Prion diseases, also known as the transmissible spongiform encephalopathies (TSEs), are a group of slowly developing neurodegenerative disorders occurring in human and animals. Prion diseases can be transferred between animals, humans, from humans to animals, and from animals to humans. As a result, the central nervous system is attacked, resulting in microglial activation, astrocytosis, prion plaque deposition, and neuronal degeneration. Prion also targets on the eye and brain visual system. In scrapie-infected sheep, chronic wasting disease (CWD)-infected mule deer, and experimental animals infected with scrapie, transmissible mink encephalopathy (TME), and Creutzfeldt-Jakob disease (CJD), damage has been found in the outer and inner nuclear layers of the retina, brain stem, optic nerve, optic tract, optic radiation and visual cortex. This article reviews the prion agent and infectivity in the eye and brain visual system, and the visual and oculomotor pathology in animal prion diseases. Effects of PrP genotypes and PrPSc types on visual and oculomotor disorders will be discussed.

Key words: Prion disease, Retinopathy, Visual degeneration, Scrapie, TME, BSE, PrP

Introduction

Prion diseases, also known as transmissible spongiform encephalopathies (TSEs), are a group of slowly developing fatal neurodegenerative disorders of humans and animals. In animals, TSEs include sheep and goat scrapie, mink transmissible encephalopathy (TME), mule deer and elk chronic wasting disease (CWD), bovine spongiform encephalopathy (BSE), and felines spongiform encephalopathy (FSE). This last not only occurs in cats, but also eland, nyala, and kudu. In humans, the TSEs include: CJD, Gerstmann-Straussler-Scheinker disease (GSS), kuru, and the familial and sporadic fatal insomnias (FFI and SFI). Many subtypes of CJD have been described which include sporadic CJD (sCJD), infectious or iatrogenic CJD (iCJD), genetic or familial CJD (gCJD or fCJD), Heidenhain’s variant CJD (HvCJD), and variant CJD (vCJD) (Greig, 1950; Gajdusek and Zigas, 1957; Dickinson, 1976; Williams and Young, 1980; Fraser and Dickinson, 1985; Carp et al., 1994; Goldfarb and Brown, 1995; Prusiner, 1998; Brown et al., 2000; Montagna et al., 2003; Ye et al., 2003; Collins et al., 2004; Cunningham et al., 2004; Johnson, 2005; Collinge, 2006).

All the above diseases are transmissible and produce spongiform degeneration in the central nervous system. All these diseases produce changes in the conformation of the normal cellular prion protein (PrPC) into an abnormally folded isoform (PrPSc). Hence, TSEs are also called prion diseases (Prusiner, 1982; 1998; Watts and Westaway, 2007). PrPSc is a protein with a high percentage of β-sheet structures and the unusual ability to recruit normal prion proteins (PrPC) into changing their shape. When a prion changes shape, or “misfolds”, it creates a cascade that causes neighboring normal prion proteins with α-helix structures to assume a β-sheet structure. As a result, the misfolded prion protein acquires new physical and biochemical characteristics including detergent insolubility and partial resistance to PK breakdown. Abnormal PrPSc accumulates causing neuronal degeneration, astrocytosis, microglial activation and spongiform changes (Hsiao et al., 1990; Bessen and March, 1992; Bruce et al., 1994; Prusiner, 1998; Collinge, 2006).

Prion agent and infectivity have also been reported in eye and brain visual system in animals infected with...
prion diseases, which cause visual and oculomotor disorders in the animals.

**Prion disease in animals**

**Scrapie**

Scrapie, an infection of sheep and goats, was the first TSE disease described in the literature and has been known in Europe for over 280 years (Greig, 1950; Dickinson, 1976; Dickinson et al., 1986; Kimberlin and Walker, 1986; Fatzer and Vandevelde, 1998; Zanusso et al., 2003). It has been reported in Asia, Africa (such as Kenya), Europe and America.

There are two different types of scrapie (Table 1). The most common is classical scrapie, which has two subtypes, subtype I: the hallmarks of its infection are pruritis and scratching. Subtype II is less common and presents with nervousness, drowsiness, and trotting, or ataxia. The second type is atypical scrapie. Recently, it has been reported that atypical scrapie cases were found in Europe. A clinical form of scrapie with unusual pathological features has been repeatedly found in Norway since 1998. This so-called Nor98 were also found in other European countries (Benestad et al., 2003, 2008; Gavier-Widen et al., 2004; Madec et al., 2004; Le Dur et al., 2005; Saunders et al., 2006). Nor98 scrapie subtype displays a distinctive PrPSc profile in immunoblots with the presence of a low molecular band at 11-12 or 7-8 kDa. PrPSc immunostaining in the medulla oblongata is much lower in Nor98 than in classical cases, with the cerebellum sometimes the only area showing immunostaining. Brain histopathology reveals neuropil vacuolation in the cerebellar and cerebral cortices (Benestad et al., 2003; 2008). Some sheep with resistant PrP genotypes (ARQ or ARR) showed ataxia, while others had no clinical signs (Benestad et al., 2003). This atypical scrapie does not

Table 1. Prion diseases in animals.

<table>
<thead>
<tr>
<th>Prion diseases</th>
<th>Infected animal</th>
<th>Clinical signs</th>
<th>Brain pathology</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Scrapie</strong></td>
<td>Sheep Goats Moufflon</td>
<td>Type I: classical scrapie subtype I: Pruritis, scratching. subtype II: Nervousness, sleepiness, drowsiness, trotting, ataxia. Type II: atypical scrapie Nor98, Ataxia or no signs</td>
<td>Vacuolation, Gliosis, Neuronal degeneration, PrPSc accumulation. Neurophil vacuolation in the cerebellar and cerebral cortex</td>
<td>Barnett and Palmer, 1971; Benestad et al., 2003; Dickinson, 1976; Gavier-Widen et al., 2004; Saunders et al., 2006; Luhken et al., 2007</td>
</tr>
<tr>
<td><strong>TME</strong></td>
<td>Mink</td>
<td>Aggressiveness, hyperesthesia, ataxia, tremors or circling, compulsive bitting of self or objects, a rough hair coat and extended tail.</td>
<td>Vacuolation in frontal cortex, hippocampus etc. gliosis, neuronal degeneration, PrPSc accumulation.</td>
<td>Bessen and Marsh, 1992; Buyukmihci et al., 1987; Hanson et al., 1971; Marsh and Hadlow, 1992</td>
</tr>
<tr>
<td><strong>BSE</strong></td>
<td>Cattle</td>
<td>1. Classical BSE (cBSE): Nervousness, aggressiveness, increased fear towards humans and head restraint, kicking at milking, nose licking, teeth grinding, tremor, ataxia, disorientation. 2. Atypical BSE: found in older animals, few or no signs of disease, a. L-type (BASE) (L-type-like); b. H-type:</td>
<td>Vacuolation, gliosis, Neuronal degeneration, PrPSc accumulation throughout the brain. Bovine amyloidotic spongiform encephalopathy (BASE)</td>
<td>Biacabe et al., 2004; Collins et al., 2004; Donnelly et al., 2002; Houston et al., 2000; Kimberlin, 1990. Sejvar et al., 2008; Comoy et al., 2008</td>
</tr>
<tr>
<td><strong>CWD</strong></td>
<td>Deer, Elk, Moose</td>
<td>Progressive weight loss, excessive saliva, polydipsia, polyuria.</td>
<td>Vacuolation, gliosis, Neuronal degeneration, PrPSc deposit in brain, olfactory cortex, and lymphoid organs.</td>
<td>Williams and Young, 1980, 1992</td>
</tr>
<tr>
<td><strong>FSE</strong></td>
<td>Cats</td>
<td>More timid, aggressive, ataxia, hypermetria and hyperesthesia to sound and touch.</td>
<td>Vacuolation, gliosis, neuronal loss or degeneration, PrPSc deposit in brain, brain stem, spinal cord, retina, and peripheral organs.</td>
<td>Demierre et al., 2002; Lezmi et al., 2003.</td>
</tr>
<tr>
<td><strong>ZSE</strong></td>
<td>Nyla, Greater Kudu, Gemsbok, Arabian oryx, Scimitar-horned oryx, Eland, American bison, Cheetah, Puma, Ocelot, Tiger, Lion, Asian golden cat, Ostriches, Non-human primate</td>
<td>TSE-infected puma showed ataxia, a hypermetrical gait, whole body tremor.</td>
<td>Vacuolation, Gliosis, Neuronal degeneration, PrPSc accumulation in brain (including spinal cord and medulla), tonsil, gastrointestinal epithelial cells and blood and lymph vessels.</td>
<td>Bons et al., 1997; Fatzer and Vandevelde, 1998; Kirkwood and Cunningham, 1994; Sigurdson and Miller, 2003.</td>
</tr>
</tbody>
</table>
appear to be as highly transmissible as classic scrapie.

**Transmissible mink encephalopathy**

Transmissible mink encephalopathy (TME) is very similar to scrapie both in its clinical signs and pathological lesions. TME was first reported in US and other countries (Marsh and Hanson, 1975; Marsh et al., 1991; Marsh and Hadlow, 1992; Marsh and Bessen, 1993). TME may also infect through ingested meat and bone meal. The disease is indistinguishable from that induced in mink by inoculation of sheep scrapie. Most of the minks on the farm died rapidly after a short encephalopathic period. The incubation in experimental situations is approximately 6-12 months (Hanson et al., 1971).

Two types of TME agents have been found in hamsters, hyper (HY) and drowsy (DY). The HY strain causes hyper-excitement and ataxia, while the DY strain causes lethargy and ataxia without hyperesthesia. DY PrPSc is more protease-sensitive than HY PrPSc, and producing different Western blot profiles (Bessen and Marsh, 1992; Bartz et al., 2000).

**Bovine spongiform encephalopathy**

Bovine spongiform encephalopathy (BSE), or mad cow disease, was identified in November 1986 as a new scrapie-like disease in UK cattle. The emerging epidemic was soon associated with the use of meat and bone meal from scrapie-infected sheep. This disease has a prolonged incubation period in cattle following oral exposure (2-8 years) and, once symptoms appear, is invariably fatal. BSE has been reported in domestic cattle in many countries (Wells et al., 1987; Donnelly et al., 2002; Collins et al., 2004; LaFauci et al., 2006).

Multiple strains of BSE exist. There are two types of BSE too, the most common one is called classical BSE (cBSE), the other is called atypical BSE. There are at least two atypical BSEs subtypes (H-type and L-type, based on the molecular weight of unglycosylated band of PK-digested PrPSc) found in Europe and Japan (Buschmann et al., 2006; Masujin et al., 2008). A higher molecular mass of unglycosylated PrPSc (H-type) was found in three French cattle (Biacabe et al., 2004), while a lower apparent molecular mass (L-type) was found in two Italian cattle. In an Italian study, isolates were obtained from two elderly (11 years and 15 years age) dairy cows without neurological symptoms. On average, most animals with BSE develop symptoms at 4-5 years of age. Spongiform changes in the brains were topographically different than seen in typical BSE or classical BSE (cBSE), and the western-blot patterns were also showing as L-type (Casalone et al., 2004). This L-type BSE is a form of bovine amyloidotic spongiform encephalopathy (BASE). L-type-like BSE was also detected in 14-year-old Japanese black beef cattle (BSE/JP24) (Masujin et al., 2008). CBSE has been shown to be responsible for human vCJD. Results suggested that BASE has similarity with a subtype of human sporadic CJD, but whether BASE relate to a subtype of human sCJD (such as MV2 subtype or MM2 subtype of sCJD) is still not clear (Casalone et al., 2004; Comoy et al., 2008; Seijer et al., 2008).

**Chronic wasting disease (CWD)**

CWD was first described clinically as a wasting syndrome in captive deer belonging to Colorado research facilities in 1967. In 1978, Dr. Elizabeth Williams reported that CWD was a TSE or prion disease. CWD has been found in captive and free ranging mule deer (Odocoileus hemionus hemionus), black-tailed deer (Odocoileus hemionus columbianus), captive and wild Rocky Mountain elk (Cervus elaphus nelsoni), and free-range white-tailed deer (Odocoileus virginianus) (Williams and Young, 1980, 1992; O’Rourke et al., 1999, 2004; Sigurdson and Miller, 2003). Recently, a wild bull moose killed by an archer had tested positive for CWD-like disease. CWD has been found in USA, Canada (Alberta and Saskatchewan) and South Korea (Williams and Young, 1992; Ye et al., 2003). Since there are two types of scrapie and BSE (classical and atypical), it is possible that the atypical CWD has not been found.

**Feline spongiform encephalopathy (FSE)**

FSE was first reported in May 1990 in a 5-year-old male Siamese cat. Since then, more than 87 domestic cats in Britain and some sporadic cases in Norway, Northern Ireland, Switzerland, and Liechtenstein. FSE has also been described in captive cheetah, puma, and ocelot, as well as a tiger in a British zoo. The epidemiology of FSE is believed to relate to the meat and bone meal fed to these animals. Attempts to find previous cases with FSE among demented or neurologically degenerate cats from the past have been unsuccessful (Demierre et al., 2002; Lezmi et al., 2003; Ye et al., 2003).

**Zoological spongiform encephalopathy (ZSE)**

Since 1986, TSE has been reported in more than 13 species of zoo animals, e.g. antelopes and large cats, including six elands (Taurotragus oryx), a nyala (Tragelaphus angasi), an Arabian oryx (Oryx leucoryx), a Scimitar-horned oryx (Oryx dammah), six greater kudus (Tragelaphus strepsiceros), a gemsbok (Oryx gazella), an American bison (Bison bison), ten cheetahs (Acinonyx jubatus), three pumas (Felis concolor), three ocelots (Felis pardalis), three tigers (Panthera tigris), four lions (Panthera leo), an Asian golden cat (Catopuma temminckii) as well as several non-human primates in British and French zoos (Kirkwood and Cunningham, 1994; Sigurdson and Miller, 2003). The occurrence of a TSE in an avian species was reported in four ostriches (Struthio camelus) in the zoos of northern
Germany but no evidence is currently available as to whether other avian species can have diseases similar to TSE (Fatzer and Vandeveld, 1998). Two animals of a non-human primate species, Eulemur fulvus mayottensis, fed over a number of years with food containing cattle meat, developed prion disease. Puma infected with TSE developed ataxia, a hypermetric gait and whole body tremor (Bons et al., 1997).

Prion agent and infectivity in the eye and brain visual system

Scrapie

PrPSc was detected in the inner and outer plexiform layer, ganglion cell layer, optic fiber layer and in nuclear layers in scrapie-affected sheep (Table 2, Fig. 1) (Hardt et al., 2000; Jeffrey et al., 2001; Greenlee et al., 2006). PrPSc was found in the retina of 19 (95%) of the 20 scrapie sheep. The highest deposit was observed in the inner plexiform and ganglion cell layers, while fine granular deposit across all layers was also found in some scrapie sheep. Spongiosis and PrPSc were detected within the visual pathways and visual cortex at the preclinical, clinical and terminal stages. PrPSc was detected in 3 (15%) out of 20 optic nerves, and 9 (69%) out of 13 optic chiasmas. PrPSc was also found in the retina of a 3-year-old female Angora goat with natural scrapie (Valdez et al., 2003).

Scrapie-infected elk

Hogan et al. (1981) inoculated homogenates of whole eyes infected with scrapie into hamsters and demonstrated that prion agent progressively increases during incubation, but is about 2 logs lower in infectivity than the brain. Later, they found that the titers of prion in brains of animals were maximal at a mean of 10 \textsuperscript{9.9} LD50 U/mL. Optic nerve and retinal titers were nearly the same at 10 \textsuperscript{8.4} LD50 U/mL. Uvea and lens had lower titers, but the lowest regional titer was found in the cornea with 10 \textsuperscript{5.4} LD50 U/mL (Hogan et al., 1981, 1986).

PrPSc immunostaining was observed in the photoreceptor segments, outer plexiform layer (OPL), inner plexiform layer (IPL), inner nuclear layer (INL), and ganglion cell layers of 263K-infected hamsters. Some PrPSc staining may have been present in the cornea (Foster et al., 1999). PrPSc immunoreactivity could not be demonstrated in the retinas of control
hamsters. Similar PrP<sup>Sc</sup> deposition patterns were also reported in the retina of ME7-infected C57, VL, VM mice, and in 79A-infected C57 mice (Foster et al., 1999).

Scrapie-infected mice

It has been reported that conjunctival or intraocular instillation of scrapie in mice can produce disease (Scott and Fraser, 1989; Scott et al., 1993). Replication of the scrapie agent was found in ocular neural tissue, which showed that scrapie infectivity rises progressively in the eye to maximal levels between 6 and 8 weeks after inoculation (Buyukmihci et al., 1980; Hogan et al., 1981).

In mice inserted with a hamster PrP transgene, PrP<sup>C</sup> immunostaining was most prominent in the inner and outer segments of rod photoreceptors, coinciding with the earliest site of pathologic changes in scrapie-infected hamsters (Chishti et al., 1997).

In another study using tgGFAP mice, after intraocular inoculation with hamster scrapie, PrP<sup>Sc</sup> was first found in retina, then dorsal lateral geniculate nucleus (dLGN), superior colliculus (SC) and other regions not associated with the visual system, including cerebral cortex, hippocampus, hypothalamus, and cerebellum, and finally the visual cortex (occipital areas). Similar results were found in 22A-infected VM mice, 87V-infected VM mice, and 79A, ME7-infected mice. Therefore, PrP<sup>Sc</sup> may be transported along the visual system pathways as well as by other mechanisms such as transport by adjacent glial cells (Scott and Fraser, 1989; Scott et al., 1992; Jeffrey et al., 1995; Kercher et al., 2004).

From all these observation, it is thought that the eyes of prion-infected animals carry prion agent and are infectious. It is not sure if the tear from prion-infected animals has prion agent and infectivity. These studies suggest that ophthalmic surgical instruments used in procedures involving the retina, cornea, optic nerve and other visual systems might represent a potential risk for iatrogenic transmission of prion diseases in animals.

### Table 2. Prion agent and infectivity in the visual system of animal prion diseases.

<table>
<thead>
<tr>
<th>Prion diseases</th>
<th>Infectivity observed in visual system</th>
<th>PrP&lt;sup&gt;Sc&lt;/sup&gt; deposition in visual system</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scrapie (Classical)</td>
<td>Brain, palpebral lymphoid follicles.</td>
<td>Brain, visual pathways, visual cortex, retina, inner and outer plexiform layer, ganglion cell layer, optic fiber layer, nuclear layer, optic nerves, optic chiasma</td>
<td>Greenlee et al., 2006; Hardt et al., 2000; Jeffrey et al., 2001; Valdez et al., 2003</td>
</tr>
<tr>
<td>Scrapie-infected elk</td>
<td>Brain, retina, inner and outer plexiform layer, ganglion cell layer</td>
<td></td>
<td>Hamir et al., 2004</td>
</tr>
<tr>
<td>Scrapie-infected hamsters</td>
<td>Brain, retina, optic nerve, lens, retinal pigment epithelium, cornea.</td>
<td>Brain, the photoreceptor layer, inner nuclear layer and inner plexiform layer, cornea.</td>
<td>Foster et al., 1999; Hogan et al., 1981, 1986</td>
</tr>
<tr>
<td>Scrapie-infected mice</td>
<td>Brain, ocular neural tissue, eye.</td>
<td>Brain, dLGN, SC visual cortex (occipital areas), retina.</td>
<td>Buyukmihci et al., 1980; Chesebro et al., 2005; Chishti et al., 1997; Hogan et al., 1981; Jeffrey et al., 1995; Scott and Fraser, 1989</td>
</tr>
<tr>
<td>TME</td>
<td>Brain, retina, cornea, optic nerve, whole eye, uvea and lens.</td>
<td>Brain</td>
<td>Marsh et al., 1991; Marsh and Bessen, 1993; Marsh and Hadlow, 1992; Marsh and Hanson, 1975</td>
</tr>
<tr>
<td>BSE-infected sheep</td>
<td>Brain, retina, internal and external plexiform layers, ganglion cell layer, aqueous humor and vitreous humor, brain.</td>
<td></td>
<td>Foster et al., 2001; Lezmi et al., 2006.</td>
</tr>
<tr>
<td>CWD (CWD-infected deer) (CWD-infected ferret)</td>
<td>Brain, the inner and outer plexiform layer of the retina, ganglion cell layer, optic nerve fibers, the oculomotor nuclei, the optic chiasm. Spongiform changes in optic chiasm in CWD-infected ferret</td>
<td>Spraker et al., 2002; Williams and Young, 1980, 1992; Sigurdson et al., 2008.</td>
<td></td>
</tr>
<tr>
<td>FSE</td>
<td>Brain, the stratum ganglionare, the stratum plexiform internum and externum, the inner nuclear layer and the rod and cone layers</td>
<td></td>
<td>Demierre et al., 2002; Lezmi et al., 2003.</td>
</tr>
<tr>
<td>ZSE (TSE-infected kudu)</td>
<td>Brain, Conjunctiva.</td>
<td>Brain</td>
<td>Bons et al., 1997; Fatzer and Vandevelde, 1998; Kirkwood and Cunningham, 1994; Sigurdson and Miller, 2003</td>
</tr>
<tr>
<td>CJD-infected guinea pig</td>
<td>Brain, cornea, anterior chamber.</td>
<td>Brain</td>
<td>Manuelidis et al., 1977</td>
</tr>
</tbody>
</table>
Transmissible mink encephalopathy (TME)

Marsh and Hanson (1975) reported prion agents in the corneal epithelium of mink with terminal TME. Corneal levels were $10^{4.8}$ median lethal dose (LD50) U/mL, and the brain was $10^{8.8}$ LD50 U/mL.

BSE-infected sheep

In experimental cBSE-infected sheep, PrPSc was found in the brain, lymphoid organs and peripheral nervous system. In the eye, PrPSc has been found in the aqueous humor and vitreous humor. In the retina, PrPSc accumulation was mainly detected in the ganglion layer, internal and external plexiform layers (Lezmi et al., 2006). PrPSc immunostaining was also detected in the inner nuclear and outer plexiform layers of retina in one of seven cBSE-infected sheep by oral transmission (Foster et al., 2001).

These studies suggested that prion agent might be found in the eye and brain visual system. It was possible that PrPSc diffused from the brain to the eye using a centrifugal model either by optic nerve (neuronal pathway) or by body fluid (extraneuronal pathway).

Chronic wasting disease (CWD)

Spraker et al. (2002) reported that PrPSc was observed in the oculomotor nuclei of all of the 11 free-ranging mule deer and 8 (89%) of 9 captive mule deer infected with CWD. This result suggested that eye movements might be affected in CWD-infected mule deer. PrPSc was found in the layer of optic nerve fibers, ganglion cell layer, as well as the inner and outer plexiform layers of the retina. PrPSc was also observed in optic chiasm of 2 (18%) of 11 free-ranging mule deer and 3 (38%) of 8 captive mule deer. Therefore, this study suggests that oculomotor and visual disorder might be

<table>
<thead>
<tr>
<th>Table 3. Visual/ocular pathology found in animal prion diseases.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Prion disease</strong></td>
</tr>
<tr>
<td>-------------------</td>
</tr>
<tr>
<td>Scrapie (Classical)</td>
</tr>
<tr>
<td>Scrapie-infected cattle</td>
</tr>
<tr>
<td>Scrapie-infected hamsters (263K-, Chandler-, 79A-, ME7- 139H- Scrapie strains)</td>
</tr>
<tr>
<td>TME-infected hamsters</td>
</tr>
<tr>
<td>Scrapie-infected mice (79A-, 139A-, ME7-, 22A-, 22L-) ME7- and RML-infected tga20 mice</td>
</tr>
<tr>
<td>CJD-infected mice</td>
</tr>
<tr>
<td>CJD-infected cats</td>
</tr>
<tr>
<td>CJD-infected squirrel monkeys and chimpanzees</td>
</tr>
<tr>
<td>CWD</td>
</tr>
<tr>
<td>BSE* (Classical)</td>
</tr>
</tbody>
</table>

present in CWD-infected mule deer.

**Feline spongiform encephalopathy (FSE)**

In FSE, PrP$_{Sc}$ deposits were found in the stratum ganglionare, and the stratum plexiform internum and externum. Weaker staining was also observed in the inner nuclear layer and in the rod and cone layers (Lezmi et al., 2003).

**Zoological spongiform encephalopathy (ZSE)**

Infectivity bioassay results for tissues from greater kuru-infected with BSE indicated that high infectivity was found in brain, brainstem, spinal cord, and lymph nodes; medium infectivity was found in spleen. Low BSE infectivity was also detected in skin, conjunctiva, and salivary gland (Cunningham et al., 2004).

**CJD-infected guinea pig**

In experimental CJD, the cornea from a CJD-infected guinea pig was divided into six and each piece was transferred into the anterior chamber of a healthy guinea pig. The brains of all six recipients showed prion infection at postmortem examination. This study confirmed that infectivity in the cornea and transmission could occur via the eye with infected cornea (Manuelidis et al., 1977).

**Visual/ocular pathology found in animal prion disease**

**Scrapie**

In the early 1970s, two scrapie-affected sheep were found with retinopathy characterized by degeneration of photoreceptor outer segments and accumulation of periodic acid Schiff (PAS) positive material in the adjacent extracellular space (Barnett and Palmer, 1971). This was the first report of visual system damage in animals infected with prion disease.

In recent studies, retinal histopathological changes and abnormal prion accumulation were further observed in naturally affected scrapie sheep (Hortells et al., 2006) and experimentally scrapie-infected sheep (Greenlee et al., 2006). The changes included loss of outer limitant layer definition, outer plexiform layer (OPL) atrophy, disorganization and loss of nuclei in both nuclear layers and Müller glial hypertrophy. In the brain visual system, dLGN, SC and occipital cortex were also affected in most scrapie sheep at clinical and terminal stages (Hortells et al., 2006). This study suggested not only that the visual receptor organ (retina) but also the visual transport system and visual processing center were affected during scrapie infection. That is to say that although animals cannot complain of visual problems to us, scrapie sheep without retinal pathological changes may also have visual problems at higher levels (Table 3, and Figure 2). In fact, some scrapie-infected animal may show “scrapie facies”, a characteristic blank stare (Williams, 2003).

**Scrapie-infected cattle**

Studies have shown that cattle infected with scrapie by intracerebral route become severely lethargic and demonstrate clinical signs of motor dysfunction, progressive stiffness, posterior paresis, general weakness and permanent recumbency. In one study, strabismus with bilateral exophthalmia was observed in 5 of 9 scrapie-infected calves (Cutlip et al., 1994). Another example of strabismus is in human prion disease called kuru (Gajdusek and Zigas, 1957, 1959; Cervenakova et al., 1998) suggesting perhaps that strabismus might relate to these prion agents target on similar region of the

---

**Infectivity and PrP$_{Sc}$ found:**

1. Pigment epithelium (PE)
2. Photoreceptors
3. Outer nuclear layer (ONL),
4. Outer plexiform layer (OPL),
5. Inner nuclear layer (INL),
6. Inner plexiform layer (IPL),
7. Ganglion cell layer (GCL)

**Pathology:**

1. Photoreceptor degeneration,
2. Macrophage infiltration,
3. ONL cell loss,
4. OPL thinner, atrophy,
5. Müller cell hypertrophy,
6. Ganglion cell vacuolation

---

**Fig. 2.** Prion protein (PrP$_{Sc}$), infectivity, and pathological changes in the retina of prion diseases reported in the literatures. The schematic view of the 3-D block of a portion of retina is from webvision.med.utah.edu (with permission).
Prion-induced visual pathology in animals

Scrapie-infected hamsters

Experimental infection with the 263K (Buyukmihci et al., 1982a,b) or Chandler (Hogan et al., 1981) strains of scrapie agent results in retinal degeneration in hamsters. Photoreceptor degeneration was also found in hamsters infected with 22C, 79A and ME7 strains of scrapie (Buyukmihci et al., 1982b). Pupillary responses to light and the ocular fundi were normal prior to the onset of the disease. During the incubation period, the pupillary responses became incomplete and the ocular fundi became pale and mottled with attenuated retinal vessels.

In another study, the initial changes were degeneration-scattered photoreceptors in the inner and outer segments, and pyknosis in the outer nuclear layer. These changes were more prominent in the central part of the retina. Macrophages lightly infiltrated the photoreceptor layer. During the incubation period, the outer nuclear layer (ONL) in the most severe cases was reduced to one incomplete row of nuclei, the outer plexiform layer (OPL) was essentially absent, but the inner nuclear layer (INL) seemed intact, as did the remainder of the retina. The retinal epithelium remained normal. There was a moderate increase in the number of macrophages (Buyukmihci et al., 1987a,b).

Scrapie-infected mice

Kozlowski et al. (1982) reported that retinal damage was found in 23% of ME7-infected C57BL mice, 28.5% of 87V-infected VM mice, but no changes were found in 87V-infected IM mice. At 6-8 weeks of age, mice were injected intracerebrally (i.c.) in the right hemisphere with a 1% homogenate prepared from brains of mice terminally ill with scrapie. At the end of incubation period, animals were killed after perfusion with aldehyde fixatives. Retinopathy in mice infected with scrapie began with a loss of photoreceptors followed by progressive loss of cells of the outer nuclear layer (ONL); the OPL became thinner. Numerous pyknotic cells were also seen in the INL. Only a few ganglion cells were damaged or lost.

Interestingly, retinal ganglion cells (RGCs) degenerate after intraocular injection of ME7, whereas amacrine cells, and retinal interneurons are more resistant. They show different susceptibility to the toxic effects of PrPSc (Russelakis-Carneiro et al., 1999).

Using transgenic mice expressing prion protein in specific cell types to study the role of different cell types in retina degeneration, it has been found that PrP expression in multiples cell types may be required for retinal degeneration, as expression in neurons alone was not sufficient. In contrast, in brain, PrP expression in either astrocytes or neurons was sufficient for degeneration (Kercher et al., 2004; Chesebro et al., 2005).

In scrapie strain ME7- and RML-infected tga20 mice (which express elevated levels of PrP protein compared to wild-type mice), most scrapie-infected mice eventually became extremely lethargic and listless, and developed a blank stare, but visual pathology was not reported (Thackray et al., 2002).

TME-infected hamsters

Photoreceptor degeneration was also found in experimental transmissible mink encephalopathy (TME) infected hamsters using the Hayward strain of the TME agent (Buyukmihci et al., 1987b). The incubation period was about 99 to 142 days, the infected hamsters showed mild head tremors and ataxia prior to becoming comatose. After anesthetization with methoxyflurane in air, 15 Syrian hamsters were injected in the temporal region of the right cerebral hemisphere with 0.05 ml of a 5% saline suspension of TME hamster brain containing 10^{-6.8} LD50 of infectivity. Pupillary responses to light and the ocular fundi were normal during the early stages of TME, however, the pupillary responses became incomplete and the ocular fundi became pale, with attenuated retinal vessels as the disease process. At the end of the TME incubation period (on day 195), eyes were fixed by immersion in cacodylate-buffered glutaraldehyde. One micrometer-thick sections were stained with azure II-methylene blue for light microscopy. Photoreceptor degeneration was evident in both eyes of all these animals.

BSE

Disorientation, an unexpected nervousness at entrances and staring at imaginary objects has been reported in cBSE (Honstead J. www.fda.gov/cvm/march96.html). It may indicate visual system disorders in BSE-infected cattle.

Chronic wasting disease (CWD)

Williams and Young (1980) reported that CWD-infected deer showed behavioral changes including episodes of apparent lack of awareness, stood for several minutes with lowered head and a fixed stare, and then reverted to a more normal state of alertness. Equivocal visual deficits occasionally were suspected.

Spraker et al. (2002) reported that the eyes of two free-ranging and one captive mule deer infected with CWD had vacuolated neurons in the ganglion cell layer. Vacuolation was also observed in the oculomotor nuclei of all of the 11 free-ranging mule deer and 5 (55%) of 9 captive mule deer.

In CWD-infected ferret, spongiosis was found in basal ganglia, thalamus, optic chiasm, midbrain and pons (trapezius nucleus) and occurred predominantly in the neuropil, but also in white matter tracts (Sigurdson et al.,...
Prion-induced visual pathology in animals

2008).

CJD-infected mice

Retinal degeneration has also been seen in mice after infection with CJD (Hogan et al., 1983). The outer nuclear layer of the eyes of normal control mice consisted of 10 to 12 cells, with some thinning towards the extremities. In the most severe cases of retinopathy in scrapie-infected mice, the outer nuclear layer (ONL) together with the inner and outer segments of the photoreceptor cells had almost completely disappeared (Foster et al., 1986). Macrophages were present in the photoreceptor segmental layer, and were more numerous in less advanced states of degeneration. In more severe cases when the outer nuclear layer was reduced to between one and four cells thick, macrophages were less obvious. Apart from the photoreceptor cell layer, other retinal elements such as the pigment epithelium, inner nuclear layer and ganglion cell layer remained intact. At no stage was an acute inflammatory reaction observed.

CJD-infected cats

Gourmelon et al. (1987) reported that 11 cats infected with experimental CJD showed behavioral changes, lack of grooming, fixed stare, dysmetria, startle reflex, myoclonus, muscular rigidity, excitability followed by immobility, and mild tremor. However, retinopathological changes were not reported. Similar fixed stare sign has been reported in BSE, CWD and scrapie-infected tga20 mice (Williams and Young, 1980; Thackray et al., 2002).

CJD-infected squirrel monkeys and chimpanzees

Zlotnik et al. (1974) reported that squirrel monkey infected with experimental CJD showed behavioral changes, severe itch with constant scratching and rubbing of the body against the bars of the cage (scrapie type I symptoms) and visual abnormalities. Roos et al. (1973) reported that chimpanzees infected with CJD developed a clinical disease with myoclonus, hemiparesis, fasciculations, somnolence and visual disturbance. New World monkeys infected with CJD showed a similar clinical disease.

Possible relationship between pathological changes and visual/ocular disorders in animal prion diseases

Retina

Retinopathy has been reported in scrapie-infected sheep (Barnett and Palmer, 1971; Hortells et al., 2006), in mule deer infected with CWD (Browning et al., 2004; Johnson et al., 2006). Retinopathy has also been found in prion-infected hamsters and mice. Hamsters and/or mice infected with certain type of scrapie agents, as well as CJD, and TME agents will develop retina pathological changes (Hogan et al., 1981, 1983, 1986; Buyukmihci et al., 1982a,b, 1983, 1987a,b; Kozlowski et al., 1982; Fraser and Dickson, 1985; Foster et al., 1986; Curtis et al., 1989; Kercher et al., 2004; Hortells et al., 2006). Hamsters injected into the right cerebral hemisphere with scrapie agent developed retinal lesions to a greater extent in the contralateral, rather than the ipsilateral, eye (Buyukmihci et al., 1985a,b).

As an extension of the central nervous system, the retina has also exhibited pathological changes in many scrapie strain-host species combinations. Retinal degeneration in experimental scrapie involves the photoreceptor layer, resulting in partial or complete loss of the outer nuclear layer. It is not known why retinopathy was found in some scrapie strain-host species combinations but not in the others.

The electroretinogram (ERG) abnormality has been reported in human prion disease. ERG has been used for detecting retina pathological changes in scrapie 79A-infected mice, (Curtis et al., 1989) as well as in human prion diseases (Richard et al., 1994). However, ERG has not been reported in other prion-infected animals. In some scrapie strain 79A-infected MM mice, abnormal ERG was detectable at 118 days post-infection (p.i.). There was a progressive reduction of b-wave amplitude without loss of retinal sensitivity or significant change in a- and b-wave peak implicit times. Extinction of the ERG observed in half the mice examined at 144 days p.i., a stage of the retinopathy with reduced the outer nuclear layer to 4 rows of nuclei (Curtis et al., 1989). In human CJD, a loss in the amplitude of b-wave in the ERG indicated pathological changes in the outer plexiform layer (OPL) of the retina (Katz et al., 2000).

In human prion diseases, damage to the retina may cause palinopsia, micropsia, macropsia, metamorphopsia and/or visual field defects. It is possible that animals having prion-induced retina pathology will have similar visual disorders found in human prion diseases.

Visual pathways

Degenerative changes in the optic nerve and optic chiasm were found in scrapie-infected sheep and experimental CJD-, GSS-, and scrapie-infected hamsters and mice (Hogan et al., 1981, 1983; Fraser and Dickinson, 1985; Scott and Fraser, 1989; Walis et al., 2003; Hortells et al., 2006) and in CWD-infected ferret (Sigurdson et al., 2008).

Vacuolation of myelinated fibers was present in the myelin sheaths, with splitting of myelin lamella. Proliferation of inner mesaxons was observed. Reactive macrophages and astrocytosis were found. It is uncertain whether optic nerve degeneration follows retinal degeneration in prion disease or vice versa. Both Wallerian degeneration and primary myelinated fiber degeneration were found in the affected animals. PrPSc and prion agent have been found in the optic nerve of prion-infected hosts (Jeffrey et al., 2001; Spraker et al., 2002). Prion agents can be transport anterogradely or
Prion-induced visual pathology in animals

retrogradely along the optic nerve (or by blood route), which may also cause pathological changes in the optic nerve and spread the prion agent.

In one study, at early stages of the disease (12 to 16 weeks), no pathological changes were observed in the contralateral dorsal lateral geniculate nucleus (dLGN) following the intraocular ME7 injection. However, 26 weeks after injection of ME7, a typical inflammatory response, characterized by T-lymphocytes and activated microglia, was present and an increase was seen in the CD68+ and CD3+ cells in the contralateral and ipsilateral dLGN and in the SC contralateral to the ME7-injected eye. Neuronal loss was observed in the contralateral and ipsilateral dLGN, although it was more severe in the contralateral nuclei. No neuronal loss could be observed in the contralateral SC or any other area of the CNS. At the end of the incubation period, PrPSc deposition and astrocytosis were associated with activated microglia and T-lymphocytes not only in the visual areas but also in non-visual areas. This study suggested that prion transport along the neuronal path (Jeffrey et al., 1991; Black et al., 1998; Russelakis-Carneiro et al., 1999).

The flash visual evoked potential (fVEP) components were moderately altered in some CJD patients. No fVEP has been reported in prion-infected animals. The increase fVEP latencies or increase amplitude may also relate to impairment of the precortical visual pathways in CJD patients. These responses are thought to relate to the damage in the SC, pulvinar and associated visual pathways (Aguglia et al., 1987, 1991, 1997; Armstrong 2006). It is possible the fVEP can be used for detecting pathological changes in visual pathway in prion-infected animals.

Damage to the visual pathway such as optic nerve, optic chiasm, LGN, and/or SC in prion-infected animals can cause visual and/or oculomotor disorders such as palinopsia, visual field defects, nystagmus, saccadic and pursuit disorders as found in human prion diseases.

Visual cortex

Mice (including gene knock out mice and transgenic mice) and hamsters are used frequently in experimental TSEs. Studies of the pathology of experimental scrapie have largely concentrated on changes in the brain and spinal cord. Spongiform degeneration is essentially a constant finding. Characteristic lesions under the light microscope consist of spongy changes in the neuropil, nerve cells and astrocytes with nerve cell degeneration and astrocytosis. These changes generally take place in the grey matter of the cerebrum and cerebellum (Kimberlin and Walker, 1986; Scott et al., 1992; Bruce et al., 1994; Jeffrey et al., 1995; Scallet et al., 2003; Ye et al., 2003; Kercher et al., 2004; Chesebro et al., 2005).

Two squirrel monkeys infected with CJD showed visual deficits. Their brain showed atrophy with very severe lesions throughout the brain. Spongy degeneration, astrocytosis and neuronal degeneration were found. Cerebral cortex, corpus striatum, globus pallidus, putamen, caudate nucleus, thalamus, midbrain, brainstem, cerebellum and spinal cord were affected (Zlotnik et al., 1974).

Damage in visual cortex and/or visual associated cortex can cause severe visual and/or oculomotor disorders (Hoyt, 2003). As in human prion diseases, such as visual hallucination, palinopsia, micropsia, macropsia, metamorphopsia, visual field defects, scotoma, visual agnosia, gaze palsies, strabismus, nystagmus, saccadic, pursuit disorders, eyelid abnormalities (ptosis, apraxia of eyelid opening and blepharoptosis), and/or pupillary abnormalities have been reported (Sato et al., 1992; Parchi et al., 1996, 1999). As in animal prion diseases, some of these visual and/or ocular manifestations such as visual deficits (Roos et al., 1973; Williams and Young, 1980; Zlotnik et al., 1974; Spraker et al., 2002), strabismus (Cutlip et al., 1994), bilateral exophthalmia (Cutlip et al., 1994), eyelid abnormalities (Bessen and Marsh, 1992), pupillary abnormalities (Buyukmihci et al., 1987a,b; Bessen and Marsh, 1992), and fixed stare (Williams and Young, 1980; Gourmelon et al., 1987; Spraker et al., 2002; Thackray et al., 2002) have been reported.

The cause of these visual and/or ocular disorders in animal prion diseases is still not clear. The causes of eyelid abnormalities in human prion diseases are numerous. They are most likely neurogenic origin. In third nerve palsies, ptosis is often the first sign of damage. The pathological mechanisms of strabismus and pupillary abnormalities are still not clear even in human prion diseases. In general, damage to neural networks in the visual cortex, thalamus, brainstem and cerebellum can cause eye movement disorders (Neetens and Martin, 1998). Therefore, the visual and/or ocular disorders in prion-infected animals can provide a model to study the mechanism of visual and/or ocular disorders in human prion diseases.

Effects of PrP genotypes and the PrPSc types on visual and/or oculomotor pathology in animal prion diseases

Scrapie-infected sheep

It has been known that prions comprise several strains that show different phenotypes (incubation period, clinical signs and pathological profiles). It has been suggested that both PrP genotypes and the PrPSc types play a role in the different phenotypes expression.

Susceptibility of sheep to scrapie is directly linked to the polymorphisms of PrP gene and is also influenced by the strain of the agent. PrP polymorphisms at codons 136 (A/V), 154 (R/H) and 171 (Q/R/H) are the main determinants of susceptibility/resistance of sheep to classical scrapie (but not atypical scrapie, Nor98).

Sheep carrying VRQ, ARQ, AHO or ARH at amino acid positions 136, 154, and 171 of PrP gene are associated with different degrees of susceptibility to
Prion-induced visual pathology in animals

Scrapie-infected mice

In mice, it has been reported that polymorphisms at codons 108 (leucine or phenylalanine) and 189 (threonine or valine) in the PrP gene play distinct roles in the control of the scrapie incubation period (Barron et al., 2005). However, in scrapie-infected mice, more than 14 scrapie strains have been reported, and they do not match the PrP genotype and the PrPSc types yet. As mentioned above, the 79A and 139A strains of scrapie produced retinal degeneration in every mouse strain tested; ME7, 22A and 22L produced changes in the retina of only certain mouse genotypes, while 22C, 87A and 87V produced minimal or no retinal pathology (Kozlowski et al., 1982; Fraser and Dickinson, 1985; Foster et al., 1986; Thackray et al., 2002). The molecular mechanisms behind these differences are still not known.

Scrapie-infected hamsters

The retinal pathology was similar to the 263K-infected hamster, which started at 3 weeks before the onset of the clinical syndrome (Buyukmihci et al., 1977). The incubation period indicating the onset of clinical signs in the 22C-infected hamsters was 160 to 181 days; it was 65-75 days for 263K-infected hamsters, 155 to 196 days for 79A-inoculated animals, and 302 to 365 days for ME7-inoculated animals. It suggests the transmission speed or the toxicity of different scrapie isolates is different.

TME-infected hamsters

In hamster-infected with TME, retinopathy has been reported in the Hayward strain of the TME-infected hamsters (Buyukmihci et al., 1987a,b), but no retinopathy was reported in the Stetsonville source of TME-infected hamsters. However, there are two phenotypes of hamster-infected with the Stetsonville source of TME. They are called hyper (HY) and drowsy (DY). They are differed with respect to incubation period (disease duration, 65 versus 165 days), clinical signs (e.g. hyperactivity and ataxia versus progressive lethargy), PrPSc PK resistance, highly resistant versus slightly resistant, two types of PrPSc were observed, the unglycosylated band of DY has a molecular weight of 19 kDa, while HY show the same band at 21 kDa. While visual pathology was not reported, a waddling gait, kyphosis, and ptosis of the eyelids were often observed in DY hamsters, but not in HY hamsters (Bessen and Marsh, 1992; Bartz et al., 2000). Since the host hamsters PrP genotype is the same, it is possible that different PrPSc types play an important role on targeting of different brain regions, which shows different visual/ocular pathology in TME-infected hamsters.

Summary and conclusion

The study of prion diseases and prion-induced visual pathology is an important issue in many aspects of biomedical science. Prion diseases have become an important public health concern because of the causal relationship between animal TSE and human TSE. Prion diseases have also become an issue in the scientific field because of the novel biological aspects of the pathogens.

It is unknown how prion infective agents cross species barriers and infectivity barriers. This is important to discover so that transmission of animal TSEs to other TSE-free animal species can be prevented. Since the prion agent is very difficult to destroy, it is important to follow the regulations when performing TSE research and when dealing with animal and human prion diseases.

Also since it has been found that the eye and other organs of prion-infected animals contain prion agent and infectivity, care is needed when dealing with tears, urine, blood (Houston et al., 2000) and other body fluid from animal with prion disease.

It is still not clear the mechanism of retinopathological changes in the animal prion diseases. Prion agent is thought to be toxic and can damage to the neuronal cells, however, it is still not clear why some types of neuronal cells such as amacrine cells, and retinal interneurons are more resistant to prion toxicity. The mechanism of prion-induced visual (and oculomotor) pathology is still unknown. For a recent review of prion pathogenesis, readers can see the detailed review by Aguzzi and Heikenwalder (2006).

Why some prion-infected animals showed retina pathology but not the others is still not clear. Host species, PrP genotypes, PrPSc types, cellular types, all play a role in the prion-induced pathology. There are several ways the prion agent might be transported into and within the CNS, such as axonal transport, uptake...
and transport via migrating cells, or cell-to-cell transfer. They may involve both non-specific phagocytosis and pinocytosis and specific receptor-binding mechanisms. Therefore, microenvironment also play a role in prion toxicity.

Another issue is the function of normal prion protein in the retina and neurons related to the visual system. The importance of normal prion protein in retina cell function and survival is unclear as well as abnormal prion-induced pathological changes in the visual system. Questions that need to be answered include which cell types play a role in protection against prion agent and/or dissemination of agent in the eyes? What are the roles of Langerhans cells (LC), dendritic cells (DC), Müller cells, macrophages, astrocytes, microglia or pigment epithelium cells in prion-induced visual degeneration? Since prion-infected animals share similar eye and brain visual pathology as human prion diseases, they are the best models for the study of mechanism of visual /ocular pathophysiology in human prion diseases.

Method of Literature Search

Literature selection for this review was based on a Medline database search (up to February 28, 2009), using the following combination of keywords: prion, PrP, PrPSc, scrapie, bovine spongiform encephalopathy, BSE, chronic wasting disease, CWD, felines spongiform encephalopathy, FSE, TME, visual system, eye, retina, Creutzfeldt-Jakob disease, Creutzfeldt-Jakob disease, Inlandmann-Straussler-Scheinker disease, Heidenhain, fatal familial insomnia, FFI, kuru, optic, ophthalmic, transmissible spongiform encephalopathy, TSE and vision. Some of the literature was derived from the author’s personal files and files from Dr. R.I. Carp.
**Prion-induced visual pathology in animals**

Comp. Neurol. 205, 153-160.


Hanson R.P., Eckroade R.J., Marsh R.F., Zu Rhein G.M., Kanitz C.L.


Parchi P., Castellari R., Capellari S., Ghezzi B., Young K., Chen S.G.,...


