REPORT OF THE COMMITTEE ON SCRAPIE

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The Committee met on October 22, 2013 at the Town and Country Hotel, San Diego, California, from 9:00 to 11:46 a.m. There were 12 members and 9 guests present. The meeting began with a review of the of the Committee purpose. Attendees did not elect to make any changes to the current language. The following presentations and reports were given.

USDA-APHIS Scrapie Program Update and Scrapie Surveillance Projects

Alan Huddleston, VMD, Associate National Scrapie Program Director
United States Department of Agriculture, Animal and Plant Health Inspection Service, Veterinary Services (USDA-APHIS-VS) *(Presented by TJ Myers Associate Deputy Administrator, USDA-APHIS-VS)*

Scrapie Eradication Program Results

- There has been a 90 percent decrease in the percent positive sheep sampled at slaughter adjusted for face color, from 0.15 to 0.015 percent, since the start of Regulatory Scrapie Slaughter Surveillance (RSSS) in FY 2003 thru September 30, 2013.
- There were 11 new infected or source flocks reported in FY 2013 as of September 30, 2013. FY 2013 is the first year since FY 2005 when a reduction in the number of new scrapie infected and source flocks was not observed. Now that the program is in the tail end of the eradication effort it is likely that the numbers will go up and down from year to year due to the difficulty in accurately measuring the frequency of uncommon events.

Slaughter Surveillance

- The number of animals sampled through slaughter surveillance in FY 2013, through September 30, 2013 was 42,888 compared to 40,776 in FY 2012; this represents an increase of 5 percent. The increase was due to increased sampling of goats.

Scrapie Surveillance Plan

- Implementation
    - States with regulatory scrapie slaughter surveillance (RSSS) collection sites will continue to sample all targeted sheep and goats.
    - States have State-of-origin sampling minimums for sheep.
    - VS plans to require annual State-of-origin sampling minimum for goats to be met once the proposed rule revising title 9, *Code of Federal Regulations* (9 CFR) parts 54 and 79 is finalized. Proposed sampling minimums were provided for FY 2013 and FY 2014.
    - The annual State-of-origin sampling minimum for sheep is 20 percent of the number required to detect a scrapie prevalence of 0.1 percent with 95 percent confidence or 1 percent of the breeding flock in the State, whichever is less. The objective is to sample sufficient sheep in a 5-year period to detect a scrapie prevalence of 0.1 percent with 95 percent confidence or 5 percent of the breeding flock in the State, whichever is less.
    - The annual State-of-origin sampling minimum for goats is determined based on the States' goat scrapie case incidence.
        - If a State has not had a goat scrapie case in the previous ten years, its annual goat sampling minimum is its prorated share of 3,000 samples, based on its proportion of the

U.S. goat population as determined by the National Agricultural Statistics Survey (NASS) Sheep and Goat annual report.

- If a State has had a goat scrapie case in the previous ten years, its annual goat sampling minimum is determined using the same method as is used for determining its annual sheep sampling minimum.
- Beginning in FY 2013, sheep and goat sampling minimums were calculated separately. As a result, a higher percentage of States will not achieve their sheep sampling minimums in FY 2013 compared with FY 2012. Approximately 40% will not achieve the sheep sampling minimums this fiscal year, compared to approximately 20% in FY 2012. States that did not meet their sheep sampling minimum in FY 2013 through RSSS but will be expected to find other sampling sources to meet the minimum in FY 2014.

Note: These are minimums. Plans are to continue to collect samples from the maximum number of targeted animals given the available budget.

FY 2014 Priorities

- VS priorities for scrapie are to focus on improving the effectiveness and cost efficiency of surveillance and to increase animal identification compliance. This will be accomplished in part by publishing a proposed rule that would address gaps in identification and require States to meet reasonable surveillance targets to remain consistent States. States must meet these targets for VS to demonstrate geographically appropriate surveillance to meet the criteria for freedom and have confidence that all of the remaining cases have been found.
- The rule would propose to:
  - Give the APHIS Administrator authority to relieve requirements for sheep and goats exposed to scrapie types, such as Nor98-like, that do not pose a significant risk of transmission;
  - Increase flexibility in how investigations can be conducted and allow the epidemiology in a specific flock to be given more consideration in determining flock and animal status;
  - Add a genetic-based approach to regulation;
  - Make goat identification requirements similar to those for sheep to support ongoing slaughter surveillance in goats (no changes will be made in the consistent State requirements regarding identification of goats in intrastate commerce);
  - Tighten the definition of slaughter channels;
  - Expand the individual identification requirement to all sexually intact animals unless moving as a group/lot (allows mixed-source groups moving in slaughter channels under 18 months);
  - Limit the use of tattoos and implants to animals not moving through markets and not in slaughter channels; and
  - Reduce recordkeeping requirements by making them similar to the current uniform methods and rules compliance guidance.
- APHIS is also revising its scrapie import regulations to bring them more in line with the World Animal Health Organization (OIE) scrapie chapter. This will ensure that we meet OIE criteria for free status and prevent the reintroduction of scrapie after free status is achieved.

Scrapie Flock Certification Program (SFCP) Standards

On May 3, 2013 APHIS announced its intention to revise the SFCP. The comment period closed June 3, and the revised program has gone into effect. The SFCP standards were revised to increase the program's ability to identify infected flocks quicker and to prevent infected flocks from becoming certified, to reduce costs associated with the program, and to increase SFCP contribution to scrapie surveillance. Scrapie program staff collected input from SFCP enrolled producers, industry representatives, and State and federal stakeholders. The public had a final opportunity to comment on the revised standards through a Federal Register notice.

In the revised SFCP the Complete category is eliminated. Additionally, the Select category is revised, and the Export category is slightly modified.

- ***Select category***: APHIS has redirected monitoring from inspections to sampling. Select category flocks do not become certified. Specifics for this category include:
  - There are no annual inspections.

- Owners must report clinical signs of scrapie.
- Herd owners follow 9 CFR 79 requirements for recordkeeping and animal ID for their flocks.
- Flock owners can acquire animals from any other flock, whether or not that flock is enrolled in the SFCP.
- The sampling and testing requirements include:
  - Sheep or goats displaying clinical signs over 12 months of age;
  - Animals of any age that either test suspect, inconclusive or positive on a live animal scrapie test or have been determined to be a scrapie suspect by a State, Federal or accredited veterinarian; and
  - A minimum of one animal per 1-3 years, depending on *flock size.*

- ***Export Category***: APHIS continues a high level of monitoring including inspections and sampling. Flocks can become Export Certified. Specifics for this category include:
  - Annual inspections are required.
  - Owners must report clinical signs of scrapie.
  - Animals must be identified with official SFCP ID.
  - Flock owners must meet rigorous recordkeeping requirements including maintaining records on every animal that leaves the flock for seven years.
  - Flock owners must have all cull animals inspected, including home slaughtered animals, for clinical signs of scrapie at least 30 days before culling.
  - Flock owners can acquire female animals and embryos only from other Export category flocks of equal or higher status.
  - Flock owners can use sheep and goat milk and colostrum and sheep and goat milk- and colostrum-derived products only from within their own flock or from other Export category flocks of equal or higher status.
  - The sampling and testing requirements include:
    - Sheep or goats displaying clinical signs over 12 months of age;
    - Animals of any age that either test suspect, inconclusive or positive on a live animal scrapie test or have been determined to be a scrapie suspect by a State, Federal or accredited veterinarian;
    - All found dead mature animals, including euthanized animals;
    - An annual sampling minimum of one test eligible animal tested for each year of status held (A flock will be removed from the program if the flock owner fails to submit at least one test eligible animal for two consecutive years.);
    - To gain six years in status, 15 test eligible animals must be sampled; and
    - The requirements for Export Certified status include:
      - seven years in status; and
      - Meet one of three sampling protocols
        - Standard: 30 test eligible animals
        - Alternative 1: test all genetically susceptible animals sold
        - Alternative 2: test all foundation flock animals.
- Participants in the Complete category had the following options: (1) join the Export category with up to 5 years of status; (2) join the revised Select category; or (3) withdraw from the program.
  - For participants who held “Certified” status in the Complete category who convert to the Export category, APHIS will continue to publish their “Certified” status on its website for 3 years following the start date of the revised program, in addition to their new “Export Monitored” status, to allow them sufficient time to become Export Certified; and
  - If instead they convert to the Select category or withdraw from the program, APHIS will not continue to publish their “Certified” status on its website.

Scrapie Surveillance Projects:

- Since the start of slaughter surveillance in 2003 the prevalence of scrapie in sheep has declined 85 percent from 0.2 percent to less than 0.03 percent. The prevalence in goats is estimated to be less than 0.02 percent.

- APHIS continues to find new approaches to increase flock level surveillance.
- In FY 2013 APHIS initiated an effort to provide information on sample collection and to encourage producer and accredited veterinarian submission of samples.
- Instructions for producers and veterinarians to submit samples are now available on the APHIS Scrapie Web Page.
- In FY 2014 APHIS will conduct pilot projects in New Jersey and Arkansas to evaluate the efficiency of working with accredited veterinarians to collect samples for scrapie testing.

Update from Agriculture Research Service, Pullman, Washington

David Schneider, DVM, PhD, Research Veterinary Medical Officer
United States Department of Agriculture, Agriculture Research Service (USDA-ARS)-Animal Disease Research Unit (ADRU) Pullman, Washington

The USDA-ARS unit in Pullman, Washington, conducts an integrated research program involving studies on scrapie transmission, diagnosis and susceptibility genetics in domestic sheep and goats. Accumulation of disease-associated prion protein (PrP^{Sc}) in the placenta of sheep is a recognized source for natural transmission of classical scrapie disease and environmental contamination. Much less is known about prion accumulation in the placenta of goats but our recent study demonstrated much less PrP^{Sc} accumulates in the placenta in goats, which calls into question its role in natural transmission. In a recent follow-up study, we now demonstrate that the placenta of goats does harbor prions infectious to other goats and sheep when exposed by the oral route. A study on Nor98-like scrapie in breeding ewes is now in its 6^{th} year. Ewes were experimentally inoculated with brain homogenate obtained from a U.S. sheep with clinical Nor98-like scrapie. Recipient ewes are bred annually to examine the placenta for evidence of a transmissible agent. Placentas shed 2009-2013 were negative. In 2013, one recipient ewe developed an unrelated disease. At postmortem examination, abundant accumulation of PrP^{Sc} was observed only in the cerebellum of this ewe with much less accumulation in the hindbrain obex. This confirms that initial inoculation of these ewes has been successful. Monitoring continues in the remaining ewes of this study. Improvements in tissue-based (rectal biopsy) live animal testing for scrapie with focus on application to goats continue. In addition, efforts toward developing a live-animal blood test have demonstrated the presence of prions (infectivity) in the blood of sheep and goats, even those with preclinical disease and within blood sample volumes routinely used in veterinary diagnostic work. A recent study also demonstrates PrP^{Sc} accumulation in lymphoid tissues of hemal nodes, small lymphoid organs that filter blood but not lymph. Collectively, these findings confirm that blood is a relevant target for continued assay development. We continue to develop methods for enriching the relevant blood fractions for assay and are now making efforts to adapt novel in vitro assays for detecting infectivity and prion-associated misfolding activity. A long term study examining the effect of prion genotype on susceptibility to goat scrapie and the effect of genetic changes on accuracy of live animal testing continue. Following oral infection at birth with placenta and brain-derived scrapie, goats with the highly susceptible genotype all developed clinical disease around 24 months. Goats with the less susceptible or long incubation genetics today remain clinically normal. Monitoring continues.

Prion Transmission Through Milk

Christina Sigurdson, DVM, PhD, Associate Professor
University of California, San Diego School of Medicine, Department of Pathology

Prion disorders are caused by misfolded proteins that are naturally transmitted, causing a fatal neurological disease in animals. In sheep with classical scrapie, prions accumulate in the follicles of lymphoid tissues in addition to the brain and spinal cord. Follicular dendritic cells (FDCs) form a network within the follicles and accumulate high levels of prions during disease. Previous work in mice has revealed that follicular inflammation in non-lymphoid organs, such as kidney, results in prion accumulation and can lead to prion shedding, such as into the urine. We have found sheep with follicular mastitis and scrapie that have accumulated prions within the follicles of the mammary gland.

In follow-up studies, we found that sheep with scrapie and lentiviral mastitis secrete prions into the milk and infect nearly 90% of naïve suckling lambs. Taken together, lentiviruses may enhance prion transmission and conceivably sustain prion infections in flocks for generations. Work by other groups has

also shown prion infectivity in all three milk fractions, cells, casein whey, and cream. Prion infectivity has also been detected in milk from sheep having the VRQ/VRQ genotype with no evidence of mastitis.

References

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2. Konold, T.*, et al.* Evidence of effective scrapie transmission via colostrum and milk in sheep. *BMC Vet Res* **9**, 99 (2013).
3. Ligios, C.*, et al.* Sheep with Scrapie and Mastitis Transmit Infectious Prions through the Milk. *J Virol* **85**, 1136-1139 (2011).
4. Ligios, C.*, et al.* PrPSc in mammary glands of sheep affected by scrapie and mastitis. *Nat Med* **11**, 1137-1138 (2005).
5. Lacroux, C.*, et al.* Prions in milk from ewes incubating natural scrapie. *PLoS Pathog* **4**, e1000238 (2008).

Committee Business:

The final response from the Committee's 2012 Resolution (26, 9 and 30 Combined) relating to the export of sheep and goats was reviewed. In this response the USDA-APHIS-VS agreed to ask the World Organization for Animal Health (OIE) to modify the Scrapie Chapter to consider options such as genotyping to qualify animals for export. USDA-APHIS-VS agreed to make this request by Spring 2014, and would expect to see the Scrapie Chapter amended in Spring 2015 or 2016 if their revisions were to be accepted by OIE.

One of the Committee members updated the group on progress related to a 2010 Resolution #48. This resolution requested USDA, Food Safety Inspection Service (FSIS) to work with USDA-APHIS-VS and industry to identify and approve appropriate sites for radio frequency identification implants for goats and sheep. As a result, both the underside of the tail and the base of the ear are now approved sites for these implants.

No new resolutions or recommendations were introduced.

The Committee briefly discussed the challenges of obtaining scrapie surveillance samples from certain flocks and herds. Several members mentioned that one barrier to sample collection is the problem that the producers have with carcass disposal after the head has been removed. Members agreed that offering options to producers to help them properly dispose of these carcasses could significantly increase voluntary participation in surveillance. Options include to transporting carcasses to diagnostic laboratories or providing payment to the producers to offset the cost of carcass disposal.