INFORMATION UPDATE ON SCRAPIE, WITH CONTROL AND ERADICATION MEASURES

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Summary: Recent scrapie scientific advances, Member Country updates and the impact of global trade agreements constitute this Scrapie update. The scientific advances in the last five years relate to diagnostic testing, genetic susceptibility and embryo transfer transmission. Seven of the Member Countries of the Americas reported on their scrapie status: Argentina, Canada, Colombia, Cuba, Mexico, Paraguay and the United States of America. Some countries also discussed their respective surveillance efforts, import policies, control and eradication programs. Finally, the impacts of the General Agreement on Tariffs and Trade (GATT) are discussed in terms of risk assessment, regionalization, harmonization, and transparency.

The scientific advances of the two forms of Prion Protein, PrP-res and PrP-sen, were discussed. PrP-res is the abnormal form of prion protein in scrapie brain. Diagnostic techniques such as immunohistochemistry were reviewed. Immunohistochemistry uses formic acid to enhance the PrP-res and to digest PrP-sen to produce reliable results. Uncertainties about the susceptibility and carrier state of different genotypes highlight the need for further research efforts in the genetic area. Finally, contradictory embryo transfer results were noted. Embryo transfer did not transmit scrapie in one project while the second resulted in scrapie transmission.

Argentina, Colombia, Cuba, Mexico and Paraguay reported scrapie-free status. Canada and the United States reported having scrapie. Some of the seven reporting Countries also reviewed such areas as surveillance efforts, import policies, control and eradication programs.

Recommendations focused on incorporating immunohistochemistry, PrP genetic factors and embryo transfer developments into Member Country scrapie policies. Recommendations were made in the following areas: diagnostic protocols, import policies, surveillance efforts, control and eradication programs. Additional research efforts were recommended on genetic factors and embryo transfer transmission. Finally, adoption of transparency, risk assessments and harmonization were recommended to support trade in live sheep and germplasm.

1. INTRODUCTION

Scrapie is a degenerative neurologic disease classified as a transmissible spongiform encephalopathy. Although the OIE has not completed a International Animal Health Code chapter on scrapie, the OIE Scientific and Technical Review included an extensive review of the disease in 1992, including geographical and temporal distribution, economic implications, aetiology, transmission and epidemiology, clinical signs, pathogenesis and pathology, diagnosis, prevention and control (2). The report cites specific articles on diagnostic testing, genetic susceptibility and embryo transfer transmission. The report also states the countries which submitted their scrapie status including: Argentina, Canada, Colombia, Cuba, Mexico, Paraguay and the United States.

Additional background information for the report includes the 1993 OIE International Animal Health Code additions on import risk analysis (Section 1.4), including chapters on general considerations (1.4.1), guidelines for risk assessment (1.4.2), and zoning and regionalization (1.4.4).

Other suggested background information for this report is an overview of concepts from the General Agreement on Tariffs and Trade (GATT) which took effect in January 1995. These concepts include transparency, risk assessments and harmonization.
2. SCRAPIE SCIENTIFIC ADVANCES

The three major scrapie scientific advances in the last five years are diagnostic testing, genetic structure and embryo transfer transmission. In each area, references to original work are cited. Suggestions to implement and use the advances follow each area.

2.1. Diagnostic testing

The standard scrapie diagnostic technique has been histopathological examination of brain tissue of brain tissue (8). The discovery of the Prion Protein has added another tool to diagnosis scrapie. The Prion Protein (PrP) occurs in two forms, one of which is associated with clinical signs of scrapie (13). PrP-res (protease resistant) is the prion protein form found in the brain of scrapie infected animals. PrP-sen (protease sensitive) is identified in brain tissue from normal animals.

Dr Janice Miller, et al, at the National Animal Disease Center, USDA, Agricultural Research Service, has developed an immunohistochemistry method to detect PrP-res from brain tissue of scrapie suspect sheep (10). Immunohistochemistry (IHC) is a technique using the antibody-antigen interaction. Primary antibody used in the IHC technique was derived from rabbits immunized with PrP-res extracted from brains of mice with experimentally induced scrapie.

Brain tissue from 21 histopathological positive sheep were examined by IHC technique. The PrP-res was widely distributed in the brain stem, with higher concentrations in neuronal cell bodies and around blood vessels. IHC detected PrP-res in four of eleven scrapie-suspect sheep not histopathologically positive. None of the fourteen clinically normal sheep displayed PrP-res from the IHC technique.

PrP-res detection by IHC is equivalent (for scrapie diagnosis), if not superior, to histopathological methods. IHC can detect PrP-res in autolytic tissue or tissue frozen prior to PLP fixation. Freezing and autolysis produce artifacts which prevent satisfactory histopathological diagnosis. Immunohistochemistry may also be useful in identifying scrapie in sheep with clinical signs without histopathological positive results or with preclinical scrapie.

The Western Blot or other immunoblotting techniques rely on PrP-sen removal by a protease digestion step in the PrP extraction protocol. The IHC technique treats tissue sections with formic acid rather than protease to destroy the PrP-sen. Formic acid also enhances the immunoreactivity of PrP-res (10).

IHC survey of formalin fixed Central Nervous System tissue sections is routinely employed at the USDA's National Veterinary Services Laboratories (NVSL) (11). The IHC detects the presence of PrP-res in samples submitted to confirm a scrapie diagnosis. The Western Blot technique, a biochemical procedure, is used at the NVSL as an ancillary test. The IHC and Western Blot procedures are collectively referred to as "The PrP Test".

2.2. Genetic structure

Identification of a specific codon of the PrP gene is the second scientific advance since 1992. This codon was identified in a project headed by Dr David Westaway(14). The nucleotide sequence variation in and around the PrP gene was first demonstrated by Dr Nora Hunter in 1989 (6).

Genes are composed of two strands of DNA building blocks called nucleotides. These units differ in the bases they carry. Bases on one strand combine with bases on the other strand to form base pairs. The base pairs are the rungs of the DNA "ladder". The base pairs specify the sequence of amino acids that must be strung together to make a specific protein. Three base pairs together form a codon. One base pair at a specific codon is exchanged for a different pair during a gene mutation(12).

A study was conducted in Suffolk sheep to determine the composition of specific codons of the PrP gene. Most (86.4%) of U.S. reported scrapie cases are in the Suffolk breed. PrP gene heterogeneity was determined at codon 171 (14). The two amino acids exchanged at this codon are glutamine (Q) and arginine (R). Many (49+/-.6% (N=69)) of nonscraped animals carry one or more Arginine (R) at codon 171 of the PrP gene.

Four scrapie affected sheep were homozygous for Glutamine (Q=susceptible) 171 codon. Thirty one additional scrapie cases were analyzed. All were Q/Q at the 171 codon. The presence of glutamine in pairs at codon 171 renders Suffolk sheep susceptible to natural scrapie. Statistical analysis indicates a strong association between PrP genotype at codon 171 and scrapie susceptibility (p < .000004).
These data imply that homozygosity for Q at 171 codon is one of at least two factors for natural scrapie to develop. The second factor is exposure to the agent.

Certain points need to be clarified before this genetic information can be practically used to control scrapie (1). Factors to note are:

a) Studies have not determined whether the Q/R or R/R at codon 171 of the PRP gene represent true resistance to infection.

b) Q/R or R/R genotype may represent a prolonged incubation period, rather than true disease resistance.

c) If the Q/R or R/R genotype prevents clinical signs, but not infection, these animals could be silent carriers and may pose a risk to susceptible animals.

d) This study used only Suffolk sheep. The results may or may not apply to all other sheep breeds.

e) This study did not address the possibility of agent "strain" differences. Foster and Dickinson in 1988 demonstrated the existence of scrapie strains (4). This work also depicted the different actions of scrapie strains. The incubation period was the main variable for the different strains.

f) In conclusion, infection and clinical signs are related yet separate and distinct events. The genotype plays an important role in the disease process. The infectious agent can be active in the absence of clinical signs. Additional work needs to be conducted on the significance of the Q/R and R/R as related to the scrapie disease process.

Producers may want to pursue developing flocks with Q/R or R/R genotypes. However, they need to understand the uncertainties of the codon 171 genotype data. Modifying the PrP genotype may not be sufficient to develop resistance. Research efforts to develop "knock-out" sheep, animals lacking the PrP gene altogether, may be a more effective means of obtaining scrapie resistant sheep.

2.3. Embryo transfer transmission

The third scientific advance is studying scrapie transmission by embryo transfer. Answering questions on the means of transmitting the scrapie agent between animals has been a long held research goal. One transmission question relates to embryo transfer - Does embryo transfer transmit scrapie? Two different embryo transfer studies reported conflicting results. The two studies are briefly reviewed with differences noted. The studies were headed respectively by Warren Foote of Utah State University, Logan, Utah (3), and J.D. Foster of The Institute for Animal Health, Edinburgh, Scotland (5).

The Foote project examined embryo transfer between scrapie-inoculated and clinically scrapie-free sheep (Cheviot and Suffolk). The study measured scrapie transmission via the embryo-offspring from embryos of scrapie-inoculated donors and clinically scrapie-free recipients. The study also measured scrapie transmission via the uterus-embryos from clinically scrapie-free donors and scrapie-inoculated recipients and offspring delivered by Caesarian Section. Under the experimental conditions, scrapie was not transmitted by the embryo or the uterus. Genotype of the sheep is not known.

The Foster study did embryo transfer between scrapie-infected and scrapie-free sheep. Semen from uninfected scrapie-susceptible rams was used to artificially inseminate the donor ewes. Embryos were transferred to recipients which had been genetically selected for low scrapie susceptibility. Six of the 26 lambs born to these recipients developed scrapie. Genotype of these sheep was well characterized.

Dr Foote's protocol specified the embryos be washed three times while the embryos were not washed in Dr Foster's protocol. Other study differences were the scrapie source and route of infection.

Obviously, embryo transfer can transmit scrapie as seen in the Foster work. Proper washing techniques and other precautions may prevent transmission as per the Foote project. A consistent protocol to prevent disease transmission needs to be developed. The safest approach would be to not use scrapie-associated animals for donors or recipients. Employing a ten-wash protocol as recommended by the International Embryo Transfer Society (7) in scrapie control efforts may prove to be a pragmatic safeguard when using embryo transfer.
3. COUNTRY REPORTS

3.1. Disease Status

Argentina, Canada, Colombia, Cuba, Mexico, Paraguay and the United States submitted disease status reports. Some also submitted reports on surveillance efforts, import policies, control and eradication programs.

Argentina, Colombia, Cuba, Mexico and Paraguay report a free status regarding scrapie. Argentina has never had a confirmed scrapie case. Colombia's last scrapie case occurred in 1976 in a lot of breeding Cheviots imported from the United Kingdom. The disease was detected in importation quarantine and all animals in the lot and contacts were euthanized. Cuba reports scrapie-free status. Mexico reports scrapie freedom based on no evidence of scrapie by clinical signs, epidemiology or histopathology. Paraguay reports freedom from scrapie.

Canada and the United States are affected with scrapie. Canada identified scrapie in three flocks in 1995. Canada has 13,000 plus flocks. The United States identified 42 scrapie cases in 1995 from a total of 90,000 flocks.

3.2. Surveillance efforts

Argentina, Canada and the United States reported on their surveillance efforts. Argentina has a surveillance and notification system through the National System of Epidemiological Surveillance (NSES). The Field Veterinary Service (GELSA) and the Inspection Service of Food Products (GIPA) support the information flow and action. A scrapie suspect would be sent to the central level by this system.

Scrapie is a reportable disease in Canada. Any person who owns or has the possession, care or control of an animal shall immediately notify the nearest veterinary inspector of the presence of scrapie or any fact indicating scrapie is in or around the animal.

Scrapie is also a reportable disease in the United States. Private accredited veterinarians are required to notify the nearest State/Federal Veterinarian of the suspicion of scrapie. Accreditation is granted to private veterinarians by the Veterinary Services of the United States Department of Agriculture. The accreditation system is the first surveillance line for foreign and domestic animal diseases. Other parts of the surveillance system are State/Federal Field Veterinarians and official diagnostic laboratories.

3.3. Import policies

Import policies often reflect a country's disease status. Argentina, Paraguay and the United States reported on their respective import policies. Argentina and Paraguay import only from scrapie-free countries. Cuba will import embryos from free regions of affected countries as well as scrapie-free countries. The guidelines of the International Society for the Transfer of Embryos will need to be followed. The United States will allow importation of germplasm from Canada and Mexico to flocks enrolled in the Voluntary Scrapie Flock Certification Program (VSFCP). The VSFCP will be discussed under the Control section. A regulation permitting germplasm from scrapie-affected countries to enter the United States is being prepared. Such imported animals will be maintained in flocks enrolled in the VSFCP. Risk of disease introduction, disease-free regions and equivalency of current control programs will be the basis for importation. The United States also imports germplasm from Australia and New Zealand without restrictions.

3.4. Control and eradication programs

Canada and the United States reported on their scrapie control and eradication programs. The control programs from the two countries are similar in diagnosis, epidemiological investigation, high risk animal designation, flock plans, cleaning and disinfecting procedures. Both countries diagnose cases based on histopathology and PrP immunohistochemistry. Both countries do an epidemiological investigation following a positive diagnosis. Both countries use a "high risk" designation for those animals most likely to show clinical signs. Both countries require flock plans and disinfecting procedures.

Differences also exist between scrapie control programs in Canada and the United States. Canada quarantines all sheep and goats on the premises where the animal last resided. The United States does not authorize such quarantines, although certain States have chosen such actions. Canada designates a "medium risk" category of animals while the United States denotes a "lower risk" category based on the epidemiological investigation. Canada has a depopulation policy and pays indemnity. The United States only requires removal of certain animals from infected flocks and does not pay indemnity. Canada has a 42 month surveillance period while the United States is now requiring up to a 60 month period.
The United States has a Voluntary Scrapie Flock Certification Program (VSFCP). The VSFCP has been described in detail in the 1992 OIE scrapie chapter (2). The main components of the VSFCP are permanent identification, record keeping, periodical flock inspections and certain movement restrictions. The VSFCP’s goal is to present flocks which have not had scrapie clinical signs or knowingly been exposed to scrapie for a minimum of five years. An enrolled flock progresses through the four phases of the program after a minimum time in each phase. The final phase, certified, is the category in which no exposure or clinical signs have been seen for a minimum of five years.

The VSFCP was started in October, 1992. There are approximately 200 flocks currently enrolled in the program. The program is a voluntary effort to control scrapie and will be a controlled means to import germplasm.

4. INTERNATIONAL TRADE AGREEMENTS

The General Agreement on Tariffs and Trade (GATT), adopted by more than 100 countries, has established new parameters for global trade. The GATT established the principle that animal health requirements for trade must be based on science. The GATT introduced risk assessment as the principal tool for evaluating the risk associated with potential trade. Regionalization was also recognized as an approach for characterizing the health status of parts of countries (zones) or groups of countries (regions). While the GATT reaffirmed the concept of national sovereignty, the trade agreement stressed the goal of harmonizing animal health requirements for trade. The OIE International Animal Health Code was recognized as the international standard for animal health requirements concerning trade. Countries implementing animal health requirements as recommended by OIE guidelines cannot be challenged. Countries retain the sovereign right to develop additional requirements; however they must be prepared to justify these additional requirements on the basis of science. Furthermore, the process by which each signator to the GATT reaches decisions regarding potential trade must be open and transparent to the trading partner.

Scrapie presents an interesting challenge within the context of GATT. The OIE has not completed a International Animal Health Code chapter on scrapie although diagnostic standards do exist (8). Therefore, each country must be prepared to justify its import requirements on the basis of science in order to avoid potential trade disputes. Fortunately, the OIE has promulgated International Animal Health Code Chapters on Import Risk Analysis (Section 1.4) which provide general guidelines for risk assessment and zoning and regionalization. Using these guidelines, countries can evaluate the risk of scrapie importation from affected regions. New Zealand has published a review of risk analysis and the importation of animals which includes a specific discussion of the application of risk analysis to scrapie (9). The discussion concludes that safeguards can be introduced to reduce the risk of scrapie importation to acceptable levels even when the prevalence of scrapie in the exporting country is unknown.

REFERENCES


