

## Imported Belgium/Netherlands Sheep Questions and Answers

**Q: Why is the U.S. Department of Agriculture (USDA) acquiring three flocks of sheep in Vermont?**

**A:** The USDA's Animal and Plant Health Inspection Service (APHIS) regulates the importation of foreign animals and works with U.S. producers to contain and eradicate animal disease. On July 10, 2000, several sheep in three Vermont flocks tested positive for a transmissible spongiform encephalopathy (TSE).

USDA intends to acquire these sheep to prevent the spread of a TSE to other livestock.

**Q: What threat do these sheep pose?**

**A:** These sheep pose a threat to the entire U.S. sheep industry. TSEs, a class of diseases, can hide in animals for a long time before the animals show signs of illness. Thus, a TSE could spread from coast to coast and no one would know until the infected sheep started to die. TSEs are almost always fatal.

Also, the type of TSE present in the Vermont sheep has not been determined. While it could be the kind that affects only sheep, they could also be carrying the TSE that affects cattle, which could destroy the cattle industry, too.

**Q: Where did these sheep come from and where are they now?**

**A:** Between May and December 1996, two shipments of sheep from Belgium were imported into the United States. These shipments included primarily East Friesian milk sheep, which originated both from Belgium and the Netherlands. A total of 65 sheep were imported. The imported sheep were originally consigned to two farms in Vermont. These sheep have been in milk production flocks since their import, and at this time the majority of the imports are between 4-5 years of age. Most of these original sheep imports bore offspring.

Since the sheep entered the United States, USDA has tracked the movements of the original sheep and their progeny. At this time, all of the imported sheep and their offspring are accounted for.

Forty-eight of the original imports are still alive. Of the 17 which have died or been euthanized, 15 were tested for TSEs with negative results. One died shortly after import and another died later.

**Q: Why did USDA allow importation of these animals?**

**A:** In the early 1990's, there was significant interest from various sources in obtaining both live sheep and germplasm from overseas. This was intended either to improve the genetic base of the domestic sheep population or to gain access to breeds not commonly found in the United States. USDA-APHIS evaluated the situation and came to the conclusion that sheep could be imported from certain countries with various restrictions that would reduce the risk of disease transmission. After this conclusion, there was a brief window of time in 1996 when imports were allowed from certain countries. This window was closed in late 1996, after information was published that outlined both the experimental transmission of BSE to sheep via oral inoculation and a wider tissue distribution of the agent than previously established.

**Q: What actions have been taken since the sheep were imported and why?**

**A:** In 1993, the first indications about bovine spongiform encephalopathy (BSE) infectivity in experimentally inoculated sheep were published. In 1996, additional research demonstrating wider tissue distribution of the agent was published. In late 1997, both Belgium and the Netherlands reported their first cases of BSE in native cattle. Subsequent to these findings, the European Union's Scientific Steering Committee (SSC) issued an opinion in 1998 that stated it is highly likely that European sheep were exposed to feed contaminated with the BSE agent.

The combination of all of these factors led USDA to the conclusion that the sheep in Vermont could possibly have been exposed to BSE while in Europe and therefore a decision was made by the State of Vermont at the request of APHIS to quarantine them. Subsequent to the quarantine, APHIS obtained information that the flocks of origin had been fed concentrates prepared at local mills. This practice has been shown as the most likely route of BSE exposure for the infected cattle in Belgium.

The two flocks of sheep imported in 1996 and their offspring have been under State quarantine in Vermont since October 1998. These sheep and their progeny are prohibited by the State quarantine from entering either the human or animal food chains. If

any of the sheep become ill or died, tissues are collected for diagnostic purposes and the carcasses are incinerated at APHIS' expense.

**Q: What tests were done? When, how, and what were the results that led to the conclusion that these animals have TSE?**

**A:** Each of the sheep that recently tested positive for a TSE underwent four separate tests. The first two tests were conducted in June 1999. The first of these was histopathology. Histopathology examines the brain of the subject for microscopic changes. The sheep tested did show some changes indicative of a degenerative neurological condition, but the changes were not diagnostic for a TSE.

The second test in June 1999 was the immunohistochemistry. This test examines sections of the brain of the subject for the abnormal prion proteins that are an indicator of TSE infection. No abnormal prion protein was detected in this test.

The third and fourth tests were conducted in June and July of this year. The third, the Western-blot test, is another method of detecting the abnormal form of prion proteins in the brain tissue of a test subject. Abnormal prion protein was detected by this method in four of the sheep. Thus, the sheep tested positive for a TSE.

The fourth test, capillary electrophoresis, detects the presence of abnormal prion protein in the blood of a subject. In this case, the same four sheep that tested positive for the Western-blot test also were positive with the capillary electrophoresis test. However, this test is still considered to be experimental; therefore, the USDA actions were not based on these test results.

**Q: Isn't more testing needed to be sure of these results? Why don't you do more testing?**

**A:** The test that was done - Western-blot analysis - is an approved test authorized by APHIS, and it was done at a USDA-cooperating laboratory. The method used for this test has been published in literature and is an accepted methodology. The tissue samples were from best location in the brain to find PrPres (an indicator of TSE infection) if it is present.

**Q: What actions are being taken by the USDA and what further actions are planned?**

**A:** As of August 1, 2000, USDA-APHIS has purchased one flock and is involved in legal actions with the owners of two other flocks. The sheep in the purchased flock were euthanized, samples were obtained, and the carcasses were incinerated.

**Q: Why is USDA taking this action?**

**A:** The test that was positive - Western-blot - cannot differentiate between scrapie and BSE. The only known method to differentiate between these two diseases requires a series of mouse bioassay systems, which take at least 2-3 years for completion. These sheep could possibly have been exposed to BSE in Europe. If they are actually infected with BSE, this would present a significant animal and human health risk. This possibility warrants the conservative actions USDA has taken to minimize any potential risks.

**Q: If the sheep have been in the country since 1996, why has USDA waited this long to take the action?**

**A:** The flocks were placed under quarantine due to the possibility of exposure to BSE that could have occurred prior to their importation. The quarantine allowed for increased monitoring and surveillance of these animals. Although many animals had been examined from these flocks prior to this date, there had not been any definitive evidence of a TSE agent. The USDA has been trying to negotiate with the flock owners to voluntarily sell the sheep to the USDA so that the risk could be removed. Without this new definitive evidence, the USDA did not have authority to move forward. At this time, we now have confirmed evidence of a TSE agent and are moving forward with more stringent actions based on this evidence.

**Q: The government must know it is BSE or why would it declare an extraordinary emergency?**

**A:** BSE cannot be differentiated from scrapie through routine methods of testing and diagnosis. The only current method differentiation uses a series of mouse bioassay systems and can take 2-3 years. The USDA does not know if the agent affecting these flocks is BSE or scrapie. However, we do know that the sheep were fed concentrate from local mills prior to being imported. This type of concentrate has been determined to be the most likely cause of BSE in cattle in those countries. Therefore, these sheep could have been exposed to BSE in Europe.

BSE in sheep has the potential to spread from animal to animal and contaminate the environment, as it appears to behave like scrapie. BSE in cattle behaves very differently and is spread primarily through feed contamination. Since BSE in sheep could be spread laterally (i.e., animal to animal) feed bans would not effectively contain or prevent new infections of BSE in sheep. The most prudent course of action, considering the greater potential of spread for BSE in sheep coupled with the devastating effect to the U.S. livestock industry should BSE become established in the United States, and the public health consequences, dictates that extraordinary actions be taken, even without confirmation of which TSE agent is involved.

In addition, the sheep were imported with numerous restrictions to prevent the introduction of a different strain of scrapie into the U.S. This action would also prevent the introduction of a different and possibly more virulent strain of scrapie.

**Q: How will USDA dispose of the sheep?**

**A:** USDA will euthanize the sheep in a humane manner, take samples for further diagnostic studies, and incinerate their remains. No tissues will enter either the human or animal food chain.

**Q: What can be done with the farms?**

**A:** USDA is evaluating various options at this time. There are some indications in research that the agent can contaminate the environment, so USDA is exploring options to address these concerns. The samples collected from the animals will provide additional information that can be used to help determine the extent of any possible contamination of the farms. This information will assist in USDA's attempt to make an informed decision of what precautions must be taken in regard to the environment on these farms.

**Q: What is the difference between BSE in sheep and scrapie?**

**A:** Both BSE and scrapie are TSEs. TSEs are forms of progressive neurodegenerative disorders that affect both humans and animals and are caused by similar uncharacterized agents that generally produce spongiform changes in the brain. In addition to BSE and scrapie, other examples of TSEs include: transmissible mink encephalopathy; feline spongiform encephalopathy; chronic wasting disease of mule deer, white-tailed deer, black-tailed deer, and elk; and in humans, kuru, Creutzfeldt-Jakob disease, Gerstmann-Straussler syndrome, fatal familial insomnia, and variant Creutzfeldt-Jakob disease (vCJD).

The common characteristics of the TSEs are long incubation periods of months to years, the presence of scrapie-associated fibrils in the brain, and the ability to transmit the disease to laboratory animals by an injection into the brain of brain tissue from the diseased animal.

Scrapie was first recognized as a disease in sheep in Europe more than 250 years ago. It was first diagnosed in U.S. sheep in 1947. Scrapie is not known to be a human pathogen.

BSE was first recognized in Great Britain in 1986 and has been considered primarily a disease of cattle. BSE has not been diagnosed in native cattle outside of Europe. There are various scientific hypotheses concerning the origin of BSE. BSE is thought to be the most likely cause of vCJD, which is a fatal human disease. There have been over 70 vCJD cases in the UK, 2 in France, and 1 in the Republic of Ireland.

BSE can be orally transmitted to sheep with as lit-

tle as one-half gram of infected brain tissue. Sheep infected with BSE showed the same signs as scrapie and routine tests cannot differentiate between the two.

There is one method of distinguishing between scrapie and BSE in the same species. This method involves conducting bioassay studies via the inoculation of infected material into mice. These mouse bioassay studies have been done to identify both BSE in cattle and strains of scrapie in sheep. When these studies were done on brain material from sheep experimentally infected with BSE, the study demonstrated that the agent was similar to the BSE agent as identified in cattle rather than the scrapie agents identified from sheep.

BSE has not been diagnosed as a natural disease in sheep to date. However, it must be pointed out that the studies to differentiate between scrapie and BSE take years and not many samples have been completed.

**Q: What causes BSE?**

**A:** Little is known about the actual agent that causes BSE and other TSEs. So far, scientists know that the TSE agent is smaller than most viral particles. It is highly resistant to heat, ultraviolet light, ionizing radiation, and common disinfectants that normally stop viruses or kill bacteria. Also, the agent does not cause the host's immune system to create detectable antibodies. The TSE agent has not yet been observed under a microscope.

Three main theories on the nature of the agent have been proposed:

- 1) An unconventional virus.
- 2) A prion or a partially protease-resistant protein that is rebuilt into an abnormal prion.
- 3) An incomplete virus (i.e., a small piece of DNA) that protects itself by using a host protein. This is called a virino.

Currently, the abnormal prion theory has gained acceptance among some in the science community. This theory suggests that the TSE agent is actually a normal host prion changed into an abnormal prion. This abnormal prion then reproduces itself by forcing other normal prions to change. It is theorized that the transformation of the prion protein may occur from mutation of the prion gene or from contact with outside abnormal prions.

BSE seems to be caused by a single strain type. This BSE strain is different from historical or contemporary isolates from sheep or goats with natural scrapie or cervids with chronic wasting disease, as determined by study of incubation periods and brain "lesion profiles" in mice.

**Q: Can BSE infect sheep?**

**A:** BSE can be transmitted experimentally to sheep through the feeding of small amounts (.5 gr) of infected cattle brain. This indicates a theoretical possibility that some sheep could have contracted BSE through the consumption of contaminated feed. Investigations on the feeding practices of sheep in Europe found that it was common practice in some countries to feed sheep meat and bone meal. Since continental European imported significant amounts of BSE-contaminated meat and bone meal from the United Kingdom, sheep in the European Union were most likely exposed to the BSE agent. Defining the natural occurrence of BSE in native European sheep will most likely take 2-3 years.

BSE in sheep cannot be differentiated from scrapie though routine methods of diagnosis (current differentiation uses a series of mouse bioassay systems and takes 2-3 years). BSE in sheep appears to cause infectivity in more tissues than BSE in cattle and may spread from one sheep to another, unlike BSE in cattle. If BSE occurs naturally in sheep and behaves like scrapie (i.e., transmits laterally), feed bans will not prevent the spread of disease.

**Q: Can the difference between BSE and scrapie in sheep be determined?**

**A:** BSE in sheep cannot be differentiated from scrapie clinically or by routine methods of diagnosis. Current methods of differentiating use a series of mouse bioassay systems that take 2-3 years to complete.

**Q: Have natural cases of BSE in sheep been detected in any country?**

**A:** Currently, there have not been any naturally occurring cases of BSE in sheep reported in any country. However, testing to differentiate between scrapie and BSE in sheep has not been done routinely. Due to the length of time required for the differentiation studies, only a small number of samples have been completed. Some work has been started in Europe to determine if any of the recent cases diagnosed as scrapie could actually be BSE, but this will take some time. The definitive test to differentiate is a mouse bioassay system that takes several years to complete.

**Q: Do the sheep imported in 1996 have scrapie or BSE?**

**A:** There is no simple laboratory test that can definitively distinguish between BSE and scrapie in animals. Mouse inoculation studies, which take 2 or more years for completion, are necessary to define the disease agent. USDA will request that such studies be initiated on samples from these sheep.

**Q: If the diseases look the same, how do we know if the U.S. sheep population has scrapie or BSE?**

**A:** We have no evidence of BSE in either our sheep or cattle populations. BSE would have to have been introduced into our sheep population through imports. Import restrictions based on both scrapie and BSE have limited the possibility of such exposure. The importation of sheep from countries affected with scrapie have been prohibited since a scrapie eradication program was started in the United States in 1952. The only exceptions to this policy have been for Canada, a country with a similar animal health status as our own, and for the brief period of time in 1996, when the policy was changed. Import restrictions due to BSE took effect in 1989, and BSE was not known to exist prior to 1986. Ten years of active surveillance of U.S. cattle has shown no evidence of BSE. The cattle population can be used as the best indicator of the possible presence of BSE in the United States, as this is the same species in which the disease naturally occurs. Our surveillance system in cattle includes a system of reporting from diagnostic laboratories, field investigations of central nervous system (CNS) disorders, testing of rabies-negative animals from public health laboratories, testing of CNS-condemned cases reported by the Food Safety and Inspection Service (FSIS), and testing of "downer" cattle.

**Q: If there is a possibility that the imported sheep have scrapie, why will the sheep be destroyed?**

**A:** Prior to the importation of these animals, efforts were made to determine that they were free of scrapie. In addition, enrollment in the Scrapie Flock Certification Program was required to ensure that monitoring continued for an extended period of time. None of this surveillance confirmed scrapie. We cannot completely rule out BSE as the possible infectious agent causing the recent evidence of a TSE infection. If these sheep were actually infected with BSE, this would present a significant animal and human health risk, as would a more virulent or different strain of scrapie.

**Q: What assurances do we have for the American public to protect their health?**

**A:** The USDA policy in regards to BSE has been proactive and preventative. APHIS has taken measures in surveillance, prevention, education, and response to protect animal and public health. For example, import restrictions have been in place since 1989 and active surveillance efforts began in 1990. APHIS actively works with State and Federal agencies, including USDA's Food Safety and Inspection Service (FSIS), the Centers for Disease Control (CDC), the Food and Drug Administration (FDA), and the National Institutes of Health (NIH), and

stakeholders to assure we are taking the proper actions in response to changing knowledge and information concerning BSE.

Additional information on public health issues can be provided by the following agencies: USDA-FSIS, FDA, CDC. The CDC can provide details about surveillance efforts for vCJD.

**Q: Is BSE in sheep a risk to humans?**

**A:** The research on BSE in sheep is too new to provide an answer to this question.

**Q: Were meat products from these animals sold for human or animal consumption?**

**A:** No meat from the four sheep that tested positive in 2000 for abnormal prions was ever sold. Approximately 28 lambs went to slaughter in 1997 and another 28 lambs went to slaughter in 1998. The meat from these lambs, approximately 2,000 lbs., was sold to consumers in the Greensboro, Vt., area. All the lamb meat was sold in the state of Vermont. No lamb product was ever exported or sold by mail.

**Q: Can this disease spread to the wildlife population?**

**A:** There is no evidence to date that BSE or scrapie can affect wildlife populations. Scrapie can be spread from animal to animal and can contaminate the environment, especially at lambing. Experiments have shown that BSE in sheep could act like scrapie, so it could also contaminate the environment and wildlife could possibly be exposed. The flocks in Vermont predominantly lambed inside, which reduces the possible risk to wildlife.

Since there is a theoretical risk that wildlife could be exposed to this agent, USDA plans to monitor this situation. Specifically, the free-ranging deer population provides a good monitor for any such exposure. In cooperation with State authorities, USDA is planning to collect samples from hunter harvest surveys to monitor whether any such exposure has occurred.

**Q: Why can't you save the sheep and study them?**

**A:** Retaining these sheep would require sufficient biocontainment holding facilities to handle the BSE agent until it can be determined which TSE agent is affecting this flock. These facilities are limited. In addition, BSE is not known to exist in the United States and because there is a potential risk that the agent affecting these flocks could be BSE, it is most desirable to remove all possible sources of this agent.

As TSEs are neurological diseases, it is necessary to study the brains of the animals. This can best be done after the animals have been euthanized.

**Q: Do genetics influence or contribute to the transmission of TSEs?**

**A:** Research has demonstrated links between genetic variations in sheep and the development of scrapie. Genetic variations among different breeds of sheep may play a role in whether sheep will become infected with scrapie and how quickly clinical signs may appear. At this time, it is not known whether genetics contributes to the development of BSE in cattle. Research into all of these subjects is ongoing.

**Q: What are the testing methods for TSEs?**

**A:** Histopathology: Bilaterally symmetrical degenerative changes are usually seen in the gray matter of the brain stem when a TSE is present. These changes are characterized by vacuolation or microcavitation of nerve cells in the brain stem nuclei. The neural perikarya and axons of certain brain stem nuclei contain intracytoplasmic vacuoles of various sizes, giving the impression of a spongy brain. Hypertrophy of astrocytes (astrocytosis) often accompanies the vacuolation.

**Electron Microscopy:** A TSE diagnosis may also be made when scrapie-associated fibrils (SAF) are detected using negative stain electron microscopy.

**Supplemental tests:** Supplemental tests are available to enhance the diagnostic capabilities for TSEs. Research shows the partially protease-resistant form of the prion protein (PrPres) is found in the brain of TSE-infected animals. Two tests that have been used routinely to detect PrPres in animals showing clinical signs of a TSE are immunohistochemistry and a Western-blot technique. In the past, if the brain tissue was not harvested shortly after the animal's death, autolysis might make it very difficult to confirm a diagnosis by histopathology, but these tests permit a diagnosis of a TSE based on finding PrPres even if the brain has been frozen or if autolysis has occurred.

Last year, the European Commission published a preliminary report on the evaluation of four companies' tests for the diagnosis of TSE in cattle brain samples. These included a modified Western-blot test developed by Prionics A.G. of Switzerland; a chemiluminescent ELISA test using a polyclonal antiPrP antibody for detection developed by Enfer Technology, Ltd., of Ireland; a sandwich immunoassay for PrPres developed by Commissariat à l'Énergie Atomique (CEA) of France; and a two-site noncompetitive immunometric procedure using monoclonal antibodies and DELFIA technology to generate a signal developed by E. G. & G. Wallace, Ltd., of the United Kingdom. The Prionics test is currently being used in Switzerland to test "fallen stock." Other countries, such as Germany and France, are going to start using the Prionics test or one of the other three tests to increase surveillance for BSE in cattle.

A number of tests have been proposed and are in the initial process of being validated for the preclinical diagnosis of TSEs in sheep. These include 1) immunohistochemistry testing of eyelid associated lymphoid tissue and tonsil biopsies, 2) use of capillary electrophoresis and fluorescent labeled peptides to detect PrPres in the blood of animals infected with a TSE, and 3) improved Western-blotting techniques with very good sensitivity to detect PrPres in blood, cerebrospinal fluid, or small pieces of biopsied tissues.

**Agent Isolation:** As the agents that cause TSEs have not been fully characterized or isolated, one method used to detect infectivity in an animal is to inoculate laboratory animals with brain material from the affected animal and monitor them for evidence of disease. This method may take more than 2 years to produce results; hence, it is not practical for routine testing. The most common animal used for this type of bioassay is the mouse. Another problem with the mouse bioassay method when testing cattle or sheep samples is that the species barrier may prevent detection of low levels of infectivity.

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