Prion Diseases : A Review

II. Prion Diseases in Man and Animals

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Abstract:
To date, a total of 13 prion diseases have been recognized in man and animals. The human diseases are: Kuru, Creutzfeldt-Jakob disease (CJD), variant CJD, Gertmann-Strausssler-Scheinker Syndrome, fatal familial insomnia and Alpers' disease. The animal diseases are: scrapie, transmissible mink encephalopathy, chronic wasting disease, bovine spongiform encephalopathy, feline spongiform encephalopathy, wild ungulates encephalopathy and spongiform encephalopathy of the ostrich. Early diagnosis and treatment of these diseases have bewildered the scientific community. The fact that the prion protein (PrP) is derived from the host – given that PrP is the sole cause of disease - makes it almost impossible to develop direct serologic tests and vaccines for the diagnosis and prevention of these diseases. At present, diagnosis is largely based on clinical and post-mortem findings, detection of abnormal prion protein by immuno-cytochemistry, Western blot, infra-red spectroscopy and biochemical examination of cerebrospinal fluid and blood of suspected patients. Methods are currently being evaluated for the identification of “capture” agents that specifically bind to misfolded prion protein (PrPSC), and for amplification of the interconversion of normal prion protein into PrPSC. No treatment is available yet for prion diseases, but several candidate drugs are being investigated that could prevent PrPSC formation, interfere with its conversion and/or metabolism or reverse it into the normally folded, harmless form.

Introduction:
During the past few years, considerable advances have been made towards better understanding of prion diseases and the development of new methods for their diagnosis and treatment. In part I of this review, we have described the etiology of these diseases, and the hypothesis that have been put forth to explain their nature and propagation. In the second part of the review, the clinical manifestations, pathogenesis, diagnosis and treatment of different types of prion disease affecting man and animals will be described.

Prion diseases, or transmissible spongiform encephalopathies (TSE) are peculiar, neurodegenerative disorders that are apparently caused by a
misfolded "infectious" prion protein, hitherto known as PrP^{SC} or PrP^{res}. These diseases are unique in that they are both infectious and inherited. All of them are characterized by a long incubation period, followed by progressive spongiform degeneration of brain tissue, with consequent dementia, motor disorders, paralysis and 100% mortality [2]. The primary lesions comprise neuronal vacuolations and loss, especially in the cerebellum and cortical parts of the brain stem. These changes are accompanied by extensive gliosis, astrocytosis [3] and accumulation of abnormal prion protein fibrils in and around the brain cells [4].

To date, thirteen types of prion diseases have been described in humans and different species of domestic and wild animals (Table 1).

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<td>Gertsmann-Straussler-Scheinker Syndrome (GSS)</td>
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Human Prion Diseases

1.1 Kuru (laughing death):

This disease appeared in the earlier part of the 20th Century among members of the "Fore" tribe, in the Eastern Highlands of Papua New Guinea, to the east of the Pacific Ocean. It reached its peak in the 1950's, killing several thousands. The disease was associated with cannibalism (eating the brain of dead humans) and spread between members of the tribe, affecting more women and children than male adults. The name "Kuru" is the local name used by Fore people to describe the condition. It means "laughing death" in their language because it is accompanied by uncontrollable laughter[5].

Kuru is an invariably fatal disease, and like other TSEs it affects both mental and motor functions. Its incubation period ranges between 2 - 40 years, but is usually several years long. However, the clinical course of the disease is relatively short – the patient dies within 3 months to one year, at the most, after the appearance of symptoms. The symptoms include: incoordination of movement, stumbling, muscle tremors, difficulty in articulating words, involuntary oscillation of the eyes (nystagmus), difficulty to swallow, inability to hold things and finally dementia and death[6].

Kuru spread among the Fore people through cannibalistic rituals. It was the practice of the Fore tribe to remove the brains of their dead relatives, apparently as an expression of respect to the deceased. This task was assigned to women, who gathered with their children, to perform it. The women would grind up the brain into a soup, heat it up and eat it[7]. It was also mentioned that consumption of the brain might have been dictated by a crave for “meat,” since the male adults treated themselves to the flesh of wild pigs hunted from the bush, while women and children were denied a share. Also the males alone ate the flesh of their deceased relatives. So, women were driven by crave for proteins to consume the extracted brains and feed their children. They also used to rub their bodies and their children’s with the “greasy” brain tissue and consequently, the infection

[1] Perez (unpublished review), however, states that Kuru means, "shivering or trembling" in the Fore language.
could also be transmitted through skin cuts, nose picking, eye rubbing or mucosal injury [8].

It has been suggested that Kuru might have originated from a single case of CJD among the Fore tribe, and was then maintained by cannibalism (Eugene Perez, unpublished review) since a nearby tribe that also performs cannibalistic rituals did not show the disease.

The name of Gajdusek, a US scientist who spent many years of his life investigating Kuru and the manner in which it is transmitted, comes to the mind whenever this disease is mentioned. In the 1950's, he travelled many times to Papua New Guinea, and stayed for extended periods with the Fore, studying Kuru and sending the brains of dead victims back to his laboratory for further investigations [9]. He gathered valuable information on the epidemiology of Kuru and proved that it was transmissible by inoculation of brain tissues into chimpanzees and other primates, as well as other laboratory animals [10]. He was awarded the Nobel Prize in recognition of his efforts.

It is generally believed that Kuru is now extinct, following laws prohibiting cannibalistic rituals, and no new cases have been reported after 1956.

1. 2. Creutzfeldt-Jacob Disease (CJD):

This is the commonest type of human TSE, with an annual incidence of about 1 per million. There are different forms of CJD: sporadic, inherited and iatrogenic - that is, infections transmitted during treatment or diagnosis. CJD was discovered in the 1920’s independently by two German physicians, Creutzfeldt [11] and Jakob [12; 13]. In contrast to Kuru, which often affects young adults, CJD is typically a disease of the elderly, the majority of cases occurring between the age of 50-70 [14].

At the onset of disease, a CJD patient shows tension or depression and insomnia, accompanied by muscle tremors and myoclonus. The patient sometimes also exhibits a slight, involuntary jerk, and there is incoordination of movement and stumbling. Other symptoms include sluggish movement, loss of speech, amnesia, dysarthria and sometimes blindness. There is progressive atrophy of the brain and dementia. The duration of the disease from the appearance of symptoms to death ranges...
from one month to >10 years, but in most cases the patient dies within 6 months after showing clinical symptoms. Diagnosis is based on clinical examination and characteristic changes in electroencephalograph [1].

Although the annual incidence of the sporadic form of CJD is 1 per million or less, there are certain, isolated groups of people in which the incidence is much higher, averaging 30 - 40 per million. This is due to the fact that about 10-15% of CJD cases have a genetic basis (inherited form). Presently, about 100 families worldwide are known to have much higher incidence of CJD than the overall average. These include Libyan and Tunisian Jews in Israel, Tunisian immigrants in France, and certain families in the Orava region in Slovakia, as well as some families from other parts of Europe, South America and Japan [15]. It was previously thought that the higher incidence of CJD among some of these families was related to their eating habits. For instance, the relatively high incidence of CJD among Jews of Libyan origin was attributed to their habit of eating sheep brains and eyes [16]. Current studies, however, showed the existence of a heritable mutation in the PrP-gene of all those exhibiting familial forms of prion disease [15].

About 1% of CJD cases are due to iatrogenic infection. Several cases were recorded following treatment with pituitary hormones extracted from human cadavers. In Britain, several people died of CJD after treatment with growth hormone collected from pituitary glands taken from soldiers killed in World War II. Similarly, in Australia, some patients treated with gonadotrophic hormones extracted from human pituitaries collected from cadavers succumbed to CJD [17]. Apart from hormonal treatment, iatrogenic CJD was also recorded following corneal grafts, meningeal grafts and the use of contaminated electrodes during EEG or contaminated surgical instruments during brain surgery [1; 18]. Like Kuru, CJD can also be transmitted experimentally to monkeys and laboratory animals [1].

1.3. Variant CJD (vCJD):

Although CJD is primarily a disease of old age, a new, zoonotic form of CJD, called new variant CJD (nvCJD) or variant CJD (vCJD), was recorded in 1995 in young adults in Britain [19; 20]. At the time of preparation of this review, a total of 126 cases of vCJD had been reported in the world: 117 from the United Kingdom, six from France, and one each from Ireland, Italy and the USA. Almost all of these patients had multiple-years exposures in
the United Kingdom between 1980 and 1996 during the occurrence of the BSE outbreak among British cattle. Compared to the classical CJD, this variant form of the disease affected a much younger age group (median age 26.5 years), and was characterized by a slower course than CJD (13 months versus 7 month) and atypical clinical manifestations. Unlike CJD, in which dementia is the most prominent sign of the disease, the early symptoms of vCJD involved psychological disturbances such as irritability, depression and aberrant behavior, which later gave way to mental disorder and neurologic abnormalities including ataxia, dementia and terminal myoclonus. On the other hand, the brain showed Kuru-like type of pathology, with amyloid deposits and spongiform changes concentrated in the cerebellum and base of the brain, and a diffusely abnormal, but non-diagnostic, electroencephalogram. In view of its unusual, previously unknown pattern, vCJD was strongly suspected to be acquired from cows infected with the BSE agent [17; 19-22]. In 1997, it was confirmed that the prion that causes BSE was the same that causes vCJD [23]. Hence, vCJD is sometimes referred to as human BSE. There is no known treatment of vCJD and, in common with all other prion diseases, it is invariably fatal.

1.4. Gertsmann-Straussler-Scheinker Syndrome (GSS):

This disease was originally described in a German family in 1928 [24; 25]. It is a very rare disease, with an incidence of 1-10 per 100 million/year [26]. Its most important clinical manifestations are incoordination of movement and mental disorders. There is involvement of the medulla oblongata and brain stem, with consequent ataxia, stumbling, dysarthria, swallowing and speech difficulties, amnesia and finally dementia. These symptoms are most frequently observed in patients at the age of 20 – 30 years, but sometimes at older age. GSS is a slowly progressive disease with duration ranging from one to ten years. The brain histopathology often reveals multicentric plaques rather than spongiform changes [26].

GSS is associated with a heritable mutation in the gene encoding for prion, and there are about 50 families worldwide affected with this disease. However, the disease has also been transmitted experimentally to animals, indicating that transmission occurs both vertically and horizontally [26; 27].
1.5. Fatal Familial Insomnia (FFI):

This disease was first described in some Italian families in 1986, and is one of the rarest types of human prion diseases, with an incidence rate of 1 per 50 million/year. So far, just over 20 kindred and 7 non-familial cases have been described worldwide. Nevertheless, like other prion diseases, it is attracting increasing attention in the wake of the “mad cow disease" epidemic in Britain [4]. FFI is a primarily hereditary prion disease, characterized by a mutation at codon 178 of the PrP gene, along with methionine polymorphism at codon 129 of the mutated allele [28]. Usually, it occurs between the ages of 40 and 60 years, and the affected person may survive for 7 to 33 months (average one year) after the onset of symptoms, depending on the type of genetic change. Patients who are homozygous for methionine at codon 129 have a shorter survival than heterozygous patients. One of the most important symptoms of FFI is progressive, incurable sleeplessness (insomnia), associated with involvement of the thalamus. The patient loses many of the brain functions relating to sleep, and exhibits hallucinations, illusions, restlessness, poor memory and inability to concentrate. Other symptoms include depression and a wide range of motor disturbances such as ataxia, dysarthria, muscle tremors, myoclonus and seizures. Profuse sweating, increased heart rate and hormonal disturbances, especially growth hormone, prolactin, melatonin and some corticosteroid hormones, have also been reported [29]. As in other prion diseases, FFI is characterized by the presence of PrP\textsuperscript{Sc} in the affected neurons. Some cases of sporadic FFI have been described in patients not exhibiting mutation at codon 178, and which could be transmitted to animals. These cases have been called the "sporadic form of fatal familial insomnia" [29; 30].

1.6 Alpers’ Disease:

Alpers’ disease, or chronic progressive encephalopathy of childhood, is an extremely rare, fatal prion disease of infants, characterized by diffuse progressive degeneration in the brain cortex, similar to CJD. In addition, there is degeneration and fibrosis of the liver. The disease has hereditary basis. It has been known since the 1930's, but so far only in very few infants and young children. The symptoms include mental disorders and dementia, motor disturbances, seizures, partial paralysis and growth retardation or early death [31; 32].
Prion Diseases of Animals

2.1. Scrapie:

This is a disease of sheep and goats. It is the first prion disease known, and is the prototype of other prion diseases. It is the most extensively studied type of prion disease, and much of the information gained on prion diseases is based on research on the scrapie agent.

Scrapie existed in Europe since four centuries. It is believed that it appeared first among Merino sheep in Spain in the 15th Century, and from there spread to other parts of Europe where it was given different names, including "scrapie," "malady of madness and convulsions," "trotting disease," "Rida" and "Le Tremblante du mouton" [33]. The disease was known in Britain since 1730, and several important outbreaks, sometimes lasting for up to twenty years, occurred during the 18th and 19th centuries [34]. Scrapie was the first prion disease proven experimentally to be transmissible, when Cuillie and Chelle in 1939 [35] transmitted scrapie to a goat by intraocular inoculation of scrapie-infected spinal cord. And when it was found that CJD was also transmissible, and could be established experimentally in chimpanzees, several researchers considered a possible link between CJD and scrapie and other forms of TSE's.

Scrapie exists in most parts of Europe and other countries of the world, with the exception of Australia, New Zealand and some parts of South America. It is endemic in Britain, especially in certain sheep breeds, such as the "Suffolk" in which the infection rate is about 30%. The mode of natural transmission of scrapie in sheep is not clear. A major outbreak of this disease occurred in sheep following their vaccination against louping ill, a viral disease of the central nervous system transmitted by ticks; the vaccine was composed of formalin-treated lymphoid tissue, probably contaminated with the scrapie agent [15; 36]. This can be regarded as iatrogenic infection. It has also been suggested that sheep could be infected by eating the placenta of infected ewes, and by contact with contaminated pastures, which probably remain contaminated for several years [31; 37; 38]. Onodera et al. [39] isolated the scrapie agent from the placenta of scrapie-infected sheep in Japan. Parry [40], on the other hand, suggested that scrapie was a genetic disease that could be controlled by appropriate breeding. Despite familial incidence, however, there is no clear evidence of vertical transmission [33].
Scrapie has been transmitted experimentally to many species of domestic and laboratory animals. Like other prion diseases, it has a relatively long incubation period, and is usually seen in sheep two or more years old. Its clinical signs include nervous symptoms, and the animal becomes easily irritated by noise or moving objects. In addition, the animal shows severe scratching, and is usually seen rubbing its back against fences, or pushing its head against walls or other solid objects and biting its limbs. The affected sheep also shows incoordination of movement, stiffness of limbs and head and neck tremors. It loses appetite and becomes gradually thin and finally dies [33; 34].

2.2 Transmissible Mink Encephalopathy (TME):

This disease occurs in minks kept in captivity for the production of fur. It was first described in the United States in 1947 on mink ranches in Wisconsin and Minnesota, where it killed thousands of minks, resulting in severe economic losses. Since then, several TME outbreaks were reported in Wisconsin, Minnesota and Idaho, and in several other countries including Canada, Finland, Germany, and the republics of the former USSR [41-43].

The last major TME outbreak occurred on a mink ranch in Stetsonville, WI, in 1985, in which 60% of a herd of 7,300 adult minks died from the disease [44]. Currently, however, TME is rarely seen and occurs only as individual cases [45]. The disease has also been reported in the Chinese hamster and some other rodents [46], but these seem to be accidental hosts.

TME is virtually limited to minks reared in commercial ranches and has not been found in the wild. For that reason, it has been suggested that the mink might have contracted the disease as a result of eating contaminated feed, such as meat of scrapie-infected sheep. The disease has been produced experimentally in minks by intracerebral injection of brain material from scrapie-infected sheep and by feeding tissues from infected sheep. It is also believed that TME could be transmitted between foxes through biting; epidemiological studies also suggest that cattle might be a source of infection [43]. There is no evidence, however, that the disease spreads by contact between unrelated minks or from mother to offspring.

TME occurs in both male and female minks, which are usually more than 1 year old. The average incubation period is more than 7 months and the most important signs of the disease are behavioral changes. At first, the
minks soil their cages, step on food and experience difficulty in eating, and then become increasingly nervous, aggressive and hyper-excitabyle by noise. They show incoordination of movement, circling, tail arching, tail chewing, stumbling and clenching of the jaw. Finally, the affected minks isolate themselves and become sleepy, inactive and unresponsive. Usually, they stop eating and may die within just one week after the appearance of symptoms, but sometimes the disease lasts for about a month before the animal dies [43].

2.3 Chronic Wasting Disease (CWD):
This disease was described in 1967 in the mule deer (*Odocoileus hemionus hemionus*) and Rocky Mountain elk (*Cervus elaphus nelsoni*) [47]. It is a rare and geographically isolated disease occurring primarily in the Rocky Mountain areas in Colorado and Wyoming, and occasionally in South Dakota, Nebraska and Oklahoma, in addition to two Canadian states, but has not been reported outside the USA and Canada. CWD usually affects captive animals but has been reported occasionally in free-ranging cervids, including mule deer, white-tailed deer, black-tailed deer and elk. The precise source of infection is unknown and no evidence has been found to indicate that it is transmitted through food. However, it has been suggested that infected captive elk and deer might have been reared in pens previously used for housing scrapie-affected sheep, while environmental contamination may also play a role in local maintenance of the disease among free-ranging animals [48]. CWD appears to be transmitted both vertically and horizontally [47]. The affected animals show progressive loss of condition and behavior changes. Prior to death, the animals show excessive salivation, thirst, polyuria, stumbling and trembling [49-51].

2.4. Bovine Spongiform Encephalopathy (BSE):
BSE or "mad cow disease" is the largest epidemic of prion disease known. It was described for the first time in British cows in 1987, two years after the first clinical cases were recorded in April 1985 [52]. BSE appeared in dairy cows fed on a ration composed of meat and bone scrap, as well as bone marrow, of scrapie-infected sheep. The feed was apparently highly contaminated with the scrapie agent, and was prepared in such a way that did not destroy the scrapie agent [53]. At the time of the appearance of BSE, there was a significant rise both in the number of sheep and in the incidence
of scrapie in them in Britain; this led to the speculation that a new “strain” of the scrapie agent might have emerged that was highly infectious to cows. The disease spread extensively in British cattle, affecting about 54% of dairy cows and more than 15% of beef cattle. A total of 2 million cattle have probably been infected, including numerous cases that were either undiagnosed or went to slaughter before showing clinical symptoms. At the peak of the outbreak in 1992-1993, more than 6,000 cows were affected monthly. Then, the incidence started to decline in the following years. This pattern supported the idea that BSE was an extended common source outbreak and was likely to disappear in due course unless it was capable of spreading horizontally from one cow to another, in which case it would become endemic [54]. Some evidence has been presented suggesting that genetic factors may be involved in susceptibility to BSE among cattle [55]. Several errors in managing the BSE outbreak facilitated its spread throughout the UK, including delay in banning the use of animal offal in cow feeds, as well as delay in slaughtering sick animals and in prohibiting the use of certain animal tissues such as nervous and lymphatic tissue in human food. Furthermore, the incubation period of the disease is long, averaging 4-5 years, which means that a large number of cattle incubating the disease went unnoticed to slaughterhouses.

Sporadic cases of BSE - all of which in cattle imported from Britain - were recorded in several other countries [56]. A large number of cows have also been slaughtered in more than 30,000 farms in Britain to bring a quick end to the disease which caused tremendous economic losses to the British beef and dairy industries.

BSE is seen in cattle aged 3 - 11 years, with the majority of cases occurring at the age of 4-5 years, and there is greater incidence of the disease in females compared to males. This is different from scrapie, which appears to affect both sexes equally. Abiola et al. [57] studied sex-related differences in four strains of mice infected with BSE, in comparison to scrapie-infected mice. They reported profound sex-specific effects in the course of primary BSE transmission, the disease being much longer in the female mice than in males. By contrast, the scrapie incubation period was similar in male and female mice in all of the four studied strains.

The clinical signs of BSE include irritability, salivation and excessive response to different stimuli like sound, light and noise. The animals may
also show aggressive behavior, frenzy and kicking [53]. There is also incoordination of movement, which is initially slight, but increases with the progression of the disease. The symptoms include stretching of limbs, high steps, running and jumping in air, paddling, circling and stumbling. In addition, there is increased breathing and difficulty in defecation and urination, and sometimes signs of tetany [58]. Unlike scrapie, however, there is no pruritis. The course of BSE lasts from 1 - 6 months during which the animal's health and production deteriorate rapidly and finally it lies on one side and dies in lateral recumbency. It is believed that the agent of BSE replicates during the incubation period in lymphoid tissue in the intestinal wall, as well as the spleen and lymph nodes, then reaches the brain where it causes spongiform changes similar to those caused by other prion diseases. These pathological changes consistently showed that the causative agent of BSE is a single, stable strain [59; 60].

Fears of the spread of BSE to humans have existed since the onset of outbreaks of this disease in British cows. However, the authorities initially assured that there was no way that BSE could be transmitted to humans, and that cows were “dead-end hosts” that could not transmit the disease; hence, the causative agent would perish with the infected animals themselves, although calls have been made to monitor the risk of the disease in veterinarians [61]. Nevertheless, as BSE outbreaks spread among cows in Britain, concerns about the risk of BSE to humans continued. The public fear was justified since BSE was acquired orally by cows feeding on scrapie-contaminated meat and bone meal, as Kuru was transmitted orally to humans in Papua New Guinea by feeding on infected human brains. Also, the fact that scrapie agent could go from sheep to cows indicated that it had crossed the species barrier and could well infect other hosts beside cattle [61]. Subsequent appearance of spongiform encephalopathy in cats and other animals that were apparently fed on infected beef added to these fears, which were confirmed in 1995 by the diagnosis of an atypical form of CJD – the vCJD - in young adults in Britain that was found subsequently to be caused by the same strain that caused BSE in cows [23; 26].

The role of sheep as a source of vCJD remains theoretical. The BSE agent has been transmitted experimentally to sheep by injecting them with brain material from infected cattle. Furthermore, sheep have most likely been exposed to some of the same infected feed that passed BSE to cattle in
the UK. Yet, it is not known whether any sheep were in fact naturally infected with BSE, since the symptoms would almost certainly be confused with scrapie, which is endemic in the UK. Recently, Ferguson et al. [62] reported that sheep posed a greater theoretical risk of spreading BSE to humans than cattle, because present control efforts are focused on protecting people from the infection in cattle. These authors, however, estimated that at worst, vCJD from sheep could kill 150,000 people, versus an estimated maximum of 50,000 if beef were the only source, and that precautions could substantially reduce the risk from sheep.

2.5. Feline Spongiform Encephalopathy (FSE):

This disease was recorded in domestic and wild cats in Britain and some other countries following “mad cow disease.” The first case was reported in a domestic cat in Bristol in 1990, and within few years, numerous additional cases were reported in domestic cats, and different species of wild cats. The disease has been transmitted from cats to mice. This has led to the establishment of the feline disease as a new type of TSE or prion disease [63; 64].

The main signs of FSE in cats are stumbling, sleepiness, vague appearance, tendency to hide in dark places, pupillary dilatation, failure to respond to light stimuli, crawling, extension of the hind legs and muscle tremors, particularly in the head region. Histological examination of the brain shows the typical changes of spongiform encephalopathies. Sporadic cases were recorded in wild felidae including cheetah, puma and ocelot in zoos in Britain, Ireland and Australia [56].

2.6. Exotic Ungulate Spongiform Encephalopathy (EUE):

From 1986 onward, many cases of prion disease were recorded in wild ungulates. Most of them were initially found in antelopes fed the same contaminated feed that caused the disease in cows. However, some cases appeared in animals born after total ban on the use of animal offal in animal feeds. The first case in wild ruminants was recorded in the nyala (Tragelaphus angasi) in 1987, around the same time as BSE. In the following year, the disease appeared in the gemsbok (Oryx gazella) and in 1988 in the Arabian oryx (Oryx leucoryx) and the greater kudu (Tragelaphus strepsicerosi). In the following years, some cases were recorded in eland (Taurotragus oryx) and single horned oryx [53; 65; 66].
These different cases appeared in animals kept at London Zoo or animals exported from London to other countries. In general, the clinical signs comprised incoordination of movement, tremors, excessive salivation, loss of weight, licking of lips, tilting of head to one side, sleepiness, vague looks and sometimes aggressive behavior. Typical spongiform changes were found in the brains of the animals [65; 66].

Sometimes the diseases in wild cats and exotic ungulates are grouped under the name "Zoological Spongiform Encephalopathies."

2.7 Spongiform Encephalopathy of the Red Ostrich:
There is preliminary evidence of prion disease in the red-necked ostrich (*Struthio camelus*). The condition was found in an adult female ostrich in a German zoological garden. The affected ostrich showed symptoms of central nervous involvement and locomotion disorders. Histological examination of the brain showed spongiform encephalopathy involving the brain stem and medulla oblongata. A male ostrich also died of similar symptoms, but no examination of the brain was made [67]. It is also not known if the condition was caused by a transmissible agent or not.

**Transmission**

Prion diseases can develop in different ways. They can be transmitted by mouth, skin wounds and mucosal surfaces as in the case of Kuru and scrapie. They can be acquired by iatrogenic infection as in iatrogenic cases of CJD acquired as a result of treatment with human-derived hormones, corneal grafts, dura matter grafts and use of contaminated medical instruments, such as electrodes. They can also be transmitted genetically e.g., GGS, FFI and inherited form of CJD. Still other cases can arise without a known risk factor such as genetic predisposition or exposure to infective material. Accordingly, Prusiner et al. [15] categorized prion diseases into (i) infectious forms resulting from horizontal transmission and including iatrogenic infections (ii) inherited forms that are invariably associated with mutation of the PrP gene and (iii) sporadic forms where the origin of the condition is unknown, and which could arise either as a result of spontaneous somatic mutation or for some other unknown cause. The sporadic form is the most common [18].
Lymphoid tissues appear to be very important for the propagation of prions. Following orally acquired infections, the ingested prions could be absorbed by the gut into the Peyer's patches. From there, they are taken to the spleen, lymph nodes and tonsils, where they replicate. In these lymphoid organs, the prions gain access to nerves. They propagate along the axons of these nerves to the spinal cord and finally to the brain [68]. These authors detected PrPSC within follicular dendritic cells (FDCs) in lymphoid tissues of humans and animals with different types of TSE’s; using scrapie-infected mouse models, they showed that the FDCs themselves produced PrP, that replication of scrapie in the spleen depends on FDCs, and that neuroinvasion following peripheral challenge is impaired in the absence of FDCs.

Infections in laboratory animals, especially with scrapie and BSE agents, could be established by different routes, including intracerebral, oral, subcutaneous, intravenous, intraperitoneal and intraocular routes [69]. However, the relative efficiency of these routes differs considerably. The best route for experimental transmission of prions is to inject the infective material directly into the brain (intracerebral inoculation) and the best source of infective prions is the brain. By contrast, infection by other routes requires tens or even hundreds of thousands greater doses than those used for intracerebral infection [56].

**Diagnosis:**

Prion diseases produce few or no symptoms until it is too late. It is therefore important to develop diagnostic methods that can detect the disease at an early stage. One of the major problems is that prion diseases do not stimulate immunity because the modified prion that causes them is host-derived. Therefore, no direct serological test is available for routine detection of these conditions in the living host. At present, diagnosis is largely based on clinical examination and post-mortem examination of the brain. Prion disease should be suspected in patients showing progressive decline in mental and motor functions [15]. Invasive clinical methods such as brain specimen biopsy can diagnose prion disease, but the only non-invasive clinical method available is electroencephalogram (EEG) in CJD. The latter disease produces a characteristic EEG wave that can be used to establish a clinical diagnosis. However, this wave pattern is limited to CJD and has not been observed in other prion diseases. The presence of abnormal
prion protein in the brains of dead humans or animals can be diagnosed by
electron microscopy and by special techniques of molecular biology,
imunocytochemistry, western blotting and infrared microspectroscopy
[70-72]. However, in some patients with inherited prion disease, there is no
detectable PrP\textsuperscript{Sc} in the brain. On the other hand, the presence of inherited
types of disease can be demonstrated by conducting genetic analysis of the
PrP gene in DNA extracts of the patient's leucocytes. This method is of little
value in the diagnosis of sporadic and infectious forms of prion disease [15].

In experimental animals, infectivity could be detected in lymphoid tissue
biopsies (spleen, lymph nodes, Peyer's patches and tonsils) prior to
involvement of the brain and appearance of clinical signs [23]. The
cerebrospinal fluid (CSF) has also been found to contain infectious prions,
and a test has been developed for early detection of the disease in the CSF;
the results are encouraging because the test is showing sensitivity and
specificity [73].

A low level of infectivity may also be detected in the blood prior to the
appearance of clinical symptoms. Schmmer et al. [74] used capillary
electrophoresis and fluorescent peptides of the prion protein to detect
misfolded prion isoform (PrP\textsuperscript{Sc}) in the blood and CSF of scrapie-infected
sheep during the incubation period, and reported that there was a good
correlation between the blood assay and the development of clinical scrapie.
In an effort to overcome the problem of detecting extremely low
concentrations of PrP\textsuperscript{Sc} in the blood, Cashmann [75] identified
protocadherin-2 (PC2) as a high-affinity cell surface receptor for both PrP\textsuperscript{C}
and PrP\textsuperscript{Sc}, and suggested that it might be used as a "capture" reagent in the
diagnosis of prion disease. On the other hand, novel, 2'-F-substituted RNA
aptamers (synthetic RNA's) have been identified and were found to bind
selectively to prion proteins from humans, mice, sheep, hamsters and cattle
but not to other proteins [76]. These aptamers were highly resistant to
nucleases and proteases and had more than 10-fold greater affinity for in
vitro refolded, PrP\textsuperscript{Sc} than for its PrP\textsuperscript{C} isoform, indicating that aptamers
could be potentially useful reagents in the diagnosis of prion diseases. More
recently, Weiss [77] selected a RNA aptamer that specifically binds PrP\textsuperscript{Sc}
and not PrP\textsuperscript{C}. A sonication procedure has also been described for cyclic
amplification of protein misfolding that allowed a rapid conversion of large
amounts of PrP\textsuperscript{C} into PrP\textsuperscript{Sc}-like form in the presence of minute quantities of
PrP\textsuperscript{SC} template [78]. This procedure, which is termed Protein-misfolding cyclic amplification; (PMCA), is basically analogous to polymerase chain reaction cycling, and could be used to detect the presence of currently undetectable levels of PrP\textsuperscript{SC} in tissues and body fluids of the patients. The PMCA may also be useful in determining whether PrP\textsuperscript{SC} replication results in the generation of infectivity in vitro.

**Treatment**

Prion diseases do not stimulate immunity. Therefore, a vaccine that prevents them is unlikely. No treatment is available either, although research in this area is progressing. Removal of the PrP gene renders mice insusceptible to intracerebral inoculation with the scrapie-agent, while PrP over-expression enhances the development of disease [79]. Therefore, it has been suggested that either gene therapy or the use of antisense oligonucleotides that interfere with replication and reduce the amount of mRNA transcription of the PrP gene might be beneficial, although the delivery of these oligonucleotides to the brain might be difficult [15]. Similarly, substances that interfere with endocytosis, exocytosis or trafficking of PrP\textsuperscript{SC} protein in the cells might be useful. Scientists have also been searching for drugs that could prevent the formation of PrP\textsuperscript{SC} without affecting PrP\textsuperscript{C} such as substances that bind to PrP\textsuperscript{C} and stabilize the $\alpha$-helix structure, interfere with PrP\textsuperscript{C}-PrP\textsuperscript{SC} interactions, or bind protein X, thereby preventing PrP\textsuperscript{SC} replication. It has been found that the amyloid stain Congo red and certain sulfated glycans strongly and selectively inhibited the production of PrP\textsuperscript{SC}, without apparently interfering with PrP\textsuperscript{C} metabolism, in scrapie-infected cells [20; 80; 81]. An Amphotericin-B derivative was also shown to prolong the incubation period and reduce the infectivity of brain tissue in hamsters infected with the scrapie agent, but does not prevent the disease from developing [15]. Anthracyclines were also found to induce amyloid plaque resorption, presumably by binding to PrP\textsuperscript{SC}, thereby preventing it from acting as a template for the formation of new PrP\textsuperscript{SC} [82] but unfortunately, these drugs are highly cytotoxic and should be modified before they can be used. It has also been found that treatment of scrapie-infected mice with a synthetic peptide homologous to a small region of the prion protein has reversed the abnormal structure of PrP\textsuperscript{SC} to its normal (PrP\textsuperscript{C}) counterpart, delayed the clinical symptoms in the infected mice significantly and reduced brain infectivity by more than 90-95% [83].
Priola et al. [84] studied the effect of three different types of cyclic tetrapyrroles on experimentally produced scrapie in mice. They reported that the drugs, when injected at different times during the “incubation period,” blocked the conversion of normal prion protein into the pathogenic isoform, thereby significantly slowing the progression of the disease. When these drugs were given concurrently with injection of the scrapie agent, they extended survival time dramatically, in some cases by 300 percent, but when given later during disease, they exerted only minimal effect. These results suggest that cyclic tetrapyrroles might delay prion diseases if given prior to or early during the course of disease, and that they might also be used to treat blood products to inactivate infectious prions. However, they are unlikely to be effective after the development of disease.

Recently, a number recombinant antibody antigen-binding fragments (Fabs) have been tested in vitro for their ability to inhibit prion propagation in cultured mouse neuroblastoma cells infected with PrP\textsuperscript{SC}. Some of these antibodies appeared to bind to a specific region in normal prion (PrPC), where the latter interacts with PrPSC, and in that manner inhibit prion propagation and clear prion clumps out of the cultured cells. These studies offer the possibility of using genetically engineered antibodies to prevent and treat prion diseases, and identify sites for drug targeting [85].

Conclusions:
There is now a wider range of prion diseases in man and animals than originally thought. Some of these diseases, like scrapie, have existed for many centuries while others have only been described recently, and still others might be added to the list. These diseases have been classified on the basis of their clinical manifestations and history, but more accurate molecular classification is becoming increasingly sought. For example, it was first thought that FFI is an exclusively inherited form of prion disease, but now sporadic cases have been diagnosed of this disease. In spite of the amount and quality of the work that has been carried out to prove that an abnormal prion protein is the sole cause of "prion diseases," and the acceptance of this theory by the majority of the scientific community, the prion hypothesis still faces some crucial problems. Many scientists argue that a definitive proof that prions could cause disease by themselves is still lacking and that an associated factor such as a virion cannot be ruled out. It
is also still difficult to explain the existence of distinct strains of scrapie on the basis that each strain is a distinct PrPC that converts to a distinct PrPSC. Until such questions pertaining to the exact cause are fully answered, attempts to develop new methods of treatment for prion diseases will be incomplete.

The emergence of mad cow disease in Britain has brought considerable prominence to other prion diseases, most of which are actually extremely rare. The research made on the molecular and genetic basis of these diseases has significantly enriched scientific knowledge, but has also exposed limitations in our ability to deal with such diseases in terms of prevention and control.

Although most prion diseases are rare, some like BSE have caused major outbreaks leading to considerable economic loss, and creating a significant risk to human health. There are also several other newly emerging diseases that represent a risk to human or animal health that should also be given similar attention. While this is highly desirable in order to protect man and animals from these emerging diseases, older maladies like tuberculosis, malaria, bilharziasis, sleeping sickness and river blindness, which are devastating millions of people and their animals in many parts of the world, should not be overlooked.
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II. Prion Diseases in Man and Animals
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منصور فارس حسين و سعود بن إبراهيم المفرج
قسم الإنتاج الحيواني - مختبر علوم الأغذية والزراعة - جامعة الملك سعود
الرياض - المملكة العربية السعودية

الملخص:

يوجد حالياً ثلاثة عشر مرضاً من أمراض البريون في الإنسان والحيوان، ففي الإنسان تجد: "مرض مكروه"، "مرض مكروه فرد"، "مرض مكروه الأسرى القائم" و"مرض الغبار الجديد"، متلازمة جرتسمان ستراسمر شنقكر، "الأرق الأسري القائم" و"مرض البر". أما في الحيوانات، فيوجد عدداً كبيراً من "مرض الرفجان"، "اعتلال الدماغ الإسفنجي المغدي في الثعلب الفضي"، "مرض النزل الزمني"، "اعتلال الدماغ الإسفنجي البشري"، "اعتلال الدماغ الإسفنجي السعوري"، "اعتلال الدماغ الإسفنجي لشوفات الحافر"، "اعتلال الدماغ الإسفنجي البشري"، و"اعتلال الدماغ الإسفنجي النعومة" وهي أمراض فريدة من نوعها، وقد حيرت العلماء طويلاً، خصوصاً فيما يتعلق بتخصصها المبكر وإمكانية معالجتها.

وبفرض نصحة نظرية البريون، فإن مصدر البروتين الضار "المحور" المسبب لأمراض البريون هو العائل نفسه، لذا يصبح من مشير تحذير احتمال مباشر للتثقيب أو لقاحات للتصحح ضد هذه الأمراض حالياً. يعتمد تشخيصها على الأعراض السريرية والصعوبات التشريحية علاوة على محاولات المتحكمن في وجود البريون أو عظام باستخدام بعض الطرق الناعمة البيولوجي/كيميائية لفحص الأنسجة والدم والسائل المخيخ الطبيعي، فضلاً عن التصوير الطيفي وغريت، ويتم حاليا تقييم بعض الموئلات التي تحدث مع البريون الضار (المحور) وبالتالي تمنع تطوره.

من ناحية أخرى لا يوجد بعد علاج لأمراض البريون، لذا تمكن عدداً كبيراً من العلاجات. تجري حالياً دراسات على تطوير علاجات فعالة على تثبيت البريون الضار (PrPSc) وعلى نفسه، وتحولهم إلى الصيغة الطبيعية غير ضارة إلى صيغة ضارة وإمكانية إعداد تشخيص وإعادة تكافؤ حالاته الطبيعية مرة ثانية.