Complex Scrapie Genotypes

Introduction

Testing for the National Scrapie Plan (NSP) has thrown up some unusual genotypes in a very small number of sheep sampled, with the result that a conventional two-allele scrapie (PrP) genotype (such as ARR/VRQ or ARQ/ARQ) cannot be confirmed.

This advice note aims to provide owners with an outline of our current understanding of complex genotypes.

How are genes normally passed from parents to offspring?

Normally, lambs will inherit two copies of the PrP (prion protein) gene, one from the ram and one from the ewe. The five recognised forms (or alleles) of the PrP gene are ARR, AHQ, ARH, ARQ and VRQ. Each is associated with a particular risk of scrapie susceptibility if inherited, for example ARR is linked to strong protection against scrapie whereas VRQ, and in some circumstances ARQ, are linked to scrapie susceptibility.

There are 15 possible combinations of the five recognised alleles and these genotypes are classified into Types 1 – 5 for sheep tested as part of the NSP schemes. The Type 1 genotype (ARR/ARR) has the lowest risk rating and Type 5 (e.g. VRQ/VRQ) the highest. The breeding restrictions (if any) imposed on an NSP animal reflects the risk category of the genotype and differs for male and female animals due to their relative influence on the risk to the flock as a whole. The genotype is an indication of the scrapie susceptibility/resistance of the tested animal itself, and more importantly, its ability to transmit high risk alleles to its progeny. The Genotype Predicting Method Chart shows how a lamb’s genotype can be derived from those of its parents.

How are complex genotypes identified?

Laboratory tests produce a ‘profile’ for each NSP sample and normally this ‘profile’ will closely match one of the fifteen standard genotype patterns. However profiles derived from blood samples of animals with complex PrP genotypes show changes from the normal pattern. These changes suggest that more than two copies of the PrP gene are present and it is not possible to classify the result as one of the standard 15 types.
How common are complex genotypes?

So far, from over 400,000 samples tested in NSP schemes, only about 400 cases of animals with complex genotypes have been identified. This represents approximately 0.1 percent, or less than 1 case per 1000 animals tested.

Could the complex genotype be a result of sample cross-contamination?

The National Scrapie Plan Administration Centre (NSPAC) were keen to rule out the possibility of this happening either at the time of the farm visit or once the sample had reached the laboratory. Cross-contamination of samples in the laboratories is extremely unlikely. Furthermore we have found in multiple cases that successive blood samples taken from the same animal on separate visits continue to yield a complex genotype. This argues strongly against sample cross-contamination. It is more likely that the complex genotype is a real biological phenomenon, and has come to light in the NSP as a result of the large number and variety of sheep tested, and as a result of the highly developed tests used by the genotyping laboratories.

Health status of complex animals

Initial investigations of sheep with complex genotypes indicate that they are healthy. However, the complex PrP genotype status is almost certainly permanent for those animals in which it is detected. Although there is no evidence that the complex PrP genotype is associated with fertility or birthing problems, inheritance of extra copies of genetic material has in some other mammalian species been associated with miscarriage. NSPAC will be monitoring this closely.

Until further research provides a definitive answer we are assuming that the complex genotype affects all tissues in the animal. As the complex genotype is part of the genetic make-up of the animal it is unlikely that unrelated animals in the same flock will be affected. Furthermore the affected animal may produce healthy progeny with normal genotypes.
Breeding for Scrapie Resistance – Special Considerations for Animals with Complex Genotypes.

Conventional PrP genotypes are transmitted to the next generation in a predictable fashion, however it is not clear how these complex genotypes will be passed on to lambs. In the normal situation (see Genotype Predicting Method Chart) each parent passes one of its two copies of the PrP gene to its direct offspring. However, in the case of animals with complex genotypes, where more than two copies of the gene may be present, it is not clear which of the multiple copies will be passed on. Furthermore, more than one of the multiple copies may be passed on from the affected animal, resulting in lambs with complex PrP genotypes.

For this reason careful consideration must be given to whether these animals should be used in a breeding plan for scrapie resistance. It is also unclear at present how the complex genotype affects the animal’s susceptibility to developing disease, if exposed to the scrapie agent.

Breeding with females

No restrictions are in place for females with complex genotypes. This is consistent with our approach to female animals with conventional genotypes (tested as part of the Ram Genotyping Schemes and the Ewe Genotyping Service). Females can therefore continue to be used in an NSP flock and may produce lambs with conventional genotypes. However, we cannot rule out the possibility that ‘complex’ females may produce progeny that also carry complex genotypes (see below).

Breeding with males

A male animal identified as carrying a complex genotype will not normally be certified for use in an NSP flock, but a controlled breeding programme may be considered if the animal is particularly valuable or if non-use causes particular difficulty for the owner. If NSPAC is satisfied that the animal does not carry VRQ as part of the complex genotype then it can be used by the owner in a non-NSP flock or sold on to a non-NSP breeder. Owners should be aware that sale of tups with complex genotypes may be compromised as no certification will be given. Finally consistent with our approach for conventional genotypes, owners of male animals where VRQ has been detected in a complex genotype will be issued with a slaughter notice for that animal.
**Progeny from animals with complex genotypes**

Females with complex genotypes will be permitted for breeding use within an NSP flock. If used, progeny from crosses of these females may or may not themselves carry complex genotypes. As it is not clear which PrP genes will be passed on by such dams, the owner will be advised to have the progeny tested for genotype at the next scheduled visit. It will be particularly important to identify any male progeny with complex genotypes, so as to exclude them from breeding use within the flock.

**Possible mechanisms to account for the production of complex genotypes.**

We are continuing to investigate the possible causes of complex genotypes. However we currently believe that the mechanisms will fall into two main categories (complex mechanisms 1 and 2 shown below). Of course, each new case may be explained by a slightly different mechanism and several processes may be operating in the same animal.

Conventional situation: Each of the parents contributes a single PrP allele to the progeny producing a lamb with a conventional genotype.

Complex mechanism (1): Each parent contributes a single PrP allele to the progeny. However as the early embryo develops it picks up extra copies of the PrP allele (perhaps from a twin). The resulting lamb (and ultimately the adult sheep) carries a complex genotype.

Complex mechanism (2): In this situation, either the sperm from the tup or the ovum from the ewe, carry more than one copy of the PrP allele. These combine to produce a ‘complex’ progeny animal with additional copies of the PrP gene.
Further research

A scientific programme has been established to explore the mechanisms underlying complex genotypes.

As part of this programme we are asking owners to complete a short questionnaire (Form VSACOMP02) concerning the family and veterinary history of the affected animal. A copy of this questionnaire is enclosed with this advice note.

Secondly, we are exploring the possibility of re-sampling easily accessed tissues from some affected animals. Analysis of such samples will help us to determine if the complex genotype is presented by tissues other than the blood sample used for testing. If the affected animal has not yet been sold or culled and is still available, we may wish to arrange a re-sampling visit at the owner’s convenience to re-sample the ‘complex’ animal and possibly other sheep in the flock.

We would be grateful if owners would complete the enclosed Additional Sampling Response form (Form VSACOMP03) to update NSPAC regarding the current availability of the animal and to indicate if the owner would be willing for NSPAC to arrange a re-sampling visit. We anticipate that the additional procedures will include the collection of additional small volumes of blood, collection of small samples of hair and swabs to be taken from the mouth or nasal passages. These procedures are expected to cause minimal discomfort to the sampled animal and should be relatively simple and quick to complete.

We will continue to send out updated advice notes to owners as our knowledge of the complex genotypes increases.