Mad Cow Disease, Creuzfeldt-Jakob Disease, Other TSEs, and Biosolids

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RECENTLY, concerns have been raised about the potential for spreading "mad cow disease" and related diseases through the application of biosolids to land. As one concerned individual recently put it, '"Livestock are also at risk from ingesting infectious prions in both Class A and Class B land applied municipal sewage sludge 'biosolids.'" The following reviews current understanding of "mad cow" and related diseases and the potential risks of infection via biosolids.

What are Transmissible Spongiform Encephalopathies?

In1997, Stanley Prusiner, a U.S. biochemist at the University of California in San Francisco, received the Nobel Prize in Medicine for the discovery of a new genre of disease-causing agents, abnormal "prions," and the elucidation of the underlying principles of prions' mode of action in causing a group of diseases called "transmissible spongiform encephalopathies" (TSEs).

The several animal and human TSEs are neurological diseases attributed to abnormal prions. These include: BSE "mad cow disease" in cattle; scrapie in sheep; chronic wasting disease (CWD) in deer and elk; kuru in humans (primarily as a result of cannibalism through the consumption of infected tissues); Creutzfeldt-Jakob (CJD) disease in humans; fatal familial insomnia (FFI), and Gerstmann-Straussler-Scheinker (GSS) [(USFDA, 2005; Collins et al., 2001)].

TSEs have the characteristic of causing tiny holes in brain tissue, making the tissue resemble a sponge when viewed under a microscope (NINDS, 2005). CJD, or Creutzfeldt-Jakob disease, the most common TSE disease in humans, was discovered by two German psychiatrists in the late 19th century; it is a rare, degenerative, and fatal brain disorder. Figures 1, 2, and 3 show a normal, a CJD cerebellum and a neocortex displaying typical effects of a TSE disease.

What are prions?

"Prion" refers to a particular kind of protein found in animal tissue. Most prions occur in a normal, harmless form, but there are abnormal or infectious forms. The normal, harmless form has the same sequence of amino acids as the abnormal form, but the abnormal, or infectious, form takes a different folded shape. In their normal, non-infectious state, it is believed that prions are involved in cell-to-cell communications and other important functions.

Unlike bacteria and viruses, prions do not contain genetic material. However, like viruses and bacteria, prions are infectious and replicate in host tissues. To cause a TSE disease, it is thought that a normal prion, called PrPc or PrP, misfolds into an abnormal prion protein called PrPSc. It is still unknown how a normal PrPc protein is transformed to an abnormal PrPSc prion. However, it is believed that when normal prions come in contact with infected prions, they are transformed into an abnormal prion. This process causes a geometric increase in the animal tissue of abnormally shaped prion proteins until the numbers of abnormally shaped prions cause a TSE illness.

Research has determined that abnormal prions are found in infected individuals mainly in neurological tissues, including the brain and spinal cord, as well as in lymph nodes, spleen, tonsils, and eyes. They have also been detected in the pancreas and adrenal gland. In experimental studies with mice, scrapie prions have been observed in muscle tissue, and evidence from laboratory studies indicates that prions have been found in blood, but only in small quantities (U. S. EPA, 2003).

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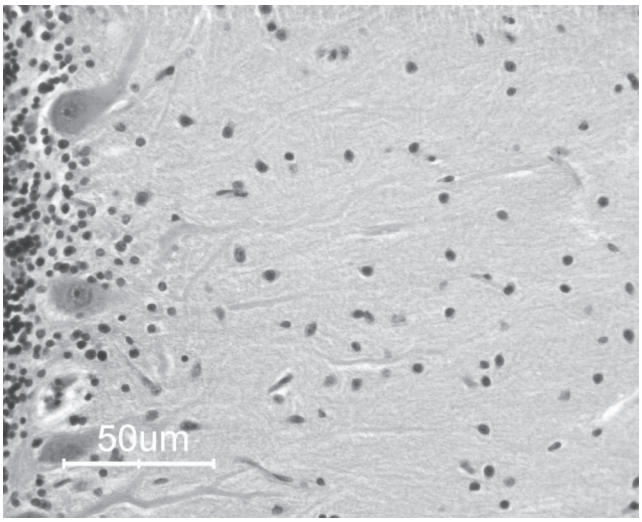

Figure 1. *Normal cerebellum (Courtesy of Drs. Barbara Crain and Honathan Epstein, Johns Hopkins Medical Center).*

Prions are not considered likely to be found in animal manures in significant quantities and are not likely to be transmitted by ingestion of manure.

Abnormal prions can survive for extended periods of time in the environment; however, there is some evidence of degradation in soils. According to Gale and Stanfield (2001), there are some forms of digestion in laboratory experiments that have been effective in degrading some forms of TSE-causing prions. In addition, some TSE-causing prions have been effectively inactivated with lime treatment. However, in general, infective prions are considered to be resistant to many conventional wastewater and sludge pathogen treatments (U. S. EPA, 2003).

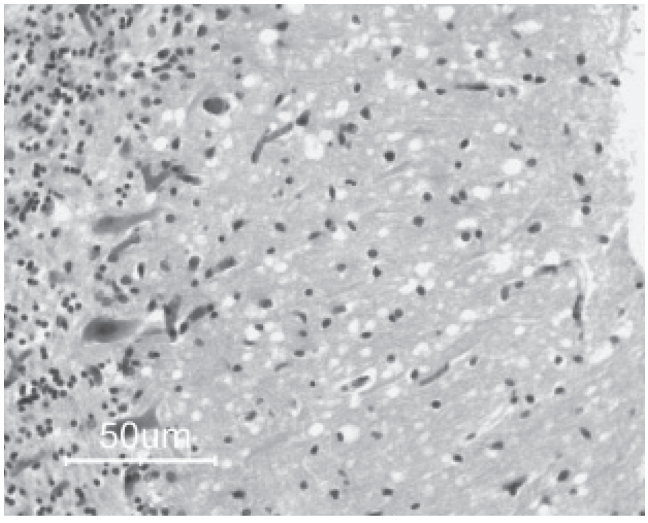

Figure 2. *CJD in cerebellum (Courtesy of Drs. Barbara Crain and Honathan Epstein, Johns Hopkins Medical Center).*

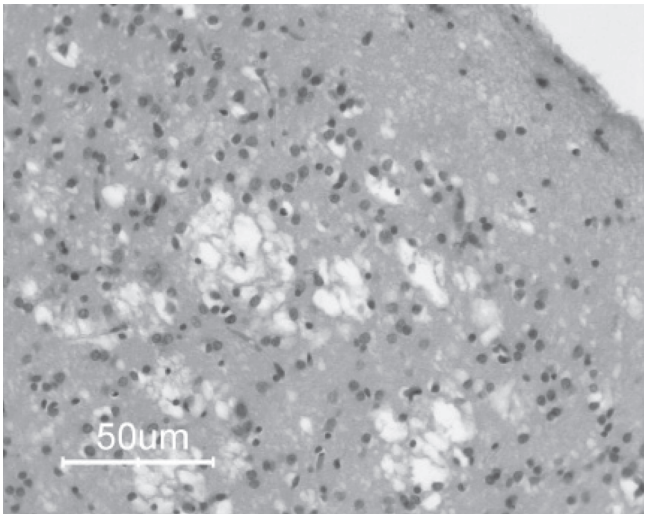

Figure 3. *CJD in neocortex illustrating how the vacuoles have a tendency to coalesce (Courtesy of Drs. Barbara Crain and Honathan Epstein, Johns Hopkins Medical Center).*

Bovine Spongiform Encephalopathy - *BSE*

Mad cow disease, or bovine spongiform encephalopathy (BSE), is a neurological transmissible degenerative disease believed to be caused by abnormal prions (Taber's Cyclopedic Medical Dictionary 2001). It is thought to have originated from scrapie, a similar disease in sheep and goats that has been recognized in Europe since the mid-18th century (Brown et al, 2001), although this mode of origin is not certain (Cohen et al., 2003). An epidemic of BSE in the United Kingdom began in 1986 and affected nearly 200,000 cattle. The disease also has been confirmed in cattle in Belgium, Denmark, France, Germany, Italy, Ireland, Liechtenstein, Luxembourg, the Netherlands, Northern Ireland, Portugal, Spain, Switzerland, Canada, and United States. The primary route of transmission of the disease is believed to be the use of slaughterhouse rendering wastes contaminated with infectious agents in the production of protein- rich nutritional supplements fed to cattle. Another theory suggests that some cases of BSE may be caused by pathogenic mutation in cattle (Brown et al., 2001). As a result of the imposition in the UK in 1988 of a ban on the feeding of ruminant protein feed to cattle, the epidemic began to be controlled. The number of BSE-infected cattle is declining worldwide.

Creutzfeldt-Jacob Disease—*CJD*

CJD is characterized by rapidly progressive dementia. Patients experience impaired memory, impaired vi-

sion, and impaired muscular coordination. They may also experience insomnia, depression, or unusual sensations. As the illness progresses, mental impairment becomes severe and blindness may occur. Eventually the patient may lose the ability to move and speak. Other infections, such as pneumonia, can set in and lead to death. Diagnosis is difficult, and the only way to confirm the disease is through a brain biopsy or autopsy. The former is dangerous and is rarely done (NINDS, 2005).

Typically, CJD affects persons 45 to 60 years of age and about 90 percent die within one year. It affects about one person in every one million people per year worldwide. In the United States, there are about 200 cases per year.

There are four different types of CJD. These are: genetic, iatrogenic, sporadic, and variant (University of Edinburgh, 2005). Genetic CJD is a very rare illness. It is not transmissible from a human being or animal. There are only a few deaths due to genetic CJD each year. Cases of iatrogenic CJD are also very rare. It occurs by transmission via infected tissues during the course of medical or surgical procedures. Sporadic CJD is more common and has been found in every country in the world where it has been looked for. It affects about one person per million of the population. Although the cause is unknown, the current theory suggests that normal prion proteins in the brain undergo spontaneous change to the abnormal form of prion, thus resulting in the disease. Sporadic CJD was first described in 1921, and there is no evidence that it is associated with food. There is no evidence that it is the result of BSE.

The fourth type of CJD is called "new variant" or "variant" CJD (vCJD). It is of most recent concern because it is relatively newly discovered and may result, in part, from the ingestion of contaminated meat products. However, according to the National Institutes of Health, "The appearance of the new variant of CJD (nv-CJD or v-CJD) in several younger than average people in Great Britain and France has led to concern that BSE may be transmitted to humans through consumption of contaminated beef. Although laboratory tests have shown a strong similarity between the prions causing BSE and v-CJD, there is no direct proof to support this theory" (NINDS, 2005).

The human prion diseases, CJD, Gerstmann-Straussler-Scheinker syndrome (GSS—genetic brain disorder), and kuru are characterized by four shared neuropathological features:

- Spongiform—the brain having the appearance or quality of a sponge
- Neuronal loss
- Astrocytosis—the proliferation of astrocytes (neuroglial cell) owing to the destruction of nearby neurons during a hypoxic or hypoglycemic episode
- Amyloid plaque formation

These neuropathological features are different between normal CJD and vCJD. CJD is the most common of all the human TSEs and is the disease most commonly mistaken for vCJD (WHO, 2002). In vCJD the neuropathological features include "daisy-shaped" areas of damage in the central nervous system (USFDA, 2005). Amyloid plaques were present in vCDJ whereas, it occurs in only 5% to 10% of sporadic CJD patients (Brown, et al., 2001)

In contrast to the classic form of CJD, the variant form, vCJD, predominantly affects younger persons (median age of about 29). Furthermore, it has its own typical clinical features including prominent psychiatric or sensory symptoms, delayed onset of neurological abnormalities, and a diffusely abnormal non-diagnostic electroencephalogram. It also has a longer than usual delay in the onset of symptoms (USFDA, 2005; CDC, 2003).

CJD has been around for a long time and the number of cases has been fairly consistent yearly at about 50 cases. On the other hand, vCJD is relatively new and is currently decreasing (Brown et al., 2001). The variant vCJD was first reported in 1996. Most of the cases have been confined to the UK. There have been six cases in France, one case in Ireland, one case in Italy, and one case in the United States.

Table 1 shows the number of cases for each of the various forms of CJD, including vCJD. These figures show the number of suspect cases referred to the CJD surveillance unit in Edinburgh, and the number of deaths of definite and probable cases in the UK, up to 3rd June 2005.

It is evident from this table that vCJD represents about 15% of the total cases reported for CJD and vCJD. Furthermore, the number of vCJD cases peaked in 2000 and thereafter have been declining. In contrast, the number of sporadic CJD cases has been fairly constant since 1997.

Some symptoms of CJD can be similar to symptoms of other progressive neurological disorders, such as Alzheimer's or Huntington's disease. Occasionally, media reports suggest all these diseases are the same or

Table 1. Number of cases for each of the various forms of CJD including vCJD reported to the CJD Surveillance Unit, University of Edinburgh, Scotland.

Referrals of Suspect CJD		Deaths of Definite and Probable CJD						
Year	Referrals	Year	Sporadic	Iatrogenic	Familial	GS0S	vCJD	Total
1990	[53]	1990	28	5	0	0	–	**33**
1991	75	1991	32	1	3	0	–	**36**
1992	96	1992	45	2	5	1	–	**53**
1993	78	1993	37	4	3	2	–	**46**
1994	118	1994	53	1	4	3	–	**61**
1995	87	1995	35	4	2	3	3	**47**
1996	134	1996	40	4	2	4	10	**60**
1997	161	1997	60	6	4	1	10	**81**
1998	154	1998	63	3	3	2	18	**89**
1999	170	1999	62	6	2	0	15	**85**
2000	178	2000	50	1	2	1	28	**82**
2001	179	2001	58	4	3	2	20	**87**
2002	163	2002	72	0	4	1	17	**94**
2003	162	2003	77	5	4	2	18	**106**
2004	114	2004	52	2	3	1	9	**67**
2005*	40	2005	13	1	1	1	2	**18**
Total Referals	**1962**	**Total Deaths**	**777**	**49**	**45**	**24**	**150**	**1045**

*As at 3 June 2005

closely related. However, CJD causes unique changes in brain tissue which can be seen at autopsy. It also tends to cause more rapid deterioration of a person's abilities than does Alzheimer's disease or most other types of dementia.

How is CJD transmitted? The most favored theory is that the normal prion protein in the brain undergoes a spontaneous change to the abnormal form resulting in the disease (University of Edinburgh. CJD Surveillance Unit, 2005). In some cases, CJD has been transmitted from grafts of brain tissue, transplanted corneas, implantation of inadequately sterilized electrodes in the brain, and other medical procedures. (As noted above, this form of CJD is called iatrogenic) (NINDS, 2005).

There has been considerable concern that CJD may be transmitted in blood. Even though millions of people receive blood transfusions each year, there are no reported cases of someone contracting CJD from a transfusion. Even among people with hemophilia, who sometimes receive blood plasma concentrated from thousands of donors, there are no reported cases of CJD transmission. "While there is no evidence that blood from people with sporadic CJD is infectious, . . . the possibility that blood from people with vCJD may be infectious has led to a policy preventing people in the United States from donating blood if they have resided for more than three months in a country or countries where BSE is common" (NINDS, 2005). As with blood donation, tissues or organs from persons affected by CJD should be avoided. CJD cannot be transmitted through the air or by person-to-person contact.

Other than the possibility of transmission of BSE via beef contaminated with neurological tissue, there are no other known cases of CJD caused by transmission of BSE or other TSEs to humans directly from any animal. Apparently, transmission of infectious prions from one species of animal or humans to another has little potential of causing a TSE disease in the different species. This is termed the "species barrier".

As an example of the species barrier, consider chronic wasting disease (CWD). CWD is the TSE that infects deer and elk. It is found in the U. S. (mostly in Rocky Mountain states to Wisconsin, but, recently, as far east as New York state) and Alberta and Saskatchewan, Canada. There has been some concern regarding the possible transmission of CWD to domestic animals, such as sheep and cattle, or to humans. There is no evidence that this occurs. Belay et al. (2004) conclude that the risk, if any, of transmission of CWD to humans is low.

Scientists are not certain how CWD is transmitted among deer and elk, but research indicates it is probably transmitted through oral exposure, although transmission may occur through indirect contact via environmental contamination with infective substances. The Region 8 (Rocky Mountain states) office of the U. S. EPA has been investigating TSEs, especially CWD, for several years. Dr. Wendy O'Brien, of that EPA office, and researchers at the University of Wisconsin have

been working to prevent any potential spread of the disease from the management of infected deer and elk carcasses. Because of the potential for laboratories that test or process carcasses to discharge wastes to wastewater, EPA Region 8 is developing guidance, under the industrial pretreatment program of the Clean Water Act, to ensure such wastes are properly disposed of by other means. Current Region 8 research is aimed at developing a method for testing for abnormal prions in environmental media, such as soils, sewage, and biosolids.

vCJD and Biosolids as a Pathway of Transmission

TSE diseases are not, at this time, common or widespread in the U. S. and Canada—with the possible exception of CWD—and they are not thought to be capable of rapid transmission. No cases of CWD transmission to cattle in the environment have been reported. In experimental studies, cattle exposed orally to CWD-affected deer brain or living in close contact with CWD-affected deer have not developed symptoms of CWD. And, as noted above, there are no reported cases of CWD transmission to humans. No cases of variant vCJD resulting from exposure to either CWD prions or scrapie prions from sheep have been identified.

As noted above, the human consumption of beef products contaminated by neurological tissue is suspected, but not proven, to have led to several cases of the variant form of CJD (vCJD) in humans.

Thus the potential transmission to humans of vCJD through the land application of biosolids is highly improbable for these reasons:

1. Abnormal prions have not been found in manure (UK DEFRA website; Smith et al., 2005).
2. There is no evidence, at the present, of the presence of abnormal prions in wastewater or biosolids. For transmission of any infectious prions into biosolids, the brain, spinal cord, and/or other neurological tissues would have to end up being discharged into a wastewater treatment plant. The rate of dilution would be enormous. Although some prions could end up in biosolids and be placed on land, the probability and potential for grazing animals or humans to consume those biosolids with the infinitesimal amount of prions is unlikely. Gale and Stanfield (2001) conducted a risk assessment on the potential transmission of BSE from land applied biosolids. They concluded that abnormal prions could end up in wastewater and would likely partition into the sludge fraction; they then estimated the risks from land applied sewage sludge based on the assumption that 1% of brain and spinal cord is lost to the sewers from slaughterhouses. Their model predicts a risk of BSE transmission of 7.1×10^{-5} cow^{-1} year^{-1} from cattle grazing on land to which sewage sludge has been applied. The authors concluded that the dose by grazing cattle is insufficient to sustain epidemic levels in the UK cattle herd.
3. There have been two confirmed cases of BSE in the United States. USDA has strict requirements and monitoring for mad cow disease/BSE that the independent Harvard Center for Risk Analysis has deemed adequate to prevent any widespread outbreak of the disease in this country. They found that only a small amount of potentially BSE-contaminated tissue is likely to reach the human food supply and be available for human consumption (Cogan et al., 2001).
4. The risks to humans through consumption of vegetable crops grown in soils to which biosolids or manures have been applied are exceptionally low (Gale and Stanfield, 2001; Gale, 2002). Gale's (2002) risk assessment showed that the risk to humans ingesting root crops would be 0.7×10^{-10} per person per year. This is infinitesimal and would be further reduced by washing soil off crops prior to consumption.
5. Dead farm animals and wild animals (deer and elk), including those in which the cause of death is unknown, are likely to remain on the farm or in the wild, and, if managed by people at all, be buried, landfilled, or incinerated. No wastes from such carcasses would end up in a sewage treatment plant. If a diseased animal ended up in a slaughterhouse or rendering plant, some portions of the brain and spinal cord tissues could be discharged into a wastewater treatment system and eventually end up in biosolids. But, as a result of increasingly strict regulations, surveillance, and changes in procedures at slaughterhouses, significant discharges of infected wastes to sewers are unlikely. Furthermore the rate of dilution would make it highly unlikely that any prions would be in sufficient concentration to be infective.
6. CWD in deer and elk is a concern in some parts of the U. S. and authorities are monitoring the situation. Research is on-going, and, because of the unknowns typical of any new area of science, EPA Region 8 and others recommend reasonable precautions be taken by any slaughterhouse, processing facility, laboratory, or other operation that handles materials con-

taminated with any possible TSE agents. According to EPA Region 8 (U. S. EPA, 2003), all potentially CWD-contaminated material that is collected should be 1) treated to inactivate CWD agent by decontamination procedures, 2) processed through alkaline hydrolysis, 3) properly incinerated, or 4) properly disposed of in landfills, as permitted by local, state, or federal law. Various guidelines exist for facilities that handle material contaminated by TSEs, such as operating rooms, autopsy rooms, research laboratories, and mortuaries (e.g. World Health Organization, 1999).

7. The infectious dose appears to be relatively large. M. D. Sobsey's summary from the recently published papers from the 2001 EPA-sponsored Emerging Pathogens workshop (Smith et al., 2005) noted: "Fortunately, prions are not highly contagious. Except for scrapie in sheep, prions do not appear to spread from an infected host to an uninfected one by normal contact. The risks of TSE transmission from water and other environmental media appear to be very low, based on a recent quantitative risk assessment (Gale et al. 1998). This is because the oral ID50 for humans (based on the oral ID50 for mice) is high (1 g of BSE-infected bovine brain or about 1013 BSE prion molecules). BSE infectivity is likely to remain bound to particulates in the aquatic environment, and through dispersion and dilution of BSE prions, consumption of drinking water, bathing water, and other environmental media would result in exposure to minute sub-fractions of an ID50."

CONCLUSIONS

Wastewater treatment facilities and biosolids management activities are not seen as likely contributors to the spread of TSE diseases. TSE-causing prions do not replicate in wastewater, sludge, or the environment. Tests for prions in environmental media are still being developed, and the effects on abnormal, TSE-causing prions of the wastewater and sludge treatment processes are still being researched.

It is important to place TSEs and their possible transmission and impacts in relation to other diseases that are either foodborne or waterborne. Consider the following:

1. The number of cases of vCJD world wide by the end of 2003 has been estimated at about 150.
2. By contrast, in the United States alone there have been 73,000 cases of infections from *E. coli* 0157:H7, with 61 deaths each year. This was primarily from uncooked contaminated beef and person-to-person transmission. Also, there have been numerous cases of death and illness from the use of raw manure on crops (Pell, 1997).
3. In 2002, there were over 3,000 reported cases of illness due to *Cryptosporidium*. In 1993, over 400,000 persons in Milwaukee became ill with this organism and over 100 died. This was primarily the result of cattle manure contaminating the water supply of the city (Mackenzie et a., 1994).
4. The recent SARs-CoV outbreak resulted in over 8,000 cases worldwide, with more than 780 deaths (29 in the U.S.) (MMWR, 2003).
5. In the United States, in 2003, there were over 17,000 cases of West Nile virus, with 650 deaths (Medline Plus, 2005)

The very few deaths worldwide from TSE diseases are insignificant as compared to other foodborne diseases. Furthermore, the number of cattle infected with BSE has decreased significantly in recent years, which has resulted in a reduction of risk to humans.

Clearly, continuing efforts to keep infective prions out of wastewater is the best approach to reducing this very small potential risk. EPA, USDA, and other agencies are properly targeting potential sources of TSE-containing wastes and establishing systems to ensure their proper capture, treatment, and disposal at the source.

REFERENCES

Belay, E. D., Maddox, R. A., Williams, E. S., Miller, M. W., Gambetti, P. and Shonberger, L. B. (2004). Chronic waste disease and potential transmission to humans. Emerging Infectious Diseases, **10** (6). Available from: http://www.cdc.gov/ncidod/EID/vol10no6/03-1082.htm.

Brown, P., Will, R. G., Bradley, R., Asher, D. M., and Detwiler, L. (2001). Bovine spongiform encephalopathy and variant Creutzfeldt-Jakob disease: Background, evolution, and current concerns. Emerging Infectious Diseases **7**, (3) Jan–Feb.

CDC. (2003) Bovine Spongiform Encephalopathy and Creutzfeldt-Jakob disease. National Center for Infectious Diseases, U.S. Department of Health and Human Services. Available at: http://www.cdc.gov/ncidod/diseases/cjd/bse_cjd_qa.htm.

Cohen, J. T., Duggar, K., Gray, G. M., Kreindel, S., Abdelrahman, H., HabteMariam, T., Oryang, D. and Tameru, B. (2003). Evaluation of the potential for Bovine Spongiform Encephalopathy in the United States. Harvard Center for Risk Analysis, Harvard School of Public Health and Center for Computational Epidemiology, College of Veterinary Medicine, Tuskegee University.

Collins, S., McLean, C. A. and Masters, C. L. (2001). Gerstmann-Straussler

syndrome, fatal familial insomnia, and kuru: A review of these less common transmissible spongiform encephalopathies. J. Clin.Neurosci., **8**(5), 387–397.

Gale, P., Young, C., Stanfield, G. & Oakes, D. (1998). Development of a risk assessment for BSE in the aquatic environment. J. Applied Microbiol., **84**, 467–477.

Gale, P. (2001). Risk assessment: Use of composting and biogas treatment to dispose of catering waste containing meat. Department of Environment, Food and Rural Affairs. DEFRA., UK.

Gale, P. and Stanfield, G. (2001). Towards a quantitative risk assessment for BSE in sewage sludge. Appl. Microbiology., **91**(3), 563–569.

Mackenzie, W. R., Hoxie, N. J., Proctor, M. E., Gradus, M. S., Blair, K. R., Peterson, D. E., Kazmierczak, J. J., Addiss, D. G., Fox, K. R., Rose, J. B. and Davis, J. P. (1994). A massive outbreak in Milwaukee of *Cryptosporidium* infection transmitted through the public water supply. New England J. Med, **331**, 161–167.

Medline Plus. 2005. Available at: www.nlm.nih.gov/medlineplus/news/fullstory_25373.html.

MMWR, 2003. Morbidity and Mortality Weekly Report Available at: www.cdc.gov/mmwr/mguide_sars.html.

NINDS. (2005). Creutzfeldt-Jakob Disease fact sheet., National Institute of Neurological Diseases and Stroke, NIH Available at: www.ninds.nih.gov/disorders/cjd/detail_cjd.htm.

Pell, A. N. (1997). Manure and microbes; public and animal health problem. J. Dairy Sci., **89**, 2673–2681.

Smith, Jr., J. E., Millner, P. D., Jakubowski, W., Goldstein, N. and Rynk, R. (2005). Contemporary Perspectives on Infectious Disease Agents In Sewage Sludge and Manure, The JG Press, Inc. Emmaus, P A, pg. 119.

Taber's Cyclopedic Medical Dictionary.

UK DEFRA website: www.defra.gov.uk.

University of Edinburgh. CJD Surveillance Unit. (2005). The different types of CJD. . CJD Surveillance Unit, Edinburgh, UK. Available at: www.cjd.ed.ac.uk/CJDtype/cjdtype.html.

U. S. EPA. (2003). Draft EPA Region 8 Recommended Approach for Treatment and Disposal of Waste Potentially Contaminated with Chronic Wasting Disease (CWD), retrieved from http://www.epa.gov/Region8/, June 9, 2005.

U.S. FDA.. (2005). Bad Bug Book: Prions and Transmissible Spongiform Encephalopathies. 2004. U.S. Food & Drug Administration, Center for Food Safety & Applied Nutrition. Available at: http://cfsan.fda.gov/~mow/prion.html.

World Health Organization. Department of Communicable Disease Surveillance and Response. 1999. WHO Infection Control Guidelines for Transmissible Spongiform Encephalopathies. Report of a WHO Consultation. Geneva, Switzerland, 23–26 March 1999.

WHO, Fact Sheet No. 80. Revised November, 2002.