Genotyping: A Tool for Controlling Classical Scrapie

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  Email: scrapie@animalagriculture.org

- There is a glossary of terms for your use in Word format to coordinate with this PowerPoint.

- This presentation is meant to be used as a guide and is accurate in its original form.
What We’re Going to Cover

- Background of Classical Scrapie:
  - History & Distribution
  - Basics of the Disease
  - Summary of the National Scrapie Eradication Program (NSEP)
- Genetics of Classical Scrapie
  - How Producers Can use Genotyping as a Tool in Preventing Classical Scrapie
- How Genotyping is Used in Classical Scrapie Infected, Exposed and Source Flocks, i.e. National Genetics Based Flock Clean-Up Plan and Flow Charts
- Your Questions
History and Distribution of Scrapie

- First case of scrapie in the United States (U.S.) was in 1947.
- First case of Nor98-like scrapie in U.S. was confirmed in February 2007.
- Only Australia and New Zealand are recognized as “Scrapie Free” by the U.S.
Basics of the Disease

Transmission & Incubation Period

- Sheep and goats that are susceptible to classical scrapie are typically infected as young lambs or kids through contact with the infected placenta and birth fluids from infected ewe(s) – not necessarily mother to offspring.
- Incubation period typically 2-to-5 years.
- Always fatal – no treatment or vaccine.
Basics of the Disease

Classical Scrapie and Rams

- Rams get scrapie.
- Infected rams are not known to transmit scrapie.
- Ram genetics will contribute to scrapie susceptibility in their offspring.
Variable Clinical Signs may include:

- Changes in behavior
  - Hypersensitivity to noise or movement
  - Scratching and rubbing
  - Diminished social interaction
- Death, possibly sudden

- Neurological abnormalities
  - Loss of coordination
  - Lip smacking
  - Gait abnormalities, i.e. bunny hopping, walking on eggshells
- Weakness, unable to rise
- Weight loss
Basics of the Disease

Diagnosis

- From dead animal
  - Brain tissue and lymphoid tissue
- From live animal
  - Lymphoid tissue of third eyelid, rectal mucosa, tonsil or lymph node
- These methods are approved for official use by USDA.
Key Aspects of the National Scrapie Eradication Program (NSEP)

Sheep

- Detect pre-clinical sheep through slaughter surveillance and live animal testing.
- Ability to trace infected animals to their flock of origin because of identification requirements.
- Genetics based clean-up strategies that allow producers to stay in business.
- Tracing and testing of exposed animals that have been sold out of infected flocks.
Key Aspects of the NSEP

Goats

- There is some evidence that goats with certain genotypes may be less susceptible than others to scrapie; however, it is unclear whether this reduced susceptibility will be adequate to prevent disease spread and thus be useful for regulatory purposes. ARS and others are researching this question.

- All goats, therefore, are currently assumed to be susceptible for regulatory purposes.
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The genotypes that control susceptibility and resistance are **THE** most important thing a producer can know about classical scrapie genetics!
Our Main Goal in This Presentation:

- Help producers understand how to use genetics to protect their flocks from classical scrapie infection.
- Not to make you into a geneticist.
Some sheep are more susceptible than others.

Genotyping is used to identify the genes that control classical scrapie susceptibility or resistance.
Genotyping is DNA testing.

A simple blood/tissue test is used to determine a sheep’s *genotype*.

- A sheep’s genotype *never* changes so only one test should be needed.
- It can be determined at *any age*.
Genotype Testing

- It’s best to use two forms of ID (two eartags, eartag and a tattoo, etc) in sheep to be tested.
- Consider using a third party (veterinarian) to collect blood.
- Collect purple top tubes early in the week so lab receives sample by Thursday, or use FTA cards which allow room temperature storage and shipping.
- Genotype results stay the same throughout the animal’s lifetime.
APHIS will only recognize the results of genotype tests if:

- The blood is drawn by an accredited veterinarian or by State or Federal personnel.
- The sheep is officially identified.
- The sample is submitted with a VS Form 5-29.
- The lab has been approved by APHIS.

Several labs are currently approved.
Labs that Perform Genotyping

- Genotyping is a key element of the National Scrapie Eradication Program.
- APHIS approves laboratories to run privately funded, official scrapie genotyping.
- Approval is subject to review and renewal.
Since USDA uses genotyping to determine whether an exposed sheep can be sold, USDA requires exposed sheep to be tested twice as a precaution against possible human error including:
- Mislabeling at the farm or lab.
- Lab test failure due to poor quality of submitted samples.
- Human error in the lab.
- Animal identification error.

The genotype test measures only susceptibility *not* whether the animal *has* scrapie.
Sheep have **one pair** of genes that affects **classical** scrapie susceptibility.

This pair of genes are known as **PRNP** genes (**Prion** Protein genes).

Each sheep has **two copies** (one pair) of the **PRNP**—one copy from each parent.
In sheep, **PRNP** produces:

- A normal cellular *prion protein molecule* **PrP**<span class="subscript">c</span>.

In infected sheep **PrP**<span class="subscript">c</span> is converted to:

- **PrP**<span class="superscript">sc</span>, *scrapie prion protein molecule*, the abnormal infectious form of the prion protein molecule.
Prion Explanation

- \( \text{PrP}^c \) exists in all animals with small differences between species.

- \( \text{PrP}^{sc} \) makes more of itself by causing misfolding of normal cellular prion protein.
Details on PRNP Gene

- All genes, including PRNP, are made up of codons.
- Each codon instructs cells to put a specific amino acid at a particular location when building a protein molecule.
- The prion protein molecule PrPc (produced by the PRNP gene) has 254 amino acids.
- The locations of the 254 amino acids are numbered 1 to 254.
In the PRNP gene, three codons affect classical scrapie susceptibility:
- Codon 171
- Codon 154
- Codon 136
Genetics & Susceptibility to Classical Scrapie in the U.S.

- **Codon 171**— Is a major determinant of classical scrapie susceptibility.
- **Codon 136**— Affects susceptibility in sheep exposed to some classical scrapie types.
- **Codon 154 & 141**—
  - Codon 154 plays a minor role in classical scrapie,
  - NOT used for regulatory purposes in the U.S. for classical scrapie,
  - Associated with susceptibility to Nor98-like scrapie.
Codons 136 & 171 and Their Specific Amino Acids

- Codon 136 programs for the amino acid Valine (V), Alanine (A) or Threonine (T).
- Codon 171 programs for the amino acid Glutamine (Q), Arginine (R), Histidine (H), or Lysine (K):
  - Q, H, and K are considered to have the same susceptibility for regulatory purposes and are reported as Q by most labs.
The genotypes of sheep in the U.S. are primarily written two ways:

- Letters of the amino acids: AA QR, AV RR, etc. (the letter placement is in numerical order Codon 136/codon 171), or

- Codon number followed by the corresponding amino acids: 171 QR, 171 RR, etc.
Sheep Genes: One From Each Parent

- Since each parent contributes one copy of the gene to the lamb, the gene can be any of the these three:
  
  **AQ, VQ, AR**

- Then, each lamb inherits two copies of the gene. The possible combinations are:
  
  **AA QQ, AA QR, AA RR, AV QQ, AV QR, VV QQ**
## Offspring Susceptibility Table

<table>
<thead>
<tr>
<th>Ewe (136/171)</th>
<th>Ram (136/171)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AQ</td>
</tr>
<tr>
<td>AQ</td>
<td>AA QQ</td>
</tr>
<tr>
<td>VQ</td>
<td>AV QQ</td>
</tr>
<tr>
<td>AR</td>
<td>AA QR</td>
</tr>
</tbody>
</table>

**Highly Susceptible**

**Rarely Susceptible**

**Resistant**
Lambs have a combination of DNA from the ewe and from the ram:

- **QQ** ewe bred to an **RR** ram will produce a **QR** lamb with **QR** placenta/birth fluids.
- **QQ** ewe bred to a **QQ** ram will produce a **QQ** lamb with **QQ** placenta/birth fluids.
Research has Found that:

- Classical Scrapie is spread to susceptible sheep (QQ) and goats through contact with infected placenta/birth fluids.
- AA QR placentas from infected QQ ewes have been negative for scrapie.
- But QQ placentas from infected QQ ewes have been positive.
- So AA QR placenta/birth fluids from infected ewes are unlikely to transmit scrapie to nearby susceptible sheep.
Importance of the Placenta’s Genotype in Classical Scrapie Transmission

171 QQ fetus + Scrapie-infected placenta = SCRAPIE
Classical Scrapie
Genotype Susceptibility Combinations

- **AA RR**—Sheep which are *resistant*
- **AA QR**—Sheep which are *rarely susceptible*
- **AV QR**—Sheep which are *much less susceptible*
- **AA QQ**—Sheep which are *highly susceptible*
- **AV QQ**—Sheep which are *highly susceptible*
- **VV QQ**—Sheep which are *highly susceptible*
The genotypes that control susceptibility and resistance are **THE** most important thing a producer can know about classical scrapie genetics!
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Selecting Rams which are **RR** or **QR**

- Each individual gets a pair of genes from each parent.

- **RR** rams will always sire **RR** or **QR** lambs (resistant or rarely susceptible).

- **QR** rams will sire either resistant, rarely susceptible, or susceptible lambs, depending on the genotype of the ewe.
Selecting Ewes which are **RR** or **QR**:

- A ewe will pass on the same resistance/susceptibility traits as a ram, but of course only to her lambs, thus affecting far fewer individuals.
- Ewe genotyping is far less cost effective than genotyping rams;
- Practical for only the most important foundation ewes.
Genotyping Should be Considered “Just One” Tool, Not the Only Tool.

- The most effective method of preventing scrapie from being introduced to your flock is to maintain a closed ewe flock.

- If your flock management system requires you to bring in ewes either;
  - Buy ewes of known background where you can be confident that the flock is free of scrapie such as Certified Flocks; or
  - Buy ewes of resistant genotypes.
If your flock type is such that you have purchased ewes from multiple unknown status sources over the years, particularly ewes from high prevalence breeds:

- **Then** consider starting a proactive genetic selection program to enhance the resistance for your existing flock, and start buying ewes of resistant genotypes; and/or

- Reduce the risk of transmission from undiagnosed ewes in your flock by using 171 RR rams.
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This plan applies only to producers who have classical scrapie in their flock (Infected or Source).

Flocks exposed to scrapie will be placed directly on a Post-Exposure Management and Monitoring Plan (PEMMP) unless scrapie is found.
How the Plan Works

- The genotypes of all individual sheep in the flock are determined.
- Sheep with susceptible genotypes are removed or placed under restricted movement.
- The flock is monitored for 5 years.

**Primary Benefit:**
- Save an estimated 60% of flock as opposed to 25% in most cases when risk was determined by exposure.
Plan Requirements

- All QQ ewes and female offspring of scrapie-infected ewes are removed from the flock, or their movement is restricted.
- Positive and suspect animals are removed.
- AV QR ewes in flocks where scrapie positive sheep with V at codon 136 have been identified are removed or their movement is restricted.
- All female goats regardless of age are removed or their movement is restricted from infected sheep flocks and/or goat herds.
All animals in the flock must be officially identified and entered into the USDA’s Scrapie National Database.

All owners/flocks must have a Post-Exposure Management and Monitoring Plan (PEMMP).
Owners Must Have a PEMMP Including:

- Official ID of sexually intact animals that are sold or acquired.
- Records of such transactions including buyer/seller contact information.
- Reporting suspect cases and deaths of mature animals and submission of above for diagnostic testing; and
- Annual inspections by state and/or federal officials.
 Owners Must Have a PEMMP (continued):

- If producers elect to retain exposed susceptible animals, restrictions will be placed on these animals and any susceptible animals that are acquired through birth or other means.
APHIS Responsibility

- Indemnification based on **commercial** market prices reported by USDA’s Agricultural Marketing Service (AMS) and additional amounts for:
  - registered animals;
  - other non-commercial classes of animals such as club lambs for which the owner can document increased value.

- Provide testing required to complete the standard National Genetics Based Flock Clean-Up Plan.

- Assistance with disposal costs.
Owner’s Responsibility

- Gathering and handling sheep
- Adequate handling facilities
- Applying Identification (ID)
- Cleaning and disinfecting
- Maintaining records
- Any additional testing
What Happens if Classical Scrapie is Diagnosed in My Flock?

- USDA will either provide genetic testing and removal of genetically susceptible breeding stock as part of the National Genetics Based Flock Clean-Up Plan, or in some cases, when agreed to by both the owner andAPHIS, depopulation of all sexually intact animals or an exposure based plan. Indemnity will be paid on sheep and goats that are removed as part of the flock plan.
The National Genetics Based Flock Clean-Up Plan offers producers the option of removing only genetically susceptible sheep, allowing the producer to retain or move RR, AA QR, and most AV QR sheep without restrictions.
The movement of ewes, rams, does, and bucks will be restricted until the owner, the state, and APHIS, agree to a flock clean-up plan and a post-exposure management monitoring plan.

When the flock clean-up plan has been completed,

- Exposed animals that are determined not to be susceptible and that are not the female offspring of a scrapie-positive female animal will be released from restricted movement.
If producers elect to retain exposed susceptible animals, restrictions will be placed on these animals and any susceptible animals that are acquired through birth or other means.
Sheep and goats that were moved out of the flock after the infection was believed to have been introduced will be traced to their new owners.

Genetic testing, scrapie testing and/or removal of exposed animals will be paid for by USDA/APHIS/VS.
While on a Monitoring Plan is it Important to Breed for Resistance?

Yes, in exposed or previously infected flocks,

- The use of **171 RR** rams to increase resistance and prevent the occurrence or recurrence of classical scrapie is recommended and

- Buy ewes of known background where you can be confident that the flock is free of scrapie such as Certified Flocks and/or

- Buy ewes of resistant genotypes.
The following flow charts describe what to expect if a scrapie exposed ewe is traced to your flock that previously resided in a flock that was subsequently determined to be an infected or source flock.

These flow charts are intended to describe the typical situation for sheep.
Trace - Exposed Female Animals

Animal is in the flock, and ID is adequate to document.

- Restrict movement of the exposed animal,
- Officially identify the exposed animals

Owner doesn’t want to keep her

Pay indemnity and submit appropriate tissues for diagnosis if $\geq 14$ months old (or younger if lambed).

Did the ewe lamb on the farm?

- Yes
  - Positive
    - Infected Flock
  - Negative
- No
  - Release exposed flock status and Release quarantine.

Next slide
Trace - Exposed Female Sheep

Animal is in the flock, and ID is adequate to document.

- Restrict movement of the exposed animal
- Officially identify the exposed animals

Owner wants to try to keep her

Genotype

QR or RR
- Genetic test again, send to different lab. If result agrees:
  - Release exposed flock status and release quarantine.

QQ
- Pay indemnity and submit appropriate tissues for diagnosis if ≥14 mos. old (or younger if lambed).

Did the ewe lamb on the farm?

- Negative
  - Release exposed flock status and release quarantine.

- Positive
  - Infected Flock
Animal is missing (done even if one female is missing in a multiple animal trace).

Did the ewe lamb on the farm?

Yes

No

Release exposed flock status.

Next slide
Yes, the ewe lambed on the farm.

- Identify and restrict animals for testing. Those animals are still on the farm that were born in the same and subsequent lambing seasons that the missing exposed animal lambed should be tested. Other sheep/goats present in the flock when the exposed ewe lambed may also be tested.

- Genotype all sheep designated.
- Live animal test all QQ sheep when they reach 14 months of age.
- If the youngest lamb crop is <12 months old, its QQs may go to slaughter rather than be tested.

If all tests are negative or invalid

Positive test result

Next slide

Infected Flock
Yes, the ewe lambed on the farm, and tests were invalid.

**Designated Scrapie Epidemiologist (DSE)** determines if sheep with invalid live animal tests must be tested at necropsy based on epidemiology of the flock and number of valid tests.

- **Positive Necropsy Result**
  - Infected Flock
  - No additional testing needed

- **Negative necropsy result**
  - Basic PEMMP, Release exposed flock status, and Release quarantine.

- **Test required**
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Question:

We understand scrapie is an always fatal disease of the nervous system and that susceptibility is controlled by genetics, but with only a few hundred reported cases in the U.S. each year, why is there so much emphasis on eradication?
Answer:

- Scrapie costs American sheep producers an estimated $10-20 million per year in lost sales abroad, in increased costs, and in lost animals.
- Scrapie is a Transmissible Spongiform Encephalopathy (TSE). Public expectation is the eradication of all TSEs.
- Many cases of scrapie go undetected. The NSEP is proving to be an effective means of controlling this disease.
Question:
What is the most important thing we need to know about **classical** scrapie genetics?

Answer:

Genotype Susceptibility Combinations

- **AA RR**—Sheep which are *resistant*
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Question:
Do I need to be concerned with the National Genetics Based Flock Clean-Up Plan?

Answer:
No, unless:
- Your flock is categorized as “Infected,” “Exposed” or “Source”.
Question: If my flock has some potential exposure to classical scrapie through purchase of ewes, what should I do?

Answer: If the flock is determined to be at risk, use of RR breeding rams to increase the percentage of more genetically resistant lambs makes sense. Also, dispose of all placentas immediately following lambing.
Question:
If my sheep do not have scrapie should I consider using scrapie genetics as a management tool?

Answer:
Yes, primarily if:

- Your flock has a high potential for classical scrapie because:
  - You have a breed in which classical scrapie is prevalent, and you have purchased ewes of unknown scrapie status.
  - You have purchased ewes from an infected flock.
  - You have observed signs of scrapie in your flock in the past.
- Customers request breeding stock to be scrapie resistant.
Questions and Answers

Question:
If I do not have classical scrapie in my flock, do I need to select for genetic resistance to scrapie?
Questions and Answers

Answer:

Yes, if you are providing breeding stock of increased genetic resistance to other flocks.

No, if the above is not one of your major concerns then managing flock genetics to optimize other traits will likely surpass the benefits of selecting for genetic resistance to scrapie.

In either case, all practices that would prevent the introduction of scrapie through purchased breeding stock should be incorporated into your flock biosecurity plan now.
Other Questions or...

For additional information:

[www.eradicatescrapie.org](http://www.eradicatescrapie.org)

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The End