

Working with Transmissible Spongiform Encephalopathy Agents

Paul Brown and Christian R. Abee

Abstract

The family of illnesses called transmissible spongiform encephalopathies (TSEs), or “prion” diseases, is composed of a small number of human and animal neurodegenerative diseases caused by unique pathogenic agents that are still not fully defined. They are best considered as “protein-misfolding diseases” (together with Alzheimer’s disease, Parkinson’s disease, and a few other rare examples) resulting from the conversion of a normal body protein into a misfolded amyloid multimer. The pathogenic agents display a unique resistance to conventional disinfection methods and an extraordinary environmental durability, which has led the US Department of Agriculture to designate the causative agent of bovine spongiform encephalopathy as a bio-terrorism security threat. In this review, precautions and regulations concerning the handling of TSE agents are discussed in relation to personnel and environmental biosafety.

Key Words: bovine spongiform encephalopathy; Creutzfeldt-Jakob disease; prion disease; transmissible spongiform encephalopathy

Introduction

The infectious agents that cause transmissible spongiform encephalopathy (TSE¹), or “prion” disease, differ from conventional pathogens in many respects. From the standpoint of laboratory research the most important are (1) their resistance to standard inactivation methods and

environmental degradation (a major disadvantage), and (2) the unlikelihood of their causing infection other than through a penetrating injury (a major advantage). The other distinctive peculiarity of TSE is the comparative slowness with which the disease evolves, requiring weeks, months, or even years of surveillance of infected animals before a bio-assay experiment can be terminated with confidence that a negative or positive result has been achieved.

The precise nature of these agents has yet to be defined. One widely accepted hypothesis is that they arise from a misfolded form of a normal body protein that acts as a “seed,” or template, to induce normally folded protein molecules to adopt its own misfolded configuration. These abnormal protein molecules then aggregate into amyloid deposits that resist cellular catabolism and ultimately destroy the cell. The hypothesis sounds straightforward (even if at odds with the dogma that nucleic acid must direct all infectious processes), but it leaves many unresolved questions, for example:

- What triggers the original misfolding?
- By what steps does the misfolding evolve?
- How does the template actually work?
- How is it possible to account for different “strains” of the agent?
- Why should TSE, alone among the misfolded protein diseases (of which Alzheimer’s disease is the most well-known example), have the property of transmissibility (i.e., infectivity)?

These puzzling issues continue to vex the prion proponents, and leave open the possibility that the protein is a cellular receptor acting in concert with a host cell-integrated or even environmental virus. However, no such agent has yet been identified, despite intensive searching.

The minimal TSE replicating unit has never been visualized, although its aggregated form can be seen under the electron microscope as a fibrillary structure that is largely composed of the misfolded amyloid protein. However, this inability to “see” the agents has not prevented the accumulation of a very large and increasing body of information about their behavior, and how best to minimize risk of infection. This basic concern underlies our discussion of diseases under investigation, animal models in current use, safety training, methods of decontamination, and regulations concerning facility biosafety levels.

Paul Brown, M.D., is the former Medical Director, US Public Health Service, and is Senior Investigator, National Institutes of Health, Bethesda, Maryland. Christian R. Abee, D.V.M., is Professor and Chair, Department of Comparative Medicine, University of South Alabama, Mobile, Alabama.

¹Abbreviations used in this article: APHIS, Animal and Plant Health Inspection Service; BSE, bovine spongiform encephalopathy; BSL, biosafety level; CDC, Centers for Disease Control and Prevention; CITES, Convention on International Trade in Endangered Species of Wild Fauna and Flora; CJD, Creutzfeldt-Jakob disease; CNS, central nervous system; CWD, chronic wasting disease; DHHS, Department of Health and Human Services; LD₅₀, mean lethal dose; PrP, proteinase-resistant protein; TME, transmissible mink encephalopathy; TSE, transmissible spongiform encephalopathy; USDA, US Department of Agriculture; vCJD, variant Creutzfeldt-Jakob disease; WHO, World Health Organization.

Diseases Under Investigation

In Figure 1, the major subtypes of TSE are presented according to known and/or presumed species inter-relationships. The first member of the family to be recognized—in the early 18th century—was scrapie in sheep. Scrapie was shown to be experimentally transmissible in 1936, and was adapted to mice in 1961 (Brown and Bradley 1998).

Creutzfeldt-Jakob disease (CJD¹) was first described in 1920, but its similarity to scrapie was not recognized until 1959, when kuru (a now extinct TSE indigenous to New Guinea) was linked neuropathologically to both scrapie and CJD. Both diseases were subsequently experimentally transmitted to primates in the 1960s. The sporadic (i.e., randomly occurring with no known cause) form of CJD accounts for the great majority of cases, but familial and environmentally acquired varieties of human disease have also been described.

Cases of sporadic CJD occur all over the world at an annual frequency of approximately one case per million population, with a peak incidence in the 50- to 75-yr-old group. The illness typically starts with memory loss, confusion, or behavioral changes either alone or in combination with cerebellar signs such as clumsiness or an unsteady gait. As the disease progresses to involve more and more of the brain, the mental deterioration and cerebellar syndrome progress to a state of disabling dementia and lack of coordination. These symptoms are typically accompanied by a wide variety of additional abnormalities involving visual deterioration, involuntary movements (especially myoclonus), tremors, rigidity, and a terminal state of akinetic mutism. Death usually occurs in 4 to 6 mo, but more rapid or prolonged illnesses are not infrequent (1 mo to more than 2 yr).

Environmentally acquired disease may present distinc-

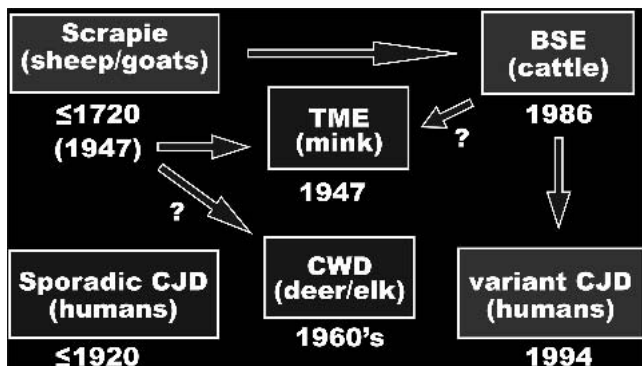


Figure 1 Known and speculative inter-relationships among human and animal forms of transmissible spongiform encephalopathy. TME, transmissible mink encephalopathy; BSE, bovine spongiform encephalopathy; CWD, chronic wasting disease; CJD, Creutzfeldt-Jakob disease; GSS, Gerstmann-Sträussler-Scheinker syndrome; FFI, fatal familial insomnia.

tive clinical features that differ from CJD and from one another. For example, patients infected by contaminated human growth hormone have a predominantly cerebellar illness, with little or no mental deterioration until the end stages of illness. Patients infected by the agent of bovine spongiform encephalopathy (BSE¹) typically present with psychiatric and sensory symptoms (this particular form of disease has been named variant CJD, or vCJD¹). These environmentally acquired forms of disease—especially the variant resulting from exposure to BSE—generated an enormous public concern and a need to study them directly, rather than rely on an assumed validity of data obtained from other model TSEs (Brown et al. 2001). The primary difficulty in studying BSE and vCJD lies in the requirement for more stringent safety measures than have historically been used for laboratory work with TSEs—biosafety level 3 (BSL¹⁻³), rather than BSL-2 conditions, to ensure the prevention of agents escaping into the environment.

The other two animal TSEs, transmissible mink encephalopathy (TME¹) and chronic wasting disease (CWD¹) of deer and elk, have received less attention. In general, the biological features of these diseases appear not to differ in any significant way from other forms of TSE, but epidemiological features may differ. Rare outbreaks of TME on mink farms have all been “dead-end epidemics.” CWD is unique in being the only animal TSE that occurs “in the wild,” and, like scrapie in domesticated sheep, is transmissible among animals indirectly through environmental contamination (Miller et al. 2004). Because CWD appears to be spreading, and it poses special problems in regard to containment and/or prevention, its study is currently experiencing a renaissance.

Research Models

Until comparatively recent times, TSE research was almost entirely limited to infectivity bioassays in animal models. The earliest era of scrapie research in the 1940s and 1950s made use of sheep and goats, and the earliest era of CJD research in the 1960s and 1970s made use of chimpanzees and monkeys. Later, these and other forms of TSE were adapted to laboratory rodents, with huge savings in time, space, and money.

Research was further facilitated by the identification in the 1980s of a proteinase-resistant protein (PrP,¹ or prion protein) as a reliable correlate of infectivity (and arguably the infectious entity) (Prusiner 2001). The ability to generate overnight results on tissues from which PrP has been extracted, and in some cases amplified, using Western blot or enzyme-linked immunosorbent assay visualization of the protein, has vastly reduced the necessity for bioassays, although they are still essential in infectivity studies of decontamination, pathogenesis, and therapy.

Currently, depending on the study goal, any of several animal species may be utilized in experimental designs. Although the use of primates has become restricted as sat-

isfactory alternatives have been developed, it is still occasionally desirable to know how a given TSE infection behaves in species closely related to humans (e.g., how chronic wasting disease of deer and elk would appear in an infected human). Therapeutic trials can also benefit from primate studies, from the standpoint of both efficacy and toxicity.

For most experimental studies, laboratory rodents—especially mice and hamsters—have been the species of choice. The widespread use of mice has been based primarily on the availability of multiple inbred strains with different susceptibilities and pathological responses to different TSE agents (features that were critical in establishing BSE and vCJD as having a single, distinctive strain of agent) (Bruce et al. 1997). Transgenic mice of various descriptions are being used increasingly in studies of genetic susceptibility and as bioassay alternatives to primates, cattle, and humans in studies of CJD, BSE, and CWD. The chief competition comes from the 263K strain of hamster-adapted scrapie, which is often preferred in pathogenesis and inactivation studies because of its comparatively short incubation period (as short as 60 days after intracerebral inoculation) and exceptionally high infectivity titer (10^{10} mean lethal dose [LD_{50}^1] per gram in brain tissue, compared with $\leq 10^6$ LD_{50}^1/g in most other strains of TSE). Guinea pigs have been used in pathogenesis studies and as bioassay animals in tests of decontamination.

Among nonrodent species, the utility of domestic cats has been restricted to electrophysiological studies, but researchers are again using sheep as subjects of blood infectivity studies in natural scrapie and ovine BSE infections. Cattle have been the subject of a huge (and still ongoing) study of BSE pathogenesis, and deer and elk are being used in the study of both naturally occurring and experimentally induced CWD.

Many investigations are satisfied by methodologies that do not require animal experimentation. Several continuous lines of cell cultures have been developed that are chronically infected with TSE agents (usually scrapie), and are used to screen the efficacy of drugs and antibodies to reduce the burden of animal-based infectivity studies. Such *ex vivo* studies are sometimes run in parallel with *in vitro* tests of normal-to-misfolded protein conversion inhibition (Caughey et al. 2001).

Employee Safety

Central nervous system tissue contains much greater concentrations of infectivity than other bodily tissues and organs that, depending on the strain of TSE strain and infected host, may or may not be infectious. Based on the accumulated evidence, tissues have recently been organized into categories of high, lower, and (to date) undetected levels of infectivity (Appendix Tables A-C) (WHO 2001). As a frame of reference, brain typically contains about one million LD_{50}^1/g , spleen may contain up to 1000 LD_{50}^1/g , and

blood contains fewer than 100 LD_{50}^1/mL . Precise infectivity levels have not been established for many peripheral tissues, but have been estimated to be low based on comparatively long bioassay incubation times (low doses of infectivity generally produce long incubation times).

The same common sense precautions taken in the handling of any infectious material, whether influenza, herpes, or human immunodeficiency virus, apply equally to the handling of TSE tissues, and are equally effective in preventing infection. The golden rule for TSE is to avoid penetrating injuries, contamination of abraded skin, and ingestion. Abundant experimental evidence has shown that at least 10 times more infectivity is needed to transmit disease by even the most efficient peripheral route (intravenous) than by direct inoculation into the brain, and an even greater differential exists for infection by other peripheral routes (e.g., subcutaneous or oral).

Thus, the standard cap, mask, gown, and glove costume, avoidance of mouth pipetting, and care in the use of sharp instruments are in fact adequate to eliminate, or at least minimize, the risk of working with TSE. Puncture-resistant or chain-mail gloves are an additional barrier to penetrating injury, but they are extremely cumbersome to use. To the extent possible, all tissue manipulations should be performed inside a negative pressure laminar flow hood, although neither experimental nor natural aerosol infections have ever been documented (for that matter, no accidental infections by any route have occurred in laboratory workers during more than half a century of experimental research).

The environmental durability of TSE agents, which can survive in soil for at least 3 yr (Brown and Gajdusek 1991), requires special care in avoiding workplace contamination. We thus recommend preventing the inevitable contacts of potentially infectious tissue with table and other surfaces by using disposable plastic coated paper sheets, which together with disposable instruments and work costumes, can be bagged before leaving the work room for eventual incineration. All nondisposable materials (e.g., instruments, bottles, and pipettes) must be treated according to the procedures outlined in the following section on decontamination.

Decontamination Procedures

The unique resistance of TSE infectivity to standard methods of decontamination requires a significant departure from the disinfection routine used for conventional pathogens (Taylor 2000). Table 1 contains a list of chemical and physical methods that have been experimentally tested on one or another form of TSE agents (usually high titer hamster-adapted scrapie, but in some cases mouse-adapted scrapie or guinea pig-adapted CJD). Of note is the inadequacy of customary techniques for decontamination. Inactivation of TSE agents requires chemical exposure to high concentrations of either sodium hypochlorite or sodium hydroxide, or physical exposure to steam or dry heat at temperatures higher than are customarily used for conventional pathogens.

Table 1 Efficacy of various chemical and physical agents to disinfect transmissible spongiform encephalopathy agents

Ineffective	Partially effective	Effective
Chemical methods Alcohol Ammonia β-propiolactone Detergents Ethylene oxide Formaldehyde Hydrochloric acid Hydrogen peroxide Peracetic acid Permanganate Phenolics	Chlorine dioxide Gluteraldehyde Iodophores Guanidine thiocyanate (4 M) Sodium dichloroisocyanurate Sodium metaperiodate Urea (6-8 M)	Hypochlorite (1-5%) NaOH (1-2 N) Formic acid (100%)
Physical methods Boiling (100°C) Microwave radiation UV radiation	Steam heat (121°C) Dry heat (300°C) Ionizing radiation (≥50 kG)	Steam heat (134°C) Dry heat (>600°C)

Historically, reusable laboratory material has been immersed in a basin containing either a freshly prepared 1:5 dilution of sodium hypochlorite, or a 1 N solution of sodium hydroxide, allowed to remain at least 1 hr (overnight if possible), then rinsed with copious amounts of tap water before being packaged for autoclave sterilization at 134°C for at least 20 min (WHO 1999). This combination of chemical and physical sterilization has been shown to be necessary to guarantee reproducible sterilization of high titer tissue such as brain, although either chemical or physical methods are probably sufficient for peripheral tissue decontamination. The point has never really been tested, so it is prudent to use the combination technique for all contaminated material.

The question of how to deal with laboratory equipment that cannot withstand these harsh disinfection measures is not easily answered. A recent report (Fichet et al. 2004) on new disinfection methods described several milder chemical formulations that significantly reduced infectivity. However, the best solution is to “dedicate” instruments that are difficult or impossible to sterilize (e.g., electrophoresis and gel transfer frames, spectrophotometers, microtomes, and electron microscopes) for exclusive use in TSE studies. Every effort should be made to restrict contact of infectious material to only those surfaces required for analytical operation.

Regulatory Environment

Government agencies in most countries have established guidelines that should be followed when working with in-

fectious disease agents. Guidelines differ from country to country, depending on many factors (e.g., in a country in which a given disease is endemic, less stringent working conditions may be permitted than in a disease-free country). In the United States, the conditions for working with infectious disease agents have been grouped into four BSLs, with the least hazardous categorized as BSL-1, and the most hazardous as BSL-4. Historically, all TSE agents have been considered to require handling according to modified BSL-2 conditions. The principal differences between BSL-2 and -3 regulations concern the risk posed by aerosol infections, and the stringency of disposal of contaminated materials. BSL-2 conditions assume that aerosol infections do not occur, and that contaminated materials are comparatively easy to disinfect; BSL-3 conditions assume that aerosol infections do occur, and that contaminated materials are difficult to disinfect. Because TSE agents are not spread by aerosol but are extremely difficult to disinfect, BSL-2 conditions have been modified to include the use of laminar flow hoods to facilitate containment of the infectious agents. Effective disinfection methods must be applied to all contaminated materials before they leave the workplace, or such materials must be placed in sealed bags for incineration outside the workplace.

With increasing concerns over the threat of bioterrorism and the steadily changing attitude regarding risks associated with handling infectious agents, regulatory requirements continue to change. The Public Health Security and Bioterrorism Preparedness and Response Act of 2002 (PL 107-188) was signed into law in June 2002. Title II of this Act, “Enhancing Controls on Dangerous Biological Agents and Toxins,” provided for the regulation of certain biological

agents and toxins by the Department of Health and Human Services (DHHS¹) and the United States Department of Agriculture (USDA¹). DHHS designated the Centers for Disease Control and Prevention (CDC¹) as the agency with primary responsibility for agents hazardous to human health, and the USDA designated the Animal and Plant Health Inspection Service (APHIS¹) to fulfill this role for agents hazardous to animals. The USDA Secretary was charged with the responsibility of establishing a list of all biological agents and toxins considered to pose a severe threat to animal or plant health, or to animal or plant products. This directive resulted in the Agricultural Bioterrorism Protection Act of 2002; Possession, Use and Transfer of Biological Agents and Toxins; Interim Final Rule (USDA/APHIS 2002). Currently, BSE is the only TSE listed as an agent determined to have the potential to pose a severe threat (select agent). Thus, despite the absence of aerosol disease transmission, BSE must be handled at BSL-3. The same containment requirements apply to vCJD, which is simply BSE passaged in humans; vCJD is not listed as a select agent, and is thus not subject to the Select Agent Regulations.

Laboratories in which work is performed with BSE must be registered with APHIS and meet a number of requirements as specified under the Act. Scientists interested in working with either BSE or vCJD should contact their institutional biosafety officer and the USDA/APHIS to obtain specific instructions on how to comply with the regulations governing possession and research use of these agents. Additional information regarding the Select Agent Program can be obtained from the CDC website (www.cdc.gov/od/sap) and from the Select Agent Regulations, 42 CFR 73.0, Interim Final Rule. All other TSEs are subject to modified BSL-2 requirements that may vary from state to state and situation to situation. For example, procedures that involve direct contact/manipulation of human tissue or a human TSE passaged through a nonhuman primate may require a BSL-3 facility, whereas tissue storage or housing of animals inoculated with these TSEs are satisfied by BSL-2 conditions (Richmond and McKinney 1999).

Although the general tenor of these guidelines is echoed in handling procedures recommended in other countries, the details differ. By way of illustration, the United Kingdom distinguishes between laboratory containment and animal containment levels, and between large and small animals. BSE and human TSEs require BSL-3 laboratory and small animal containment, and scrapie requires BSL-2 laboratory and small animal containment. All TSEs require only BSL-1 conditions for large animal containment. Clearly, before setting up a laboratory to study TSE, it is mandatory to consult governmental guidelines issued by the country in which the research is to be carried out.

Laboratory Environment

Containment of agents and prevention of accidental exposure are accomplished by attention to three successive bar-

riers. The first barrier is protective clothing such as disposable gloves, gowns, masks, and glasses. The second is the laboratory area in which the agent is used, and the third is the building in which the laboratory is located. Most biomedical research laboratories meet facility design requirements for working at BSL-2, but more stringent facility design requirements must be met for work requiring BSL-3.

BSL-2 facility requirements include placement of the laboratory away from public areas, and lockable doors to prevent accidental entry of unauthorized personnel. Each room within the laboratory should have a sink for hand-washing that can be operated "hands-free." All spaces in the laboratory should be easily accessible for cleaning and disinfection, and bench tops must be impervious to water and resistant to chemicals. Biological safety cabinets should be designed so that fluctuations in the room air supply or exhaust do not prevent the cabinet from operating within its design parameters. There should be an eyewash station readily available, and lighting should be designed to avoid glare that could impede vision. There are no specific ventilation requirements at the BSL-2 level.

BSL-3 conditions are required for all work with BSE and vCJD. In addition to the BSL-2 requirements described above, the following facility design requirements must be fulfilled (Richmond and McKinney 1999):

1. Access to the laboratory must be restricted to authorized personnel only, and the facility must be separated from areas of unrestricted traffic flow within the building. At least two sets of self-closing doors must be located at the entrance into the laboratory. The outer set of doors must be lockable. A clothes change area must be provided either in the passageway between the two sets of doors or in a separate room accessible after people enter the facility.
2. All surfaces in the laboratory, including walls, floors, and ceiling, must be sealed and impervious to water and chemicals used in the laboratory. Any seams in finished surfaces must be sealed. It is preferable that the laboratory is devoid of windows, but if windows are present, they must be closed and sealed.
3. The laboratory must contain a ducted exhaust air ventilation system designed to create directional airflow that draws air from clean or uncontaminated areas toward contaminated areas. Exhaust air is not recirculated, but must be dispersed away from occupied areas and supply air intakes, or, if this is not possible, exhaust air must be high-efficiency particulate air filtered. Visual monitoring devices that verify proper air flows must be visible to laboratory personnel.
4. BSL-3 facility design and operational procedures must be documented. The facility must be tested, and the fulfillment of operational parameters must be verified prior to operation. Reverification should be carried out annually.

Before beginning studies that may require BSL-3 condi-

tions, it is wise to consult with the CDC and APHIS/USDA to assure compliance with currently accepted practices and applicable legislation regulating the use of the specific pathogen to be studied. In addition to facility design requirements, there are Standard Microbiological Practices, Special BSL-3 Practices, and Specific Safety Equipment (Primary Barrier) requirements that must be met (Richmond and McKinney 1999).

Shipment of Specimens

It often happens that infectious tissues must be transported from the place of collection (e.g., a hospital or agricultural field station) to one or more laboratories with different areas of analytical expertise. Regulations governing the shipment of biologically hazardous materials have been substantially modified in recent years, due in part to the threat of bioterrorism; and they are under continual review with frequent changes that can significantly delay the exchange of tissues at both national and international levels. For example, a recent change extends biohazard shipping regulations to noninfectious recombinant PrP and antisera raised against TSE agents!

An additional level of regulation must be met if specimens fall under the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES¹). The United States and most other countries are CITES signatories, and require a series of measures for international shipment of tissues and biological fluids from endangered and threatened species, including all nonhuman primates. The US Department of Interior, Fish and Wildlife Service, Division of Management Authority is responsible for regulating such shipments.

At a minimum, all such shipments must be packaged and shipped in containers that meet international standards for biohazardous materials, and be preapproved with all appropriate documentation by both the shipping and receiving country. Furthermore, because regulations (and their interpretation by regulators) are continually changing, and there is usually a stipulated time limit for completing the shipment, it may happen that the entire process of approval and documentation will have expired before the shipment can actually be sent, requiring the entire process to be repeated. Therefore, it is strongly recommended that appropriate state and federal agencies be consulted well in advance of any intended shipments, and that a professional courier company experienced with international shipment of CITES specimens be used for pick-up, trans-shipment and delivery.

References

Brown P, Bradley R. 1998. 1755 and all that: A historical primer of transmissible spongiform encephalopathy. *Br Med J* 317:1688-1692.

- Brown P, Gajdusek DC. 1991. Survival of scrapie virus after 3 years' interment. *Lancet* 337:269-270.
- Brown P, Will RG, Bradley R, Asher DM, Detwiler L. 2001. Bovine spongiform encephalopathy and variant Creutzfeldt-Jakob disease: Background, evolution, and current concerns. *Emerg Inf Dis* 7:6-16.
- Bruce M, Will RG, Ironside JW, McConnell I, Drummond D, Suttie A, McCordle L, Chree A, Hope J, Birkett C, Cousins S, Fraser H, Bostock CJ. 1997. Transmissions to mice indicate that "new variant" CJD is caused by the BSE agent. *Nature* 389:498-501.
- Caughey B, Gregory RJ, Callahan MA, Wong C, Baron GS, Xiong L-W. 2001. Interactions and conversions of prion protein isoforms. *Adv Protein Chem* 57:139-169.
- Fichet G, Comoy E, Duval C, Antloga K, Dehen C, Charbonnier A, McDonnell G, Brown P, Lasmézas CI, Deslys J-P. 2004. Novel methods for disinfection of prion-contaminated medical devices. *Lancet* 364:521-526.
- Miller MW, Williams ES, Hobbs NT, Wolfe LL. 2004. Environmental sources of prion transmission in mule deer. *Emerg Infect Dis* 10:1003-1006.
- PL [Public Law] 107-188. 2002. Public Health Security and Bioterrorism Preparedness and Response Act of 2002. Washington DC: GPO. June 12, 2002.
- Prusiner SB. 2001. Shattuck lecture—Neurodegenerative diseases and prions. *N Engl J Med* 344:1516-1526.
- Richmond JY, McKinney R, eds. 1999. Biosafety in Microbiological and Biomedical Laboratories. 4th ed. Washington DC: GPO.
- Taylor DM. 2000. Inactivation of transmissible degenerative encephalopathy agents: A review. *Vet J* 159:10-17.
- USDA/APHIS [US Department of Agriculture/Animal and Plant Health Inspection Service]. 1999. Agricultural Bioterrorism Protection Act of 2002: Possession, Use, and Transfer of Biological Agents and Toxins, Interim Final Rule. Washington DC: GPO.
- WHO [World Health Organization]. 1999. Infection Control Guidelines for Transmissible Spongiform Encephalopathies. Report of a WHO Consultation WHO/CDS/CSR/APH/2000.3. Geneva: WHO, March 23, 1999.
- WHO [World Health Organization]. 2003. Guidelines on Transmissible Spongiform Encephalopathies in Relation to Biological and Pharmaceutical Products, ECBS. Available online (<http://www.who.int/biologicals—Meeting Reports, TSEs>).

APPENDIX

Distribution of Infectivity and PrP^{TSE} in Tissues of Humans and Animals with TSE

The information in Appendix Tables A-C represents an updated version of data compiled by a World Health Organization (WHO) expert committee that convened in 2003. The data are based exclusively on observations of naturally occurring disease, or primary experimental infection by the oral route. Not included are data on models using strains of TSE that have been adapted to experimental animals, because passaged strain phenotypes can differ significantly and unpredictably from those of naturally occurring disease. In addition, because immunohistochemical and/or Western blot detection of misfolded host protein (PrP^{TSE}) have proven to be reliable indicators of infectivity, PrP^{TSE} testing results have been presented in parallel with bioassay data. Tissues are grouped into the following three major infectivity categories, irrespective of the stage of disease:

- A. High-infectivity tissues: Central nervous system (CNS¹) tissues that attain a high titer of infectivity in the later stages of all TSEs, and certain tissues that are anatomically associated with the CNS.
- B. Lower-infectivity tissues: Peripheral tissues that have tested positive for infectivity and/or PrP^{TSE} in at least one form of TSE.
- C. Tissues with no detectable infectivity: Tissues that have been examined for infectivity and/or PrP^{TSE} with negative results.

Although the category of lower risk tissues almost certainly includes some (e.g., blood) with a lower risk than other (e.g., lymphoreticular) tissues, there are so few data

about infectivity levels in these tissues that no attempt was made to subdivide the category into different levels of lower risk. It is also evident that the placement of a given tissue in one or another category can be disease specific, and subject to revision as new data accumulate. Data entries are shown as follows:

- + Presence of infectivity or PrP^{TSE}
- Absence of detectable infectivity or PrP^{TSE}
- NT Not tested
- NA Not applicable
- ? Controversial or uncertain results
- () Data limited to one or two tested specimens (human tissues)

Appendix Table A High-infectivity tissues

CNS tissues that attain a high titer of infectivity in the later stages of all TSEs and certain tissues anatomically associated with the CNS

Tissues	Human TSEs				Cattle		Sheep & goats	
	vCJD		Other TSEs		BSE		Scrapie	
	Infectivity ¹	PrP ^{TSE}	Infectivity ¹	PrP ^{TSE}	Infectivity ¹	PrP ^{TSE}	Infectivity ¹	PrP ^{TSE}
Brain	+	+	+	+	+	+	+	+
Spinal cord	+	+	+	+	+	+	+	+
Retina, optic nerve	+	+	+	+	+	NT	NT	+
Spinal ganglia	NT	+	NT	+	+	NT	NT	+
Trigeminal ganglia	NT	+	NT	+	+	NT	NT	+
Pituitary gland ²	NT	+	+	+	–	NT	+	NT
Dura mater ²	NT	–	+	–	NT	NT	NT	NT

¹Infectivity bioassays of human tissues have been conducted in primates, mice, or both; bioassays of cattle tissues have been conducted in cattle, mice, or both; and most bioassays of sheep and/or goat tissues have been conducted only in mice. In regard to sheep and goats, not all results are consistent for both species.

²No experimental data about infectivity in human pituitary gland or dura mater have been reported, but cadaveric dura mater patches and growth hormone derived from cadaveric pituitaries have transmitted disease to nearly 350 recipients and must therefore be included in the category of high-risk tissues.

Appendix Table B Lower-infectivity tissues

Peripheral tissues that have tested positive for infectivity and/or PrP^{TSE} in at least one form of TSE

Tissues	Human TSEs				Cattle		Sheep & goats	
	vCJD		Other TSEs		BSE		Scrapie	
	Infectivity ¹	PrP ^{TSE}	Infectivity ¹	PrP ^{TSE}	Infectivity ¹	PrP ^{TSE}	Infectivity ¹	PrP ^{TSE}
<i>Peripheral nervous system</i>								
Peripheral nerves	NT	+	(-)	+	-	+	+	NT
Enteric plexuses ³	NT	+	NT	(-)	NT	+	NT	+
<i>Lymphoreticular tissues</i>								
Spleen	+	+	+	+	-	-	+	
Lymph nodes	+	+	+	-	-	-	+	+
Tonsil	+	+	NT	-	+	NT	+	+
Nictitating membrane	NA	NA	NA	NA	+	-	NT	+
Thymus	NT	+	NT	-	-	NT	+	NT
<i>Alimentary tract</i>								
Oral cavity ⁴	NT	-	NT	-	-	NT	-	+
Esophagus	NT	-	NT	-	-	NT	NT	+
Fore-stomach (ruminants only)	NA	NA	NA	NA	-	NT	NT	+
Stomach/abomasum	NT	-	NT	NT	-	NT	NT	+
Duodenum	NT	-	NT	NT	-	NT	NT	+
Jejunum ⁵	NT	+	NT	-	-	NT	NT	+
Ileum ^{5,6}	NT	+	NT	-	+	+	+	+
Large intestine ⁵	NT	+	NT	-	-	NT	+	+
<i>Reproductive tissues</i>								
Placenta ⁷	NT	-	(+)?	-	-	NT	+	+
<i>Other tissues</i>								
Lung	NT	-	+	-	-	NT	-	NT
Liver	NT	-	+	-	-	NT	+	NT
Kidney	NT	-	+	-	-	-	-	-
Adrenal	NT	+	-	-	NT	+	+	NT
Pancreas	NT	-	NT	-	-	NT	+	NT
Bone marrow	NT	NT	(-)	-	+	NT	+	NT
Skeletal muscle ⁸	NT	+	(-)	+	-	NT	-	+
Blood vessels	NT	+	NT	+	-	NT	NT	+
Olfactory mucosa	NT	NT	NT	+	-	+	+	+
Cornea ⁹	-	-	+	-	NT	NT	NT	NT
<i>Body fluids</i>								
CSF	NT	-	+	-	-	NT	+	NT
Blood ¹⁰	+	-	-	-	-	NT	+	-

³In cattle, limited to the distal ileum.

⁴In humans with sVJD or vCJD, tested negative tissues included dental pulp, gingiva, and salivary glands; in sheep with scrapie, only tongue (positive) and salivary glands (negative) were tested; and in cattle with BSE, only the salivary glands (negative) were tested.

⁵In vCJD, positivity is limited to gut-associated lymphoid tissue and enteric plexuses (mucosa, smooth muscle, and serosa are negative).

⁶In cattle and sheep, only the distal ileum has been bioassayed for infectivity.

⁷A single report of transmission of CJD infectivity from human placenta has never been confirmed and is considered improbable.

⁸Intracerebral inoculation of muscle homogenates has not transmitted disease to primates from humans with sCJD, or to mice or cattle from cattle with BSE. However, older reports described single instances of transmission from goat and hamster muscle, and recent published and unpublished studies have reported the presence of PrP^{TSE} in skeletal muscle of mice and hamsters orally infected with either scrapie or BSE, and in humans with both sCJD and vCJD. It remains to be seen whether PrP^{TSE} will be associated with infectivity.

⁹Because only one or two cases of CJD have been plausibly attributed to corneal transplants among hundreds of thousands of recipients, cornea is categorized as a lower-risk tissue; other anterior chamber tissues (lens, aqueous humor, iris, conjunctiva) have been tested with a negative result both in vCJD and other human TSEs, and there is no epidemiological evidence that they have been associated with iatrogenic disease transmission.

¹⁰Early reports on the transmission of disease to rodents from the blood of patients with vCJD have not been confirmed, and evaluation of the ensemble of experimental and epidemiological data relevant to TSE transmission through blood, blood components, and therapeutic plasma products fails to suggest transmission from blood of patients with any form of "classical" TSE. However, two highly probable transmissions from packed red cell transfusions donated by patients in the presymptomatic phase of vCJD have recently been reported in the United Kingdom; and in genotypically susceptible sheep with natural scrapie or experimentally induced BSE, transfusion of large blood volumes has transmitted disease, as have blood and blood components from experimentally infected rodents with both animal and human strains of TSE. Fetal calf blood has not transmitted disease.

Appendix Table C Tissues with no detected infectivity

Tissues with no detected infectivity

Tissues	Human TSEs				Cattle		Sheep & goats	
	vCJD		Other TSEs		BSE		Scrapie	
	Infectivity ¹	PrP ^{TSE}	Infectivity ¹	PrP ^{TSE}	Infectivity ¹	PrP ^{TSE}	Infectivity ¹	PrP ^{TSE}
<i>Reproductive tissues</i>								
Testis	NT	–	(–)	–	–	NT	–	NT
Prostate/epididymis/ seminal vesicle	NT	–	(–)	–	–	NT	–	NT
Semen	NT	–	(–)	–	–	NT	NT	NT
Ovary	NT	–	NT	–	–	NT	–	NT
Uterus (nongravid)	NT	–	NT	–	–	NT	–	NT
Placenta fluids	NT	NT	(–)	NT	–	NT	NT	NT
Fetus ¹¹	NT	NT	NT	NT	–	NT	–	NT
Embryos ¹¹	NT	NT	NT	NT	–	NT	?	NT
<i>Musculo-skeletal tissues</i>								
Bone	NT	NT	NT	NT	–	NT	NT	NT
Heart/pericardium	NT	–	–	–	–	NT	–	NT
Tendon	NT	NT	NT	NT	–	NT	NT	NT
<i>Other tissues</i>								
Trachea	NT	–	NT	–	–	NT	NT	NT
Skin	NT	–	NT	–	–	NT	–	NT
Adipose tissue	NT	–	(–)	–	–	NT	NT	NT
Thyroid gland	NT	–	(–)	–	NT	NT	–	NT
Mammary gland/ udder	NT	NT	NT	NT	–	NT	–	NT
<i>Body fluids, secretions and excretions</i>								
Milk ¹²	NT	NT	(–)	NT	–	NT	–	NT
Colostrum ¹³	NT	NT	(–)?	NT	NT	NT	–	NT
Cord blood ¹³	NT	NT	(–)?	NT	–	NT	NT	NT
Saliva	NT	–	–	NT	NT	NT	–	NT
Sweat	NT	NT	–	NT	NT	NT	NT	NT
Tears	NT	NT	–	NT	NT	NT	NT	NT
Nasal mucus	NT	–	–	NT	NT	NT	NT	NT
Urine ^{13,14}	NT	NT	–	–	–	NT	NT	NT
Feces	NT	NT	–	NT	–	NT	–	NT

¹¹Embryos from BSE-affected cattle have not transmitted disease to mice, but no infectivity measurements have been made on fetal calf tissues other than blood (negative mouse bioassay). Calves born of dams that received embryos from BSE-affected cattle have survived for observation periods of up to 7 yr, and examination of the brains of both the unaffected dams and their calves revealed no spongiform encephalopathy or PrP^{TSE}.

¹²Evidence that infectivity is not present in milk includes temporospatial epidemiological observations failing to detect maternal transmission; clinical observations of more than 100 calves nursed by infected cows that have not developed BSE; and experimental observations that milk from infected cows has not transmitted disease when administered intracerebrally or orally to mice. Experiments are in progress in which large volumes of milk from experimentally infected cows are concentrated and tested for the presence of PrP^{TSE}.

¹³Single reports of transmission of CJD infectivity from human cord blood, colostrum, and urine have never been confirmed and are considered improbable.

¹⁴An atypical form of PrP was recently reported to be present in the urine of sporadic and familial CJD patients, but has since been determined to be an artifact of urinary bacterial contamination.