



Notes Section of *Genotyping: A Tool for Controlling Classical Scrapie*

Slide 1

This presentation is designed to assist in educating producers, who have varying amounts of knowledge, on how and when it may be applicable to use genotyping as a tool to control **classical** scrapie in their flock. Genetic selection will **not** prevent Nor98-like, non-classical scrapie.

We have added notes to each slide to assist you in understanding what the creators of this slide presentation were trying to convey with a slide, or in some cases, additional information to assist you in explaining or answering questions that producers may have.

There is a glossary of terms for you to use as a handout, in Word format, included on this CD.

If you have questions regarding this presentation, email: scrapie@animalagriculture.org. Check the National Institute for Animal Agriculture's (NIAA) National Scrapie Education Initiative website: www.eradicatescrapie.org for updates to this presentation.

For the convenience of the presenter we have divided the presentation into 5 stand alone parts.

1. Slides pertaining to "Background of Classical Scrapie" have a lavender colored background.
2. Slides pertaining to "Classical Scrapie Genetics" have a green colored background.
3. Slides pertaining to "How Producers Can Use Genotyping as a Tool in Preventing Classical Scrapie" have a dark green colored background.
4. Slides pertaining to "How Genotyping is Used in Classical Scrapie Infected, Exposed and Source Flocks, i.e. National Genetics Based Clean-Up Plan and Flow Charts" have a cream colored background.
5. Slides pertaining to "Your Questions" have a blue colored background.

Slide 2

Click to add your notes.

Slide 3

This is an overview of what is going to be covered in this section of the presentation. The font color of the bullet heading is the same as the background color of the slides in this section of the presentation.

As this is the start of the "Background of Classical Scrapie" section, all the slides will have the purple tinted background.

Slide 4

Scrapie was first recorded in the UK in 1732. Scrapie has an almost worldwide distribution, only Australia and New Zealand are recognized as "Scrapie Free." Scrapie cases have been identified in many breeds of sheep and goats and in both sexes. In the early 1950's scrapie research intensified with the importation of scrapie-infected sheep into Canada, Australia and the United States.

Since the first case of Nor98-like scrapie was confirmed in February 2007 in a sheep originating from Wyoming, other non-related cases of non-classical scrapie have also been diagnosed in the U.S. Non-classical scrapie was first identified in Norway in 1998,

thus the name Nor98. It is known from experience in Europe that non-classical scrapie infects goats as well as sheep. Research in the United States and Europe is being conducted to learn more about this disease.

- Slide 5** Classical scrapie is NOT an inherited disease. The disease is passed in the placenta and birth fluid. Genetically susceptible sheep or goats may become infected with scrapie if they are exposed to an infected placenta or birth fluids.
Please stress that SUSCEPTIBLE sheep and goats have to be in contact with infected sheep or goats that are lambing/kidding or with contaminated lambing/kidding areas. This is the current understanding of classical scrapie transmission.
- Slide 6** Semen and seminal fluids are not known to “carry” scrapie. New studies are warranted as new technologies with increased sensitivity for detecting the scrapie agent (PrP^{sc}) are developed.
- Slide 7** Clinical signs are variable but can include changes in behavior (hypersensitivity to environment and scratching and rubbing, decreased social interaction often occurs early on), other neurological abnormalities (loss of coordination, lip smacking, gait abnormalities), weight loss, weakness, unable to rise, and death, possibly sudden. Common age of onset of clinical signs is 3-5 years old; however, clinical cases have been seen in sheep ranging from 1 to 12 years of age.
Other ailments to rule-out that may present similar clinical signs include abscesses or other masses in the nervous system; viral and bacterial central nervous system (CNS) infections such as rabies and *Listeria monocytogenes*; nutritional disease such as excessive intake of grain and vitamin deficiency; toxicities such as lead poisoning; and metabolic conditions such as pregnancy toxemia.
Some scrapie-infected sheep rub uncontrollably against whatever object they can find, sometimes to the point where abrasions and scabs occur. The name scrapie comes from this observation. Similar intense rubbing can also be seen with lice infestations.
- Slide 8** Diagnostic tests include:
1) Immunohistochemistry (IHC), histopathological examinations, and/or Western blot on brain tissue.
2) IHC and/or Western blot on lymphoid follicles/tissue.
In 2008, USDA approved the rectal biopsy as a live animal test for scrapie program use. Compared to the third eyelid test, it is easier to obtain a sufficient number of lymphoid follicles for testing, easier to perform, and appears to be less stressful to the sheep and unlike third eyelid may be used in goats. At this time (June 2008) only state, federal and authorized accredited veterinarians are approved to collect third eyelid or rectal biopsy samples for program use. Sample collection and submission will be directed by the Area Veterinarian in Charge (AVIC) in each state. Unfortunately, there is not a blood test currently available to diagnose scrapie.
The United States Department of Agriculture, Animal and Plant Health Inspection Service, Veterinary Services (USDA/APHIS/VS) administers the National Veterinary Accreditation Program (NVAP). This voluntary program certifies private veterinary practitioners to work cooperatively with federal veterinarians and state animal health officials. Accredited veterinarians are instrumental in increasing the USDA/APHIS/VS capability to perform health certifications and to maintain extensive disease surveillance and monitoring.
If veterinarians would like more information on accreditation, the NVAP website is:
http://www.aphis.usda.gov/animal_health/animal_diseases/scrapie/accredited-vets.shtml.

- Slide 9** For further information regarding ID requirements visit the NIAA website at www.eradicatescrapie.org or the USDA/APHIS website at http://www.aphis.usda.gov/animal_health/animal_diseases/scrapie/ or call the NIAA @ 270-782-9798 for print materials regarding Scrapie Eradication Program Sheep ID requirements.
It is extremely important to check the requirements not only of your home state but also those of any states you may be shipping to or through.
NOTE: At this time, there is no genetic based clean-up plan for Nor-98-like scrapie because it is known to affect most genotypes of sheep including RR sheep.
- Slide 10** As scientific information is incomplete, all exposed female goats on scrapie infected farms are depopulated or restricted. The USDA/APHIS/VS completed a study to determine the prevalence of scrapie in the adult slaughtered goat population. No scrapie cases were found indicating that the prevalence of scrapie in cull goats is likely to be less than 0.1 percent.
ARS – Agricultural Research Service of USDA
- Slide 11** This is an overview of what is going to be covered in this section of the presentation. The font color of the bullet heading is the same as the background color of the slides in this section of the presentation.
As this is the start of the “Genetics of Classical Scrapie” section, all the slides will have the green tinted background.
- Slide 12** The genotypes that control susceptibility and resistance are the main points about which we want to educate the producers.
Many of the following slides pertain primarily to producers who feel their flock is at risk for classical scrapie infection and also where market forces demand classical scrapie risk reduction.
- Slide 13** *Italics and **bold** styles are used to stress important words/points.*
Geneticist: a scientist who studies genetics.
As the science of genetics is complicated and uses a lot of ‘scientific’ terminology, we will explain what those terms mean as we go along. It is important to understand that the following **pertains only to sheep.**
At this time no resistant genotypes have been identified in goats. All goats, therefore, are assumed to be susceptible.
- Slide 14** **Genotyping is the identification/labeling of an individual’s genetic composition.**
Some literature will refer to genotyping as DNA scrapie susceptibility testing.
Sheep with susceptible genetics may have other qualities that are desirable to preserve. The aim of this presentation is NOT to remove all susceptible animals, but instead is to lower the risk of contracting classical scrapie given exposure.
- Slide 15** For the benefit of this presentation genotyping is defined as DNA testing.
DNA/Genotyping of an individual sheep does not change over time. The genotype (genetic foundation/DNA) the sheep is born with will always be the same.
- Slide 16** It is advisable to use duplicate ear tags in case the sheep loses one ear tag prior to receiving genotyping results. You must use an “official” form of ID in order for USDA to accept the result as official.
Test results are less likely to be questioned by recipients if the blood is drawn and submitted by an objective third party. Also, collection by an accredited veterinarian is required for USDA to accept the result.
The blood needs to be drawn into purple top tubes or placed on FTA cards. Immediately after the blood is drawn, the tubes need to be gently rocked back and forth 3 times to

prevent the blood from coagulating. Then the tubes need to be placed in a cooler and transferred to a refrigerator.

The blood should be shipped in a cooler with ice packs or ice. The lab should receive the sample within 48-72 hours of sample collection. Practically speaking, the blood should be drawn on a Sunday or Monday and shipped by Tuesday via 2nd-day or next day delivery.

In lieu of tubes, samples may also be submitted on FTA cards. These cards stabilize the DNA and protect the sample from contaminants and environmental degradation such that they can be stored at room temperature for years. They don't require any special handling for mailing to a lab. If using FTA cards make sure the cards are dry before closing. Do NOT store cards that are not completely dry in plastic bags as it may result in mildew growth or degradation of the DNA.

Slide 17 The labs that are approved by APHIS are on the Official USDA Scrapie Website: http://www.aphis.usda.gov/animal_health/animal_diseases/scrapie/app-labs-genotype-test.shtml.

Slide 18 **Please read and follow each lab's sampling and handling instructions.**
Handling the sample, in general:
The blood needs to be drawn, into purple top tubes. Immediately after the blood is drawn the tubes need to be gently rocked back and forth 3 times to prevent the blood from coagulating. Then the tubes need to be placed in a cooler and transferred to a refrigerator. The blood should be shipped in a cooler with ice packs or ice. The lab should receive the sample within 48-72 hours of sample collection. Practically speaking, the blood should be drawn on a Sunday or Monday and shipped by Tuesday via 2nd-day or next day delivery.
Alternatively FTA cards may be used to allow room temperature storage and shipment of samples.

Slide 19 Why two blood tests? Human error could be mislabeling the purple top tube at the farm or in the lab, the quality of the sample submitted, as well as animal identification. The two samples are taken and sent to 2 separate laboratories. If the results are different, a third test is completed.
Genotype testing is NOT testing for scrapie infection, only for genetic susceptibility. Later in the presentation, we will indicate the specific genotypes of exposed sheep that can be sold.

Slide 20 This slide might be one that is difficult to explain. The intent is to explain that there is only one pair of genes (two copies) that affect classical scrapie susceptibility. The slide then explains, genetically, the basis of how each sheep receives that gene with one copy coming from each parent.

Slide 21 Normal prion protein is all folded the same way. The abnormal prion protein molecule is misfolded from the normal protein. The abnormal prion protein is believed to make more abnormal prion proteins by causing the normal prion proteins to be misfolded.

Slide 22 **PrP^c** or normal prion protein is species dependent.
The DNA is the "engineer." The codon is the "construction manager." The cell is the "laborer," and the amino acids are the "building blocks". The prion is a functional finished product. If the finished product is not constructed correctly it will not work the way the engineer intended.

- Slide 23** The following information is intended to be helpful to the presenter in understanding, if needed, the above slide. It is not intended for the audience.
Codons are composed of 3 bases, that in combination instructs the cell which specific amino acid is placed in each particular position. The combination of amino acids creates the specific protein. The PrP^c protein in sheep is composed of 254 amino acids.
For a more detailed discussion please visit this website:
http://www.aphis.usda.gov/animal_health/animal_diseases/scrapie/downloads/scrapie_genetics.pdf to access the paper titled “The Genetics of Scrapie Susceptibility”.
- Slide 24** While there are 254 codons that build the PRNP gene, **only four** of these codons are known to affect classical scrapie susceptibility. Susceptibility to different strains of scrapie is determined in part by different codons. In the U.S., susceptibility to classical scrapie is determined primarily by codon 171. In the other countries codon 136 also plays a large role.
- Slide 25** The codon we will concentrate on is 171 since this codon is the primary determinant of susceptibility in U.S. sheep.
There have been relatively few cases in U.S. flocks where codon 136 has been important in determining susceptibility in those sheep. Codon 154 only provides partial resistance to classical scrapie and is therefore not used in the U.S. regulatory program. **Codons 154 and 141 are believed to be involved in susceptibility to Nor98-like scrapie; however, no genotype has been shown to be fully resistant. Research is being done to learn more about the genetic aspects of this strain.**
- Slide 26** For the remainder of the presentation sheep that have H or K at codon 171 will be considered the same as sheep that are Q at 171.
So, when Q is used it will represent sheep that are Q, H or K at codon 171. The actual susceptibility of K is unknown due to its rarity.
T at 136 is very rare and of unknown significance, so for the remainder of the presentation sheep that have T codon 136 will be considered the same as sheep that are V at 136.
So, when V is used it will represent sheep that are V or T at codon 136.
- Slide 27** The genotypes of sheep are primarily written two ways: Letters of the amino acids: AA QR, AV RR, etc. (the letter placement is in numerical order 136/171) or codon number followed by the corresponding amino acids: 171 QR, 171 RR, etc.
NOTE: In the scientific literature it is common to see the amino acids on each allele written separately for example ARQ/ARR which is the same as 136 AA, 154 RR, 171 QR.
- Slide 28** If both parents contribute an AQ gene then the lamb will be AA QQ.
If one parent contributes an AR gene and the other contributes an AQ gene then the lamb will be AA QR.
If both parents contribute an AR gene then the lamb will be AA RR
If one parent contributes an AQ gene and the other contributes a VQ gene then the lamb will be AV QQ
If one parent contributes an AR gene and the other contributes a VQ gene then the lamb will be AV QR
If both parents contribute a VQ gene then the lamb will be VV QQ.
The VR gene combination is very rare and has not been found in the US.
For a more detailed discussion please visit this website:
http://www.aphis.usda.gov/animal_health/animal_diseases/scrapie/downloads/scrapie_genetics.pdf to access the paper titled “The Genetics of Scrapie Susceptibility”.

- Slide 29** “AV QR” sheep represent a small percentage of the total infected U.S. sheep population. Therefore testing for AV QR is generally not cost-effective. Selection for 171 RR and 171 QR sheep is more than 99% effective for classical scrapie prevention. We have chosen to mark the highly susceptible sheep in red, a warning color, to alert producers that these sheep are susceptible to classical scrapie. The green color is resistant to classical scrapie and therefore a “go” for choice in genetics.
NOTE: Two cases of classical scrapie have been reported in 171 RR sheep, one in France and one in Germany. It is unclear what if any significance this will have for the US.
- Slide 30** This is background information for the next slide.
- Slide 31** Classical scrapie is spread primarily to susceptible QQ sheep and goats through contact with infected placenta/birth fluids (QQ) from infected ewes (QQ). AA QR placentas rarely test positive when in the same horn as a positive QQ placenta. AV QR ewes infected by the Valine associated (V) form of classical scrapie can produce positive QQ placentas.
NOTE: In anticipation of producers’ questions, “much less susceptible” is further explained as follows. A few cases of classical scrapie have been found in U.S. sheep with the AV QR genotype. These cases are thought to be infected with a strain of classical scrapie for which susceptibility is determined primarily by Valine at codon 136. This Valine (V) associated scrapie type is rare compared to the Glutamine (Q) scrapie type that causes the vast majority of U.S. scrapie cases for which susceptibility is determined by codon 171.
- Slide 32** Genetically susceptible sheep have to be in contact with infected sheep, usually during lambing or with contaminated lambing areas, to become infected with classical scrapie.
- Slide 33** In anticipation of producers questions, “much less susceptible” is further explained as follows. A few cases of classical scrapie have been found in U.S. sheep with the AV QR genotype. These cases are thought to be infected with a strain of classical scrapie for which susceptibility is determined primarily by Valine at codon 136. This Valine (V) associated scrapie type is rare compared to the Glutamine (Q) scrapie type that causes the vast majority of U.S. scrapie cases for which susceptibility is determined by codon 171.
- Slide 34** We are repeating this slide because of its importance!
- Slide 35** This is an overview of what is going to be covered in this section of the presentation. The font color of the bullet heading is the same as the background color of the slides in this section of the presentation.
As this is the start of the “How Producers Can use Genotyping as a Tool in Preventing Classical Scrapie” section, all the slides will have the green tinted background.
- Slide 36** This section has a green background and is for producers who do not have a known classical scrapie infection and would like to use genetics to increase resistance in their flock.
It is not our goal to rid flocks of all QQ sheep. QQ sheep may have other specific traits that the producer would like to continue.
This slide and the following slide are self-explanatory. They compare the outcomes of using genotyped rams versus ewes.
- Slide 37** This slide should be preceded by the ram genotype slide. Using genotype testing in a ram is more cost-effective because of the large number of lambs that are affected. Refer to **slide 5** regarding the interaction between susceptible genetics and transmission.

- Slide 38** If maintaining a closed flock is not practical or has not been done because of flock management style:
- Reduce the risk of introducing scrapie by purchasing ewes only from fully monitored flocks in the Scrapie Flock Certification program; or
 - By the addition of only 171 QR and 171 RR ewes (resistant genotypes); or
 - Reduce the risk of transmission from undiagnosed ewes in your flock by using 171 RR rams.
- Slide 39** Monitoring flocks for scrapie infection can be done by submitting suspect animals (see **Clinical Signs, slide 8**) for necropsy or by conducting live animal testing using lymphoid tissue (rectal or third eyelid) in flocks at risk. Contact your veterinarian, State Veterinarian or local USDA/VS Area Office @ 866-873-2824 for more assistance.
- Slide 40** This is an overview of what is going to be covered in this section of the presentation. The font color of the bullet heading is the same as the background color of the slides in this section of the presentation. As this is the start of the “How Genotyping is Used in Classical Scrapie Infected, Exposed and Source Flocks, i.e. National Genetics Based Flock Clean-Up Plan and Flow” section, all the slides will have the cream tinted background.
- Slide 41** Exposed flocks are those that purchased ewes from flocks that were subsequently determined to be infected flocks or source flocks.
Because there is insufficient knowledge on the genetic aspects of Nor98-like scrapie, flock clean-up procedures are based on depopulation and exposure-based clean-up plans when Nor98-like scrapie occurs.
For audiences interested in USDA exposed flock policy see slides 56-60 of this presentation.
- Slide 42** Until 2003, with the exception of a few states with pilot projects, risk in flocks infected with classical scrapie was determined primarily by exposure to the disease. This resulted in a large percentage of exposed breeding animals being destroyed, prohibited from movement or sale, or restricted to movement by permit only. Now, under a national pilot project called the National Genetics Based Flock Clean-Up Plan, individual sheep that are found to be genetically resistant to classical scrapie through genotyping, and whose owners have met various other conditions, can be moved and/or sold in a normal manner. APHIS personnel estimate that on average 60% of a flock is preserved through the application of the current program and is often much higher in flocks where genetic selection has been used. This is in contrast to a figure of 25% of the flock being preserved through the system that was previously in effect.
- Slide 43** Current policy requires removal of **QR** offspring from infected ewes. By studying the offspring, more is learned about scrapie.
- Slide 44** A Post-Exposure Management and Monitoring Plan (PEMMP) plan is explained in the following 2 slides.
- Slide 45** Click to add your notes.
- Slide 46** This is only done with SPECIAL PERMISSION by USDA/APHIS/VS. That is why this slide stands alone!
- Slide 47** Click to add your notes.

- Slide 48** Click to add your notes.
- Slide 49** Indemnity is limited to animals in the flock at the time indemnity is first offered.
- Slide 50** Click to add your notes.
- Slide 51** Click to add your notes.
- Slide 52** This is only done with SPECIAL PERMISSION by USDA/APHIS/VS. That is why this slide stands alone!
- Slide 53** This explains tracing animals that are not currently in the flock.
- Slide 54** **Advisement to Producers:**
Request a copy of genotype test results to be sure that sheep under considerations for purchase are as represented.
- Slide 55** This is an overview of what is going to be covered in this section of the presentation. As this is the beginning of National Genetics Based Flock Clean-Up Plan Flow Charts for USDA Exposed Flock Policy, all the slides will have the brown background.
- Slide 56** The following 5 slides are what were referred to in at the beginning of the National Genetics Based Flock Clean-Up Plan.
NOTE: These slides address typical situations, they do not address sheep potentially exposed to “V” associated scrapie or to goats exposed to scrapie or other unusual circumstances.
This slide explains the process if you have an exposed identified (i.e. ear tagged or other official ID) ewe and don’t want to keep her.
- Slide 57** This slide explains if you have an exposed identified (i.e. ear tagged or other official ID) ewe and want to keep her.
NOTE: This slide does not address sheep potentially exposed to “V” associated scrapie or to goats exposed to scrapie.
- Slide 58** Slides 58, 59 and 60 go together and discuss various categories of how previously lambed ewes should be *handled*.
- Slide 59** This slide is a continuation of information from slide 58, “Trace – Exposed Female Animals”.
- Slide 60** A Designated Scrapie Epidemiologist (DSE) is a regulatory veterinarian in each state or region whose job is to investigate cases of scrapie and determine when the infection was introduced and which animals were exposed.
NOTE: Now that the rectal biopsy test has been approved it is usually possible to get a valid live animal test.
This slide is a continuation of information from slides 58 and 59, “Trace – Exposed Female Animals”.
- Slide 61** This is an overview of what is going to be covered in this section of the presentation. The font color of the bullet heading is the same as the background color of the slides in this section of the presentation. As this is the start of the “Question and Answers” section, all the slides will have the green tinted background.

- Slide 62** Click to add your notes.
- Slide 63** *Scrapie* is a Transmissible Spongiform Encephalopathy (TSE) such as BSE in cattle and Chronic Wasting Disease (CWD) in elk and deer. While there is no scientific evidence that scrapie can spread from one species to another under normal conditions, it is only prudent to eradicate the disease.
NSEP - National Scrapie Eradication Program
- Slide 64** Click to add your notes.
- Slide 65** Click to add your notes.
- Slide 66** Click to add your notes.
- Slide 67** Maintaining a closed-ewe flock can substantially reduce the risk of introducing scrapie without eliminating any sheep of QQ genetics.
- Slide 68** Selecting for increased resistance to classical scrapie could be thought of as a non-traditional production trait. Selection for this trait is not a concern in flocks which have not found classical scrapie assuming that their present biosecurity practice mitigates their risk of introducing scrapie.
- Slide 69** Click to add your notes.
- Slide 70** For additional information visit the above websites or contact the National Institute for Animal Agriculture.
- Slide 71** If you have questions regarding this Power Point presentation contact: scrapie@animalagriculture.org.
For an updated version of this Power Point presentation and more material on Eradicate Scrapie!, please visit the following website: www.eradicatescrapie.org.