypes are described for the three codons (136, 154 and 171) which represent a polymorphism known to be linked to Scrapie susceptibility. At these codons, named after the amino acids they encode, the following alleles are distinguished: ARR, AHQ, ARH, ARQ and VRQ in order of increasing susceptibility. The aim of this paper is to present the initial results of an investigation of the PrP allele frequencies in Sicilian Valle del Belice dairy sheep and to compare these to cross-bred animals in Sicilian flocks with Scrapie outbreaks. Furthermore the genotyping for PrP offers opportunities for pedigree verification and reconstruction. This work is part of a broader project aimed at pedigree reconstruction and genotype errors in dairy sheep.

MATERIAL AND METHODS – Data consisted of two datasets, both originating from Sicilian dairy sheep flocks. Dataset 1 is from seven flocks with pure-bred Valle del Belice sheep participating in a pilot study and containing complete PrP genotypes of 1064 animals.

Furthermore, Dataset 2 consists of allele frequencies of four flocks having a Scrapie outbreak. These four flocks had 2240 cross-bred sheep.
Using Dataset 1 allele and genotype frequencies were calculated per flock and overall flocks together. The Hardy-Weinberg equilibrium was verified using Genepop 3.4 software (Raymond and Rousset, 1995).
To obtain approximate confidence intervals of the allele frequency estimates a simulation was undertaken. The simulation assumes that the number of sires and dams from all animals in a flock were 8% and 60% of the flock size, respectively. Note that all animals, together result from several mating seasons. Each sire contributed equally to the offspring and each dam as well. There was no selection and no mutation affecting PrP allele frequencies. Therefore the distribution of the allele frequencies depended only on drift, resulting from a limited number of parents producing a limited number of offspring. A total of 1 million repeats were undertaken including all flocks in each dataset.

RESULTS AND CONCLUSIONS – Table 1 shows the Valle del Belice allele frequencies found per flock and overall. The following overall PrP allele frequencies were estimated ARR (32,1%), AHQ (6,6%), ARH (1,1%), ARQ (59,0%) and VRQ (1,2%). The table clearly shows that in all flocks firstly ARQ and secondly ARR were the most present alleles. The most susceptible allele VRQ appears to have a very low frequency and therefore it can be eradicated from the population relatively fast. If necessary all carriers could be culled. Also ARH is at such a low frequency that it could be eradicated easily. The second most susceptible allele ARQ is however the most frequent allele found. This allele can only be removed in several generations. The eradication of ARQ can be achieved by using only sires which are not carriers of ARQ and understandably not of ARH and VRQ either. Palhiere et al (2003) reported allele frequency estimates in 29 French breeds. In 27 of those breeds ARR and ARQ were the two most frequent alleles. The frequency of VRQ was at most 25%, but most often below 10%. Hence Valle del Belice allele frequencies fit within this common pattern.
The 95% confidence interval indicates where the allele frequencies are expected to be in the next generation if no selection and mutation on PrP occurs. For example the VRQ allele is highly likely to remain at a low frequency in each flock because its frequency could only be substantially increased if several of the sires used are carriers. The chance of that occurring is low. In fact the probability of the VRQ allele disappearing within one generation interval due to drift is 0.3% in flock 2, 8.1% in flock 3, 65.8% in flock 5 and 12.1% in flock 6 together resulting in a probability of 0.014% for the four flocks with VRQ together. Note that there are just 14 VRQ alleles in flock 2, 6 in flock 3, 1 in flock 5 and 5 in flock 6.

Table 1: Valle del Belice relative allele frequencies with 95% confidence interval

		Allele				
Flock	**Animals**	**ARR**	**AHQ**	**ARH**	**ARQ**	**VRQ**
1	**88**	29,5%	6,8%	0,0%	63,6%	0,0%
		16-44%	*1-15%*	*0-0%*	*49-78%*	*0-0%*
2	**269**	39,6%	6,5%	1,7%	49,6%	2,6%
		31-48%	*3-11%*	*0-4%*	*41-59%*	*0-5%*
3	**261**	36,0%	1,3%	1,0%	60,5%	1,1%
		28-48%	*0-3%*	*0-3%*	*51-69%*	*0-3%*
4	**130**	40,4%	2,7%	1,2%	55,8%	0,0%
		29-54%	*0-6%*	*0-4%*	*44-69%*	*0-0%*
5	**90**	25,0%	26,7%	3,3%	44,4%	0,6%
		12-38%	*13-40%*	*0-9%*	*30-60%*	*0-2%*
6	**101**	18,8%	7,9%	0,0%	70,8%	2,5%
		8-30%	*1-16%*	*0-0%*	*58-84%*	*0-6%*
7	**125**	16,8%	6,0%	0,0%	77,2%	0,0%
		8-28%	*0-12%*	*0-0%*	*66-88%*	*0-0%*
Total	**1064**	**32,1%**	**6,6%**	**1,1%**	**59,0%**	**1,2%**
		28-36%	*5-9%*	*0-2%*	*55-63%*	*0-2%*

Table 2 shows the genotype frequencies found. The genotypes VRQ/AHQ, VRQ/ARH, VRQ/ARQ and VRQ/VRQ are considered the highly susceptible genotypes, followed by VRQ/ARR. In total these genotypes include 2,3% of the population. The resistant animals are ARR/ARR, ARR/AHQ, ARR/ARH and ARR/ARQ, which together comprises 53,9%. The remaining 43,8% is considered less resistant to Scrapie. In total 83,4% of the animals carriers at least one ARQ allele and 54,8% carries an ARR allele.

Table 2: Valle del Belice relative genotype frequencies

		Allele 1				
		ARR	**AHQ**	**ARH**	**ARQ**	**VRQ**
Allele 2	**ARR**	9,4%				
	AHQ	3,8%	0,6%			
	ARH	0,2%	0,4%	0,0%		
	ARQ	40,5%	7,9%	1,5%	33,5%	
	VRQ	0,9%	0,0%	0,1%	1,2%	0,1%

Verification of Hardy-Weinberg equilibrium resulted in a Chi-square of 15,7 with 12 df and a probability of 0,20. Therefore the population in these flocks was not in disequilibrium. Hence we can conclude that selection so far does not strongly affect the PrP locus.

Table 3 shows the relative allele frequencies found in the four flocks with cross-bred sheep and Scrapie infection. Comparison with Table 1 shows that the allele frequencies have the same order of frequency. However the VRQ allele had a more than twice as high frequency in the outbreak farms. This confirms that the VRQ allele shows the largest risk. The second most susceptible allele, ARQ, was the most frequent allele in both populations.

Table 3: Infected cross-bred flocks relative allele frequencies with 95% confidence interval

		Allele				
Flock	**Animals**	**ARR**	**AHQ**	**ARH**	**ARQ**	**VRQ**
1	**676**	40.2%	1.5%	3.0%	53.0%	2.3%
		35-46%	*0-3%*	*1-5%*	*47-58%*	*1-4%*
2	**530**	40.1%	4.4%	2.1%	50.4%	3.0%
		34-46%	*2-7%*	*1-4%*	*44-56%*	*1-5%*
3	**560**	43.1%	2.9%	2.2%	49.2%	2.6%
		37-49%	*1-5%*	*1-4%*	*43-55%*	*1-5%*
4	**474**	30.7%	2.8%	0.3%	63.6%	2.5%
		25-37%	*1-5%*	*0-1%*	*57-70%*	*1-5%*
Total	**2240**	**38.9%**	**2.8%**	**2.0%**	**53.7%**	**2.6%**
		36-42%	*2-4%*	*1-3%*	*51-57%*	*2-4%*

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