Feline Spongiform Encephalopathy: A study of the clinical presentation and pathology

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ProQuest LLC.
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106 – 1346

Abstract

Feline Spongiform Encephalopathy (FSE) was first reported in 1990 by Wyatt *et al.*, over three years after Bovine Spongiform Encephalopathy (BSE) was first identified. At present only limited published clinical and pathological descriptions of FSE are available. The aims of this thesis are therefore to review the current literature on FSE and describe the clinical and pathological features of the cases in this study.

The nine naturally occurring cases of FSE included in this thesis were presented to Glasgow University Veterinary School during the period of June 1990 to April 1998. A set of clinical criteria was identified allowing a presumptive ante-mortal diagnosis of FSE. These included: progressive behavioural changes, hyperaesthesia, ataxia, hypersalivation and intermittent pupillary dilatation, although not all where present in every case. No evidence of a breed or sex predilection, geographic clustering, reliable routine diagnostic test or successful treatment modality could be identified.

The nature and distribution of the pathological changes are very similar in all cases in this series, with the significant changes confined to the central nervous system (CNS). The changes include vacuolation of grey matter neuropil, vacuolation of individual neurones, Wallerian-type degeneration and a reactive astrocytosis. Neuropil vacuolation is widespread, but certain regions (including the cerebellar granule cell layer, deep cerebral layers, thalamus, basal nuclei and septal nuclei) appear to be preferentially affected. Vacuolation of individual neurones is selectively present in the dorsal nucleus of the vagal nerve and raphe nuclei, and occasionally present in the hypoglossal nucleus, vestibular nuclei and reticular formation. Characteristic patterns of pathological PrP accumulation in the CNS are evident on PrP immunocytochemistry, using monoclonal antibody 3F4 raised against hamster PrP.

Aspects of the spongiform changes and PrP immunocytochemistry in these cases bear strong similarities to new variant CJD (vCJD), although with some differences. These similarities are consistent with circumstantial evidence suggesting that both FSE and vCJD originated from ingestion of material contaminated with the infective agent responsible for BSE (Bruce *et al.* 1997; Collinge *et al.* 1996).

Dedication

This thesis is dedicated to:

Professor Ian Griffiths
with many thanks for all the patient input into this thesis

and

Carly
with love

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Declaration

I, Jacques Penderis, do hereby declare that the work carried out in this thesis is original, was carried out by myself or with due acknowledgement, and has not been presented for the award of a degree at any other University.

26 April 1998.

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1. INTRODUCTION

1.1 Review of Feline Spongiform Encephalopathy

1.1.1 History

The first case of feline spongiform encephalopathy (FSE) was that reported by Wyatt *et al.* (1990), over three years after BSE was identified. The cat, a five year old male neutered Siamese with a history of progressive ataxia and neurological signs, was presented to the University of Bristol Veterinary School. Attempted treatment did not result in any improvement and the cat was subsequently euthanased and a diagnosis of a spongiform encephalopathy was made, based on the histopathological appearance. A retrospective review of reports and tissue sections from cats presented with neurological disease was performed both at the University of Bristol Veterinary School and the University of Liverpool Veterinary School (Pearson *et al.* 1993). Cases going back to 1975 were reviewed and revealed no evidence of a similar disease. The only cases that had evidence of spongiform changes were two kittens with congenital spongiform changes of the white matter (Kelly and Gaskell 1976). FSE was, therefore, judged to be a novel disease.

Experimental infection of cats with FSE has been achieved, with the first successful transmission occurring as early as 1972 when a 34% transmission rate was reported on inoculation of cats with human brain suspension from patients with Creutzfeldt-Jakob Disease (CJD) (Amyx *et al.* 1983). In addition cats have been used as experimental models to study transmissible spongiform encephalopathies (TSE's) (Amyx *et al.* 1983; Gourmelon *et al.* 1987; Mitrova and Mayer 1977). The clinical signs of experimental and naturally occurring FSE are similar, although sleep pattern abnormalities were reported more frequently in the experimental cases in which only discrete to minimal spongiosis was detected microscopically.

Subsequent to its demonstration in domestic cats, FSE has also been reported in a number of captive exotic felidae (Willoughby *et al.* 1992; Kirkwood and Cunningham 1994a,b), all of which can be traced back to the UK.

1.1.2 Occurrence

1.1.2.1 Domestic Cats

FSE has been reported from all regions of the UK, including Northern Ireland and some of the smaller offshore islands. To the 1st of January 1998, 81 cases of FSE had been reported in the UK. In addition to the cases reported in the UK, single cases have been reported in Norway (Bratberg *et al.* 1995), Italy (Zanusso *et al* 1998), Liechtenstein and the Republic of Ireland. The case in Norway had been fed a variety of commercial, dry cat foods imported from the UK, but no other contact with the UK was evident, either directly or genetically.

Clinical details for the first 24 cases reported in the UK are available (Pearson *et al.* 1993), which includes one of the cases from Glasgow Veterinary School (Leggett *et al.* 1990). Additionally the clinical details of the FSE cases from Norway and Italy have been published (Bratberg *et al.* 1995; Zanusso *et al* 1998). The age and sex distribution of the 26 reported cases of FSE in domestic cats are shown in Table 1.

Based on the initial sex distribution of the 24 reported UK cases, a male predilection was suspected but the number of cats in the series was considered too small for significant conclusions to be made (Pearson *et al.* 1993).

The reported University of Bristol Veterinary School cases originated from a wide geographic distribution including Yorkshire, the Midlands, South Wales, the South West and Guernsey. More extensive clinical details for these 11 cases (Pearson *et al.* 1993) and for a number of the individual case reports (Bratberg *et al.* 1995; Leggett *et al.* 1990; Synge and Waters 1991; Zanusso *et al* 1998) are summarised in Table 2.

1.1.2.2 Captive Wild Felidae

Up to the 1st of January 1998, FSE has been reported in seven cheetahs (which includes one each in Australia, the Republic of Ireland and France) (Kirkwood and Cunningham 1994a,b; Peet and Curran 1992), three pumas (Willoughby *et al.* 1992), one tiger and two ocelots.

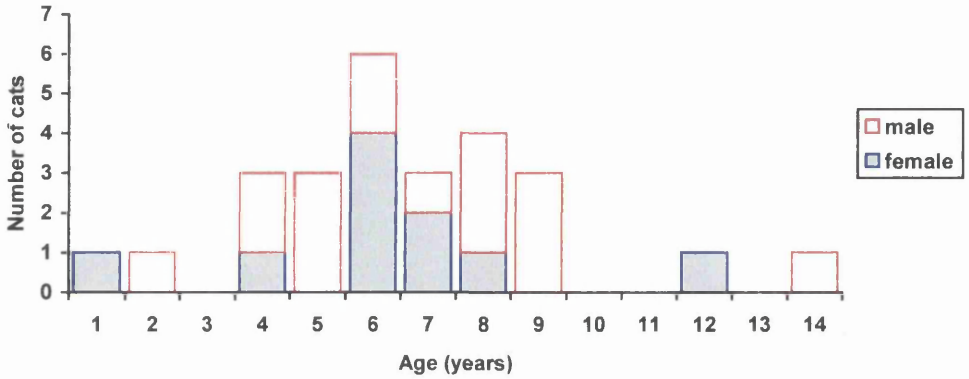


Table 1 Age and sex of the 26 published cases of FSE in domestic cats.

Case	Breed	Age (years)	Sex
Bristol: 1	Siamese	6	MN
2	Domestic shorthair	8	MN
3	Domestic shorthair	8	MN
4	Domestic shorthair	5	MN
5	Domestic shorthair	4	MN
6	Domestic longhair	5	MN
7	Domestic shorthair	10	FN
8	Domestic shorthair	8	MN
9	Domestic shorthair	2	MN
10	Persian	4	F
11	Domestic shorthair	9	MN
Glasgow	Domestic shorthair	7	FN
Shetland	Not stated	4	MN
Italy	Domestic shorthair	7	FN
Norway	Domestic shorthair	6	FN

Table 2 Available clinical details of published FSE cases in domestic cats.

A second tiger tested positive for FSE, but was not showing relevant clinical signs at the time of death. One of the Ocelots was presented to Glasgow Veterinary School. The three cheetahs, which developed FSE outside the UK, had all originally been exported from the UK where it is likely that they were exposed to the disease.

1.1.3 Clinical Presentation

1.1.3.1 Domestic Cats

In all except one of the reported cases (Bratberg *et al.* 1995; Gruffydd-Jones *et al.* 1991; Leggett *et al.* 1990, Pearson *et al.* 1993, Synge and Waters 1991) the clinical signs were characterised as being insidious in onset and slowly progressive. In the majority of cases, altered behaviour was the first clinical sign although some owners reported ataxia as the initial abnormality. The altered behaviour included timidity, manifested as fear of human contact or fear of going outside or aggression directed at the owners or other household pets. In addition to the altered behaviour, all the cases developed hind-limb ataxia, which progressed to involve the forelimbs in advanced cases. The ataxia was characterised by a rapid, crouching, hypermetric gait and an inability to judge distances. In some cases, a fine continuous tremor especially of the head and ears was noted.

Further signs observed in a variable number of cases included the presence of hyperaesthesia, either to auditory or tactile stimuli, dilated but responsive pupils, altered grooming, polyphagia and polydipsia, persistent or intermittent hypersalivation and head pressing.

In the case from Italy (Zanusso *et al* 1998) the reported clinical features differed slightly, with the onset being more rapid and initially characterised by the presence of frenzy, twitching of the body and hyperaesthesia. This was followed by the development of ataxia.

The clinical sings observed in the first 11 cases presented to the University of Bristol Veterinary School are summarised in Table 3.

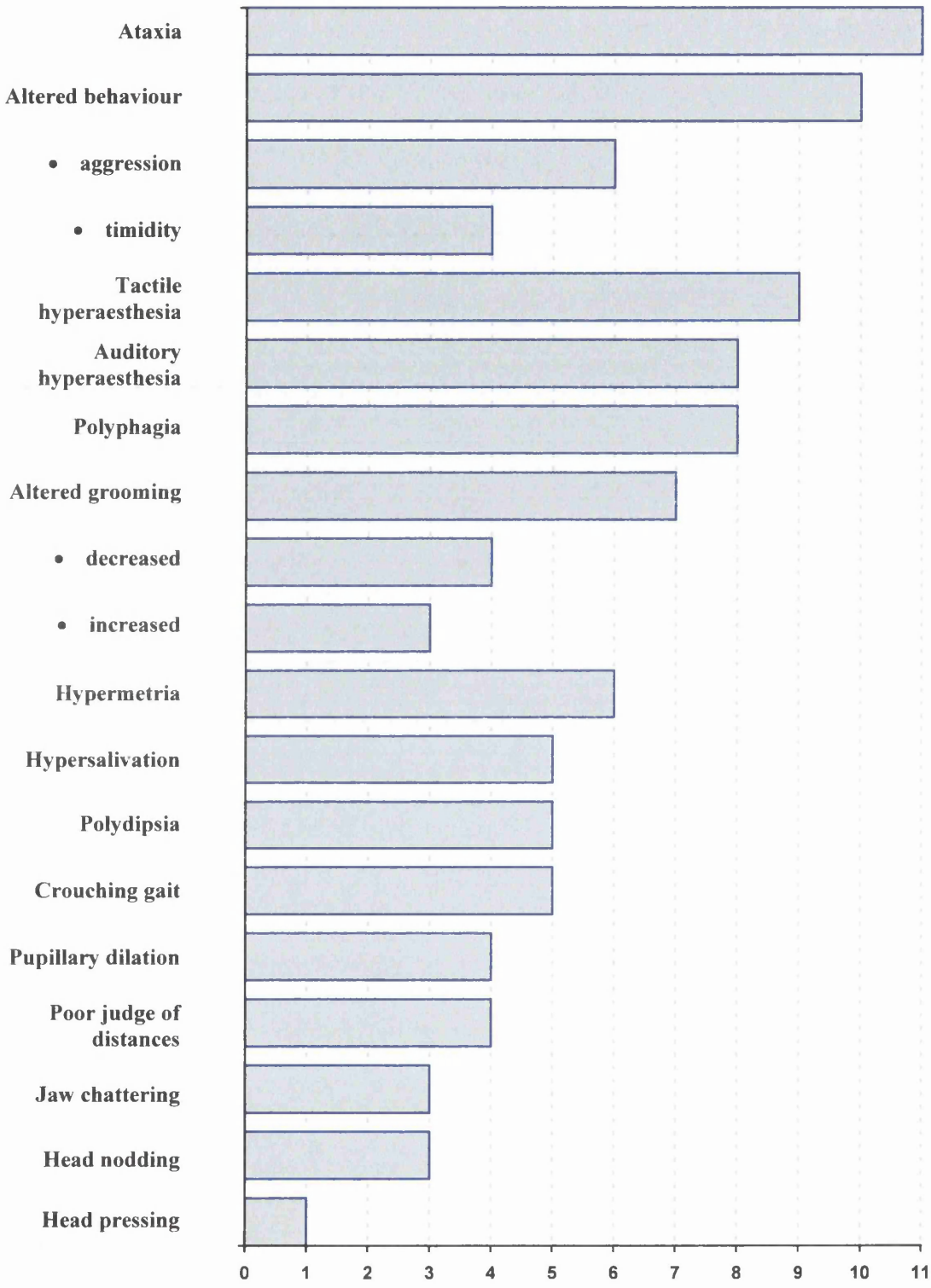


Table 3 Clinical signs observed in 11 cases of FSE (Pearson *et al.* 1993)

1.1.3.2 Captive Wild Felidae

FSE in captive wild felidae is characterised by the development of ataxia and progression of the clinical signs over a three-week to eight-week period (Bradley 1997). In addition to ataxia, the cheetahs exhibited variable signs including hyperaesthesia, weight loss, falling and muscle spasms (Kirkwood and Cunningham 1994), while the puma showed apprehension, difficulty balancing and a fine, whole-body tremor (Willoughby *et al.* 1992).

1.1.4 Pathology

No laboratory methods are currently available for the antemortem diagnosis of the TSE's and therefore the principle method of diagnosis relies upon the histological examination of the brain.

Histopathological changes in the TSE's are confined to the central nervous system and include vacuolation of the neuronal perikarya and neurites, neuronal degeneration and loss, gliosis (principally an astrocytosis) and amyloidosis (Wells and McGill 1992). Primary degenerative changes of the white matter, unrelated to the neuronal degeneration and loss, have not been recognised in the naturally occurring TSE's of animal species but have been described experimentally in mice. The vacuolation of the neurites gives the appearance of a spongiform change within the grey matter neuropil. The vacuolation of the neuronal perikarya and spongiform changes characteristically have a bilaterally symmetrical distribution and the pattern of these changes appears to be strongly conserved where the same prion strain and the same species are involved. The appearance and distribution of the neuronal perikaryon vacuolation and the spongiform changes can be regarded as pathognomonic (Wells and McGill 1992).

The neuronal loss in the animal TSE's may not be obvious in every case and the vacuolated perikarya may appear otherwise normal. The astrocytosis has been regarded as either a purely secondary phenomenon or one that is closely associated with agent replication. Amyloidosis has been described in all TSE's occurring in animal, apart from transmissible mink encephalopathy and feline spongiform encephalopathy, although in other species it is not a constant feature.

1.1.4.1 Domestic Cats

In none of the reported cases of FSE were any significant gross abnormalities visible at autopsy. The reported histological findings in all the domestic cats with FSE are similar and characterised primarily by vacuolation of the grey matter. The authors of the case report from Italy (Zanusso *et al* 1998) suggested that the neuropathological changes in their case was at variance with other reported cases of FSE, but did not publish a detailed neuropathological description.

In comparison to other TSE's in animals, with particular reference to Bovine Spongiform Encephalopathy (BSE), FSE has been reported to have severe vacuolar changes accompanied by an intense astrocytic and microglial reaction (Wells and McGill 1992). The vacuolar changes in FSE appear to have a pattern of distribution different from that of BSE with the cerebral cortex, corpus striatum, thalamus and medial geniculate body more prominently and consistently involved (Wyatt *et al.* 1991).

In the review of the first 24 cases of FSE in the UK, Wyatt *et al.* (1991) report that the vacuolar changes of the grey matter consisted of two types: spongiform change and vacuolation of the perikarya of neurons.

The predominant type of grey matter vacuolar change was spongiform change, which was evident as small, discrete spaces with no visible content in the grey matter neuropil. The spongiform changes were present through the central nervous system, but were consistently more evident in certain areas which included the medial geniculate body, the corpus striatum and the thalamus. In addition to these areas, in most cases the cerebellum and the deeper layers of the cerebral hemispheres were moderately severely affected.

Vacuolation of neuronal perikarya was evident as large, single or multiple vacuoles with a clearly defined margin. The predilection areas for this type of change were the raphe nucleus, the dorsal nucleus of the vagus nerve, the vestibular nuclei and the red nucleus. Occasional vacuolation of the neuronal soma was reported in the spinal cord with other neuronal changes being infrequent.

In all cases, a glial reaction (predominantly of astrocytes, but also microglia) was present. In some instances vacuolation and axonal degeneration was present in the white matter, particularly in the pyramidal tracts.

Fresh brain tissue from five cases of FSE was examined for the presence of scrapie-associated fibrils and PrP^{Sc} (protease-resistant disease related isoform of prion protein) (Pearson *et al.* 1992), which can be considered as molecular markers confirming the presence of a TSE. The PrP^{Sc} is extracted from the brain tissue by detergent followed by protease treatment, resulting in a protease-resistant core in which scrapie-associated fibrils can be demonstrated by negative stain electron microscopy. In all five cases of FSE, the brain tissue was positive for the presence of scrapie-associated fibrils and PrP^{Sc}. In addition, one other cat with neurological signs, but no histological evidence of spongiform changes, was positive for scrapie-associated fibrils and PrP^{Sc}. It was suggested that this was a pre-clinical case of FSE. Scrapie-associated fibrils in the absence of PrP^{Sc} were demonstrated in two other cats, one with neurological signs and a histologically confirmed meningioma and a second with no neurological signs or histopathological brain abnormalities.

In the single case report from Italy the authors support their claim of a new variant of FSE on the basis of the differing clinical presentation, the uncharacteristic neuropathology and the presence of type-1 PrP^{Sc}, rather than the expected BSE-associated type-4 PrP^{Sc}.

1.1.4.2 Captive Wild Felidae

Pathological findings have been published for one of the cheetahs (Peet and Curran 1992) and one of the pumas (Willoughby *et al.* 1992) which developed FSE.

The central nervous system of the cheetah was characterised by the presence of severe spongiform changes in the grey matter, especially in the corpus striatum, thalamic regions and mid-brain. The spongiform changes were characterised by the presence of small ovoid spaces in the neuropil and vacuolated neurons, similar to those described by Wyatt *et al.* (1991). No evidence of spongiform changes or vacuolated neurons was seen in the grey matter of the spinal cord. In the cheetah there was extensive axonal degeneration and demyelination of all spinal cord tracts, with the changes extending up the pyramidal and other white matter tracts of the medulla as far rostral as the internal capsule region. Macrophages were visible in some of the empty myelin cylinders of the spinal cord white matter.

Post mortem evaluation of the puma did not reveal any significant gross abnormalities, but histopathology demonstrated abnormalities throughout the neuroaxis. The spongiform changes were characterised by single or multiple large

vacuoles, some of which contained floccular or globular eosinophilic material, in neuronal perikarya and multiple smaller, round, empty vacuoles in the grey matter neuropil. The vacuolation of the neuropil was most marked in the caudal colliculus and the granular and molecular layers of the cerebellar cortex. In the spinal cord there was some evidence of Wallerian type degeneration with an associated astrocytosis. Astrocytes and microglia were present at high concentrations in the grey matter neuropil of the brain, but due to the absence of species controls the degree of gliosis could not be quantified. Further changes included occasional microglial nodules and occasional perivascular lymphocytes in the neuropil and meninges.

PrP (prion protein) immunostaining was performed on sections from the medulla and spinal cord of the puma. The PrP deposition was evident as granular deposits in the neuronal perikarya and grey matter neuropil. The granular PrP immunostaining in the perikarya appeared localised discretely in the cytoplasm in both vacuolated and non-vacuolated neurons of the affected grey matter areas. The immunostaining in the grey matter neuropil was either scattered or occasionally arranged in linear arrays suggestive of deposition along cell processes. The most intense immunostaining was present in the reticular formation, vestibular nuclei, nucleus ambiguus and the inferior olives. Occasional ventral horn neurons in the spinal cord showed intense granular immunostaining.

1.1.5 Epidemiology

1.1.5.1 Domestic Cats

Although there are at present insufficient cases of FSE with adequate histories to allow accurate analysis of the epidemiology of FSE, there is strong circumstantial evidence to suggest a link with BSE. This link is likely to be either through a common origin or from cats being exposed to BSE infected tissues or a product of BSE infected tissues. Certainly the temporal and geographic occurrence of FSE suggests an origin from BSE rather than scrapie (Bradley 1997). The weight of evidence suggests that the source of infection in cats is likely to be related to the feeding of BSE-contaminated feed. The quantification of the relative risks of different components of the diet of domestic cats has not been possible as the feed intake of these cats tends to be much more variable as a result of their free ranging nature. A number of studies have demonstrated a link between the agent causing

BSE, new variant CJD (vCJD), FSE and the TSE's of exotic ungulates in the UK. This agent appears to be distinct from that causing iatrogenic and sporadic CJD. One study included evaluation of two dairy farmers with sporadic CJD, who had recorded cases of BSE in their cattle herds (Collinge *et al.* 1996).

Transmission of FSE to mice (Fraser *et al.* 1994) has demonstrated similar incubation periods, lesion profile and percentage of affected mice as that of BSE but distinct from that of scrapie. The authors concluded that FSE and BSE are likely to have arisen from a similar source.

Western blots of PrP^{Sc} after proteolytic cleavage, in the sporadic and iatrogenic forms of CJD, allow differentiation between the type-1, type-2 and type-3 forms by evaluation of different band sizes. Type-4 CJD, or vCJD, while having similar band sizes to type-3 CJD, can be clearly distinguished from the other three forms of CJD by a characteristic pattern of band intensities (Collinge *et al.* 1996; Aguzzi and Weissmann 1996). Similar western blot analysis of BSE, naturally occurring FSE and experimental BSE in a macaque revealed an almost identical pattern of band intensities, which was distinct from that of type-1, type-2 and type-3 CJD. From this it was concluded that vCJD, BSE and FSE are all due to infection from a common source, with the same strain of PrP^{Sc}.

Differentiation between the different strains of agent which cause the TSE's can be achieved by the disease characteristics they cause in experimentally infected animals, in particular, the incubation period and neuropathology (including the lesion profile of spongiform changes and distribution of PrP^{Sc} within the central nervous system). A study by Bruce *et al.* (1997) demonstrated striking similarities in the lesion profile for RIII mice after transmission into panels of these mice of new variant CJD from three cases, BSE from four cattle, FSE from two cats and a spongiform encephalopathy from a nyala and a kudu. This lesion profile was distinct from that generated by transmission into panels of these mice of scrapie from six sheep and sporadic CJD from two cases. As a further part of the same study, which is still on-going, a strong correlation has been demonstrated between the incubation periods in four strains of mice after transmission of vCJD from three sources, BSE from eight sources and FSE from two sources. This correlation is again distinct from that obtained by transmission of scrapie from six sources into the same panel of mice. The authors concluded that it is likely that the same agent strain is responsible for vCJD, BSE, FSE and the TSE in captive exotic ungulates in the UK.

In the single case report from Italy (Zanusso *et al* 1998) the authors suggest that this case of FSE differs from other reported cases. They suggest that the source may be either an example of horizontal transmission from the owner (who was diagnosed as suffering from the sporadic form of CJD), infection from an unknown source or the chance occurrence of sporadic FSE in a cat. As evidence of this they described a differing clinical presentation, uncharacteristic neuropathology and the presence of type-1 PrP^{Sc}, rather than the expected BSE-associated type-4 PrP^{Sc}.

1.1.5.2 Captive Wild Felidae

Due to the controlled environment and feeding history of the captive wild felidae the probable source of infection can be more clearly defined. The documented diet of the captive puma with FSE (Willoughby *et al.*, 1992) consisted of chicken and rabbit carcasses and parts of cattle carcasses fed fresh. The cattle material all came from a single supplier and consisted of meat on the bone including split spinal columns, but not offal as the puma appeared to find these unpalatable. The puma additionally occasionally caught and ate wild pigeons, rodents and insects and on one occasion (three months before the development of clinical disease) was given meat from a cull of two eland from within the collection.

Dietary details are available from three of the first four cases of FSE reported in cheetahs (Kirkwood and Cunningham 1994). Two were fed horse meat and cattle parts, including entire or split spinal columns, as part of their diet. The third case was occasionally fed entire stillborn calves in addition to a variety of cattle and horse meat, although no spinal columns from adult animals were included in this cheetah's diet. It is interesting to note that the cheetah that was exported to Australia and subsequently developed FSE left the UK 26 months prior to the first clinical signs attributable to FSE (Peet and Curran 1992). This would imply that if that cheetah had contracted the disease in the UK, as is believed, then the incubation period must have been at least 26 months.

The first two cheetahs to develop clinical signs of FSE did so in 1992, with the third and fourth cases occurring in 1993. The captive adult puma described by Willoughby *et al.* (1992) developed clinical signs in 1991. Numbers of species and individual animals kept by the National Federation of Zoological Gardens of Great Britain and Ireland are available for the 31st of December 1989 (Bennett 1990). At that time (Kirkwood and Cunningham 1994) there were 23 different species of Felidae being

kept, including populations of 16 pumas (*Felis concolour*) and 42 cheetahs (*Acinonyx jubatus*).

1.1.6 Control

The control methods suggested assume that FSE originates from a feed source and that no other origin of the disease exists. The basis of disease control involves implementing an effective ban on the entry of specified bovine offal into cat feed, or by ensuring adequate processing of potentially infected bovine tissues before use in cat feed. The carcasses of affected cats should be disposed of safely, preferably by incineration, and there should be no risk of their entering the food chain. Bradley reported in 1997 that so far no case of FSE had been reported in a cat born after the imposition of the specified bovine offal ban in September 1990. It is now widely believed that an effective ban on the incorporation of specified bovine offal to any species of mammal or birds was not instituted until after the recognition of new variant CJD in 1996 (personal communication, Williams 1998).

Control of TSE's in zoos in the UK and the Republic of Ireland has been attempted by informing all the said zoos of the occurrence, clinical sings and pathological findings in exotic species. Zoo authorities have been made aware of the potential risks in exporting animals and attempting to release animals back into the wild.

1.2 Review of Transmissible Spongiform Encephalopathies in Animals

1.2.1 Scrapie

Scrapie was the first transmissible spongiform encephalopathy to be reported in animals, having been described in England as early as 1732. It affects sheep and goats (although less common in goats) (Harcourt and Anderson 1974), and in the UK has been reported to occur in moufflon (*Ovis musimon*), a primitive breed of sheep (Wood *et al.* 1992). It has been diagnosed in all of the major sheep raising areas of the world except Central America and the Caribbean, although it has successfully been eradicated from Australia, New Zealand and possibly some European countries (Bradley 1997).

1.2.1.1 Epidemiology

The epidemiology of scrapie appears to be more complex than that of the other transmissible spongiform encephalopathies. This is in part due to the sheep *sip* gene, which plays an important role in determining susceptibility, and partly due to the apparent absence of a dietary origin. Investigations into transmission of A-group and C-group strains of scrapie have demonstrated that the polymorphisms of the two domains of the PrP gene, codons 136 (valine versus alanine) and 171 (arganine versus glutamine), determine the relative susceptibility to different scrapie strains. These susceptibility effects reverse between A-group and C-group strains of scrapie (Bradley 1997).

The demonstration of susceptibility effects is of significance as they allow selection of sheep with low susceptibility for the scrapie agent (Dawson *et al.* 1998). That these susceptibility effects reverse, depending on whether the scrapie strain is of the A-group of C-group is important, as the field scrapie strain has to be determined prior to selection. Additionally, if a ram, with the opposite PrP gene polymorphisms, is introduced into a flock resistant to the local strain of scrapie, offspring susceptible to the local strain of scrapie will be bred. If these offspring are kept for breeding stock an apparent outbreak of scrapie will occur. This explains outbreaks of scrapie in an apparently scrapie-free flock where a new ram, itself from a scrapie-free flock, was introduced.

Infectivity has been demonstrated in the placenta (Pattison *et al.* 1974), CNS and lymphoreticular systems, but no other tissues have been demonstrated to be infective, including semen, skin and a variety of other bodily fluids and tissues (Hadlow *et al.* 1982). Once a scrapie-infected sheep has been introduced into a flock there is rapid horizontal spread and evidence of vertical spread. The source of the horizontal transmission may be through eating infective placentas and contamination of pastures with the infective agent (through contact with infected placentas).

1.2.1.2 Clinical presentation

The clinical signs associated with scrapie are variable and animals do not necessarily display all the clinical signs (Clark and Moar 1992). Scrapie may cause sudden death, without preceding clinical signs (Clark *et al.* 1994). The disease is characterised by an insidious onset, with gradual progression, resulting in death a few weeks to months after the first clinical signs were noticed.

The first clinical sign to be noticed is altered behaviour. Affected sheep will tend to lag behind or walk ahead of the flock and may not respond normally to sheep dogs. Pruritis, from where the disease's name is derived, is the main feature of the disease once it is fully established, although this is not a consistent finding. The pruritis manifests as scratching against objects, biting and in horned breeds, using their horns to scratch their flanks and backs (Linnabary *et al.* 1991). Ataxia, hypermetria and hyperaesthesia may also be present (Bradley 1997). Decreased rumination has been reported, although feeding is normal and the majority of sheep display polydipsia and polyuria (Austin and Simmons 1993).

1.2.1.3 Pathology

Scrapie displays all the classical features of a transmissible spongiform encephalopathy with vacuolation of the neuronal cytoplasm and grey matter neuropil, marked astrocytosis, neuronal cell loss and amyloidosis. Particularly apparent is the neuronal cytoplasmic vacuolation, particularly in the mid-brain, pons, medulla and ventral and lateral grey matter of the spinal cord (Bradley 1997). Amyloid plaques were present in over 50% of cases (Gilmour *et al.* 1985). In a review of brains, affected by sheep scrapie, submitted to the Central veterinary Laboratory in Weybridge, certain breed specific patterns of disease were apparent.

1.2.2 Bovine Spongiform Encephalopathy

BSE was first reported in 1987 in the UK as a novel disease (Wells *et al* 1987), with the first identified case dating back to April 1995. Following this initial report increasing numbers of BSE cases were reported resulting in epidemiological studies commencing in 1987. Cases in the UK peaked in 1992 with 36682 confirmed cases in that year. As of the 13th of February 1998 a total of 170555 confirmed cases have been recorded, since the start of the BSE epidemic. In addition to the UK, BSE has been reported in a variety of European countries, with (as at the 1st of February) the highest number of cases in the Republic of Ireland (275 cases), Switzerland (272 cases), Portugal (93 cases) and France (33 cases).

1.2.2.1 Epidemiology

Epidemiological studies have demonstrated strong circumstantial evidence that the BSE epidemic has arisen through feed contaminated with the infective agent. It was originally thought that the prion protein strain responsible for the BSE epidemic was as a result of one of the thermostable scrapie strains becoming pathogenic for cattle. The recycling of cattle and sheep carcasses into cattle feed in the form of meat and bone meal led to the perpetuation of the epidemic.

A possibility is that the BSE agent may originally have arisen from a single case of sporadic BSE (similar to sporadic CJD in humans), that subsequently found its way into the cattle feed chain as meat and bone meal. Supporting this is that the BSE strain does not resemble the 20 scrapie strains to which it has so far been compared, although there is still a possibility that BSE may have arisen from a less common, but more thermostable, scrapie strain (Bruce *et al* 1997). The change in the method of processing of the meat and bone meal immediately preceding the BSE epidemic was originally suspected to be the cause of the epidemic, which may have allowed a more thermostable strain of scrapie to survive at an infective level. This theory has been refuted (Taylor 1996) with no demonstrable difference in the level of infectivity of feed samples derived from different processing methods.

Transmission studies have demonstrated that oral transmission of the BSE agent is feasible and the experimental host range of the BSE agent via the oral route includes sheep, goats, mice, mink (Bradley 1997) and calves (Matthews 1996). Experimental transmission via the parenteral route has been demonstrated in cattle, sheep, goats, pigs, mice, marmosets and mink, but not in hamsters and chickens (Bradley 1994;

1997; Dawson *et al.* 1990). Studies (Bruce *et al.* 1997; Collinge *et al.* 1996) have demonstrated that the agent strain responsible for BSE is similar to that causing new variant CJD, FSE and the spongiform encephalopathies of captive exotic ungulates in UK zoos, and distinct from agent strains resulting in sporadic CJD and of the scrapie strains tested to date.

Identification of the BSE epidemic and the linkage to the feeding of meat and bone meal led to the introduction of the ruminant feed ban of July 1988 in the UK. By 1996, 27000 cases of BSE, born after the introduction of the feed ban, had been identified in the UK (Matthews 1996). Some of these cases can be ascribed to the continued feeding of meat and bone meal, which had been stockpiled before the ban was introduced. Identification of cross contamination of ruminant feed in feed mills with meat and bone meal destined for poultry and pigs led to increased controls being introduced in 1995 and 1996. This included the introduction of a complete ban on the use of mammalian meat and bone meal on farms and for feeding food producing animals, horses and farmed fish. As a result of the control measures, the number of confirmed cases has dropped from 2000 a week at the height of the epidemic to below 200 a week at present.

1.2.2.2 Clinical presentation

BSE is a progressive disorder which eventually results in extreme loss of condition, paraplegia and is invariable fatal. Following the widespread knowledge about BSE, the disease is now usually identified early on and affected animals are euthanased before displaying advanced clinical signs. The clinical features associated with BSE (Wilesmith *et al.* 1992) include the development of altered behaviour, altered sensation, bradycardia (Austin *et al.* 1996), decreased rumination (Austin and Simmons 1993) and postural abnormalities. The clinical features are often exacerbated by stress, explaining the higher incidence associated with calving and management changes.

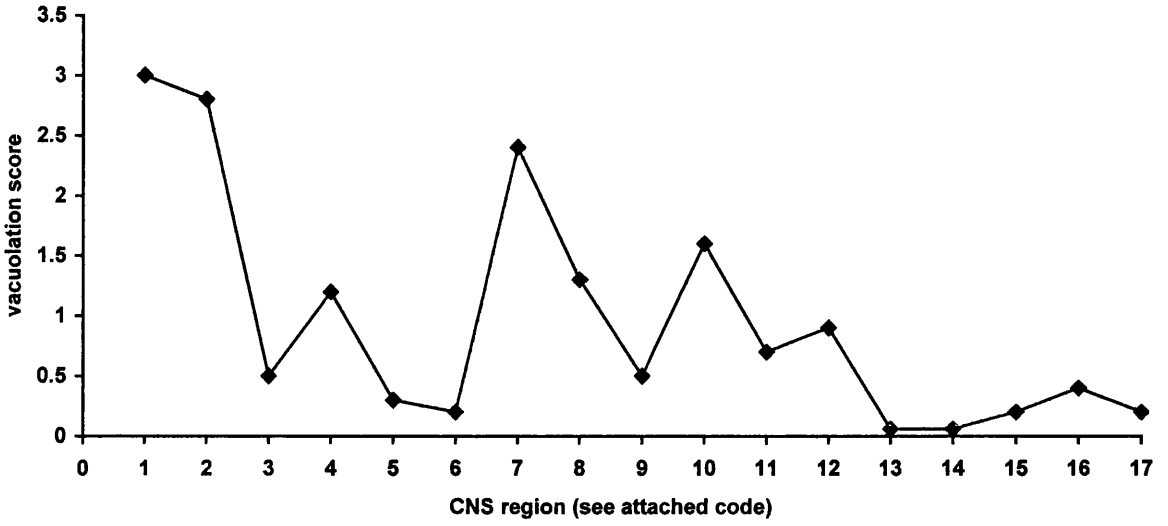
Not all the clinical features are present in every case. The behavioural changes include the development of frenzy, nervousness of entrances, teeth grinding, abnormal ear position, inappropriate behaviour, altered temperament and apprehension. Altered sensation is apparent as blindness, head pressing or rubbing, head shyness, excessive licking, kicking in the milking parlour and hyperaesthesia. The postural abnormalities

that may be apparent include ataxia, paresis, tremors, abnormal head carriage, falling, recumbency, knuckling on the fetlocks and circling.

1.2.2.3 Pathology

The pathology associated with BSE is characteristic of the transmissible spongiform encephalopathies and includes vacuolation of the grey matter neuropil, single or multiple vacuoles in the neuronal perikarya (Wilesmith and Wells 1991), astrocytosis seen as astrocyte hyperplasia (Wells *et al.* 1991), neuronal cell loss and the presence of PrP^{Sc}. The neuronal cell loss is most noticeable in the vestibular cell complex (Jeffrey 1992). The single strain hypothesis has been supported by the extremely consistent distribution of the spongiform changes seen throughout the BSE epidemic (Bruce *et al.* 1994; Simmons *et al.* 1996). This consistency of distribution of the spongiform changes allows reliable diagnosis of BSE on a single section of the medulla through the obex (Wells *et al.* 1989) and a suitable sample can be collected through the foramen magnum, therefore reducing costs and improving safety.

Lesion profiles performed on at least a thousand random BSE cases taken at two different time-periods during the BSE epidemic have been published (Simmons *et al.* 1996). A summary of these findings is included in Figure 1.



CNS regions:

1. nucleus of solitary tract
2. nucleus of spinal tract of Cn. V
3. hypoglossal nucleus
4. vestibular nuclei
5. cochlear nucleus
6. vermis of cerebellum
7. central (periaqueductal) grey matter
8. rostral colliculus
9. medial geniculate nucleus
10. hypothalamus
11. dorsomedial thalamic nucleus
12. ventrolateral thalamic nucleus
13. frontal cortex
14. septal nuclei
15. caudate nucleus
16. putamen
17. claustrum

Figure 1 BSE lesion profile in domestic cattle in the UK (adapted from Simmons *et al.* 1996)

1.2.3 Spongiform Encephalopathies of Exotic Ungulates in the UK

Associated with the BSE epidemic, spongiform encephalopathies have been diagnosed in a number of captive exotic Bovidae kept in British zoos. As at the 1st of January 1998, six kudu, one gemsbok, one nyala, two oryx and six eland have been diagnosed with spongiform encephalopathies. Epidemiological and transmission studies are consistent with the spongiform encephalopathy in these animals being related to the BSE epidemic.

1.2.3.1 Epidemiology

The source of the infectious agent in these cases is likely to be through contaminated feed, although in one case (Kirkwood *et al.* 1992) there was no evidence of an affected greater kudu having direct exposure to contaminated feed, although this animal's dam had died of a spongiform encephalopathy. Epidemiological analyses have demonstrated a link with BSE. Firstly the temporal and geographic occurrence is identical to that of BSE. Secondly in all the affected animals where details are available there was either direct exposure to potentially contaminated feed, or the affected animal was born into a herd which had been exposed to potentially contaminated material (Kirkwood and Cunningham 1994*a*). Thirdly transmission of brain homogenate from one affected kudu and the affected nyala into mice resulted in a similar pattern of neuropathological changes to that of BSE (Bruce *et al.* 1997). Based on the suspected dietary origin of the disease, control in the EU has been instituted under the ban on feeding mammalian protein to ruminant animals.

1.2.3.2 Clinical presentation

The disease course in all the affected animals was reasonably rapid, varying from seven to fifty-six days. Two of the kudu were culled for managemental reasons before they were demonstrating any clinical signs and a diagnosis of a spongiform encephalopathy was made on post-mortem examination. The clinical presentation varied in the affected species, from episodic collapse in the gemsbok, to more classical sings of dullness, drooling, muscle tremor, hypermetria and ataxia. Not all the kudu demonstrated all the clinical signs, but all of the clinically affected kudu had the presence of a head tilt (Bradley 1997; Kirkwood and Cunningham 1994*b*).

1.2.3.3 Pathology

In all the spongiform encephalopathies of captive wild Bovidae, the pathology resembles that of BSE. In the nyala (Jeffrey and Wells 1988) the vacuolation of the neuropil was most severe in the medulla, diminishing rostrally and caudally, although changes were present as far cranial as the hippocampus. In particular, the dorsal nucleus of the vagus nerve and nucleus of the spinal tract of the trigeminal nerve were severely affected. Cytoplasmic neuronal vacuolation was demonstrated in the dorsal nucleus of the vagus nerve. No astrocytosis and no amyloid plaques were demonstrated. The neuropathology in the other species was similar, although the severity of the changes in the brain stem of the oryx was less severe (Bradley 1997).

1.2.4 Spontaneous Spongiform Encephalopathies of Captive Primates

Spongiform encephalopathies have been diagnosed in a two lemurian zoo primates (*Eulemur fulvus mayottensis*) (Bons *et al.* 1997) and three rhesus monkeys (*Macaca mulatta*) (Bons *et al.* 1996). In all cases the affected animals were maintained on a diet containing animal protein (including a bovine source), which would suggest that the spongiform encephalopathy in these animals is related to the BSE epidemic.

1.2.4.1 Epidemiology

The source of the infectious agent in these cases is likely to be through contaminated feed, although no confirmatory epidemiological evidence has been published. All of the affected animals were maintained on a diet containing animal protein. The three rhesus monkeys came from the same zoo and all the cases originated from zoological gardens in France.

1.2.4.2 Clinical presentation

The clinical presentation in both species was characterised by the development of dramatic neurological signs. The affected rhesus monkeys presented with a progressive neurological disorder with behavioural abnormalities. The affected individuals deteriorated physically and died.

1.2.4.3 Pathology

A detailed pathological examination was performed on one rhesus monkey with the reported changes similar to those seen in monkeys following experimental transmission of CJD. The changes were characterised by an astrocytosis and

vacuolation of neuronal cell bodies and processes. The disease-related isoform of PrP, PrP^{Sc}, was demonstrated by immunocytochemistry.

In the lemurians, microscopy of the brain revealed vacuolation of neurones with demonstration of the disease related isoform of PrP by immunocytochemistry. In the lemurians, in addition to the demonstration of PrP^{Sc} in the brain, prion immunoreactivity was demonstrated in the tonsils, gastrointestinal epithelial cells and blood and lymph vessels.

1.2.5 Transmissible Mink Encephalopathy

Transmissible mink encephalopathy (TME) is a spongiform encephalopathy affecting ranch-raised, adult mink. The disease was first described in 1947 in the USA and has since been described in Finland, Germany and the former Soviet Union (Summers *et al.* 1995).

1.2.5.1 Epidemiology

Scrapie-contaminated products of sheep and goats are though to be the source of infection in transmissible mink encephalopathy, although this has not definitely been confirmed. In one reported outbreak of TME (Marsh *et al.* 1991), the mink on the affected ranch were fed only on food derived from cattle sources, predominantly from sick and downer cows, in the absence of any sheep or goat-derived feed products. Transmission studies in this outbreak demonstrated that the disease could be transmitted from the mink to bull calves by intracerebral injection, and back into mink, raising the question whether there is a sub-clinical spongiform encephalopathy in cattle in the USA. Experimental inoculation of central nervous system (CNS) tissue from transmissible mink encephalopathy cases into goats and various strains of mice (Barlow and Rennie 1970) resulted in the development of a disease that was clinically and pathologically indistinguishable from Scrapie. Transmission of TME into hamsters has lead to the identification of two distinct strains of the causative agent, namely hyper (HY) and drowsy (DY), based on biological properties (Bessen and Marsh 1992). The introduction of infection into a group of mink is likely to be through contaminated feed, but whether the route of infection is through oral ingestion or intradermal inoculation as a result of fighting is unclear. Additionally the role of cannibalism in the spread of the disease is not clear.

1.2.5.2 Clinical presentation

Based on experimental and epidemiological information the incubation period has been determined to be in the region of eight to twelve months (Summers *et al.* 1995). The disease is characterised by a relatively short clinical course of a few weeks, although this can vary. Initially the mink display behavioural abnormalities, including the development of aggression, hyperexcitability and hyperaesthesia as well as inappropriate defecation and feeding patterns, resulting in soiling of their coats and loss of condition. The behavioural abnormalities are initially relatively subtle and are followed by the development of progressive ataxia, debilitation, somnolence and eventually death. Blindness in the terminal stages is common. In some cases self-mutilation occurs and this may be severe enough to result in death due to blood loss (Bradley 1997).

1.2.5.3 Pathology

Pathologically the appearance is that of a classical spongiform encephalopathy with bilaterally symmetrical grey matter spongiform changes and an astrocytosis. The most marked changes are found in the prosencephalon, particularly in the gyri of the frontal lobes, the amygdaloid nucleus, corpus striatum and thalamus. The spinal cord and cerebellum are usually spared (Bradley 1997; Marsh and Hadlow 1992).

1.2.6 Chronic Wasting Disease

Chronic wasting disease is a transmissible spongiform encephalopathy which has been reported to affect mule deer (*Odocoileus hemionus hemionus*), Rocky Mountain elk (*Cervus elaphus nelsoni*), black tailed deer (*Odocoileus hemionus columbianus*) and hybrids of captive mule deer and white-tailed deer (*Odocoileus virginianus*) in wildlife facilities in Colorado and Wyoming (Williams and Young 1980; 1982). The disease was first recognised in 1967 in captive mule deer.

1.2.6.1 Epidemiology

Epidemiological analyses of data from facilities for captive deer and elk in Wyoming and Colorado have demonstrated no evidence that chronic wasting disease is related to a dietary origin. The disease is though to be spread via horizontal contact. Vertical transmission may play a role, but does not explain the cross species transmission that has been reported. Experimentally, the disease has been successfully transmitted to

other deer and ferrets, but the primary source of the disease is not known (Williams and Young 1982; 1992; Spraker *et al.* 1997).

1.2.6.2 Clinical presentation

The disease in both deer and elk is characterised by being chronic and progressive over a period of several months, with affected animals usually in the 3 to 5 year age range (Summers *et al.* 1995). In both deer and elk the clinical signs are characterised by weight loss, teeth grinding, behaviour abnormalities (including decreased association with other animals in the same group and keepers, blank appearance and increased somnolence), excessive salivation and is invariably fatal. In deer, additional clinical signs that are evident include polyuria and an underlying polydipsia, difficulty swallowing, oesophageal dilation, regurgitation and aspiration pneumonia (usually occurring terminally). In elk, in addition to the clinical signs discussed above, hyperaesthesia and hyperexcitability are common and hind limb ataxia is occasionally seen (Bradley 1997).

1.2.6.3 Pathology

The pathological distribution is similar to that of scrapie and BSE, with spongiform changes of the neuropil, single or multiple intracytoplasmic vacuoles in the neuronal perikarya and associated astrocyte hyperplasia and hypertrophy (Guiroy *et al.* 1993). The spongiform changes of the neuropil are especially evident in the spinal cord grey matter, medulla oblongata, pons, mesencephalon, thalamus, hypothalamus, cerebral cortex and cerebellar cortex. In some cases amyloid plaques have been demonstrated in association with the other CNS lesions (Guiroy *et al.* 1991*a, b*). These amyloid plaques are readily visible in deer on routine histopathological examination, but in elk are only demonstrable by PrP immunocytochemistry.

1.3 Review of Transmissible Spongiform Encephalopathies in Humans

A number of human varieties of TSE's have been described, including Kuru, CJD, Gerstmann-Sträussler-Scheinker Disease (GSSD or GSS), Fatal Familial Insomnia (FFI) and atypical prion dementias. The human TSE's can be divided into three categories: inherited, acquired and sporadic forms. Recently, a new form of CJD, vCJD, has been identified with an age of onset, pathology and clinical picture distinct from the other forms of CJD. There is strong evidence linking vCJD to the recent BSE epidemic.

1.3.1 Inherited TSE's

The development of molecular genetics has identified specific mutations associated with the inherited TSE's, allowing molecular classification of the different syndromes in addition to the traditional phenotypic distinctions. These diseases are now preferentially referred to as inherited prion diseases. Furthermore, the recognition of specific mutations has allowed classification of phenotypes that are not easily allocated under the existing descriptions of CJD and GSS. It has provided an explanation of families where CJD and GSS syndromes co-exist and has allowed demonstration of prion diseases in the absence of characteristic pathology (Collinge *at al.* 1990). Once disease has developed due to the presence of a mutation in the human PrP gene it can be transmitted by iatrogenic and experimental means.

The human PrP gene is a single copy gene spanning 16kb and containing two exons, located on the short arm of chromosome 20. The complete open reading frame of 759 nucleotides is found in the larger second exon, which comprises the majority of the 2.4 kb mRNA. Both the familial forms of CJD and GSS have an autosomal dominant pattern of disease segregation. The mutations described to date in the human PrP gene can be classified into two groups (Collinge and Palmer 1997):

1. Point mutations within the coding sequence resulting in the substitution of amino acids in the PrP sequence or in one case the production of a stop codon resulting in the expression of a truncated form of the normal protein.
2. Insertional mutations, with additional integral numbers of repeat elements within the normal series of tandem octapeptide repeats near the N terminal.

1.3.1.1 Common phenotypes associated with PRNP mutations

Although a variety of atypical phenotypes have been described, including atypical prion dementias and peripheral neuropathies the three main phenotypes are CJD, GSS and FFI.

1.3.1.1.1 CJD phenotype

The classic clinical presentation for CJD (Collinge and Palmer 1997) is a rapidly progressive multifocal dementia with myoclonus. Frequently, pyramidal, cerebellar and extra-pyramidal signs are present. The clinical course is rapid with progression to akinetic mutism and finally death. The duration of illness is usually less than 12 months, with many cases dying within two to three months. The age of onset of clinical disease is advanced, for example, in the PrP $Glu^{200}Lys$ mutation the average age of onset is 55 years (Brown *et al.* 1992*a*). The inherited forms of CJD do not usually display the characteristic EEG changes seen in sporadic CJD, although the neuropathology is typical. CJD is characterised by the presence of spongiform changes, neuronal loss and an astrocytosis, but PrP amyloid plaques are absent. Protease resistant PrP can be demonstrated by immunoblotting of brain homogenates (Collinge and Palmer 1997).

1.3.1.1.2 GSS phenotype

GSS is characterised by a chronic cerebellar ataxia with pyramidal features (Seitelberger 1962). The development of dementia tends to occur later and the disease has a much more prolonged clinical course than CJD. Onset of clinical signs is usually in the region of 30 to 50 years, with the mean duration being about five years. In addition to the spongiform change, neuronal loss, astrocytosis and white matter loss, typical of the prion diseases, there are multicentric amyloid plaques.

1.3.1.1.3 FFI phenotype

The first reported cases of FFI described a rapidly progressive disease with untreatable insomnia, dysautonomia and motor signs (Lugaresi *et al* 1986; Medori *et al.* 1992*a*, *b*). Neuropathological changes are characterised by selective atrophy of the anterior-ventral and medio-dorsal thalamic nuclei, marked thalamic astrocytosis, mild spongiform changes in some cases and the presence of protease-resistant PrP by immunoblotting.

1.3.1.2 Genotypes associated with PRNP mutations

The reported genotypes have been listed and only those of particular interest or relevance are discussed in more detail.

1.3.1.2.1 Polymorphic variations in PRNP which are not associated with clinical disease

A number of polymorphic variations in the human PrP gene (PRNP) have been described which have not been associated with clinical disease. These include: PrP Met^{129}Val (which has an important role in selection of susceptibility , selection of the individual phenotype and determination of the age of onset of the respective spongiform encephalopathies); PrP GGC^{124}GGG (Hsiao *et al.* 1992; Collinge and Palmer 1997); PrP GCA^{117}GCG (seen in 2.5% of Caucasians), PrP CAC^{177}CAT (Ripoll *et al.* 1993); PrP Glu^{219}Lys (occurring at an allele frequency of about 6%in the Japanese population) (Furukawa *et al.* 1995); and a PrP 24 bp deletion (one less octapeptide repeat) (Laplanche *et al.* 1990; Palmer *et al.* 1993).

1.3.1.2.2 Missense Mutations

The site of reported point mutations within the coding sequence of the human prion protein gene, the amino acid substitutions involved and the reported associated clinical syndrome are shown in Figure 2.

1. PrP Pro^{102}Leu (Hsiao *et al.* 1989)
2. PrP Pro^{105}Leu (Kitamoto *et al* 1993*b*)
3. PrP Ala^{117}Val (Dohura *et al.* 1989; Hsiao *et al.* 1991; Collinge and Palmer 1997)
4. PrP Tyr^{145}STOP

This mutation is of interest because it results in the formation of a truncated form of the PrP protein. The investigators demonstrated that the truncated form of PrP was expressed, but more importantly, all deposited PrP consisted of the truncated form with none of the wild type present. This is consistent with the belief that for successful interaction of the host PrP with the PrP^{Sc} the two proteins should be homologous (Kitamoto *et al.* 1993*a*).

5. PrP Asp^{178}Asn

The original described mutation resulted in a CJD-like phenotype (Goldfarb *et al.* 1991*b*). Subsequently, it has been demonstrated that this mutation is also responsible for Fatal Familial Insomnia (FFI) (Lugaresi *et al.* 1986; Medori *et al.*

1992*a,b*). To date all the cases demonstrating the CJD-like phenotype have the mutation on a valine 129 allele, while all the FFI-type phenotypes have the mutation on a methionine 129 allele (Goldfarb *et al.* 1992). The 129 allele appears to select the phenotype of the mutation. Further studies are needed to ensure that the CJD and FFI phenotype are not just extreme ends of the normal disease spectrum. An alternative explanation is that the presence of the valine or methionine 129 allele may be due to the selection of related subjects for the study, who incidentally have that respective coding on the 129 allele. Western blot analysis of proteinase K treated PrP, extracted from these cases has demonstrated different sized PrP bands, suggesting that these FFI and CJD cases may be related to different forms of PrP^{Sc} (Monari *et al.* 1994).

6. PrP $Val^{180}Ile$ (Farlow *et al.* 1989; Ghettie *et al.* 1989; Kitamoto *et al.* 1993*b*).
7. PrP $Phe^{198}Ser$ (Farlow *et al.* 1989; Dlouhy *et al.* 1992)

Codon 129 appears to play a role in age of onset in this disease, with individuals heterozygous at this site having a later onset of clinical disease than homozygous individuals.

8. PrP $Glu^{200}Lys$ (Golfarb *et al.* 1990; Brown *et al.* 1992*a*)

Since the development of PrP gene analysis, further cases of this mutation with an atypical clinical presentation (including a peripheral neuropathy) have been described (Neufeld *et al.* 1992).

9. PrP $Arg^{208}His$ (Mastrianni *et al.* 1995)

This mutation has been identified in a single patient with CJD, with no published clinical or pathological history.

10. PrP $Val^{210}Ile$ (Davies *et al.* 1993)
11. PrP $Gln^{217}Arg$ (Hsiao *et al.* 1992)
12. PrP $Met^{232}Arg$ (Kitamoto *et al.* 1993*b*)

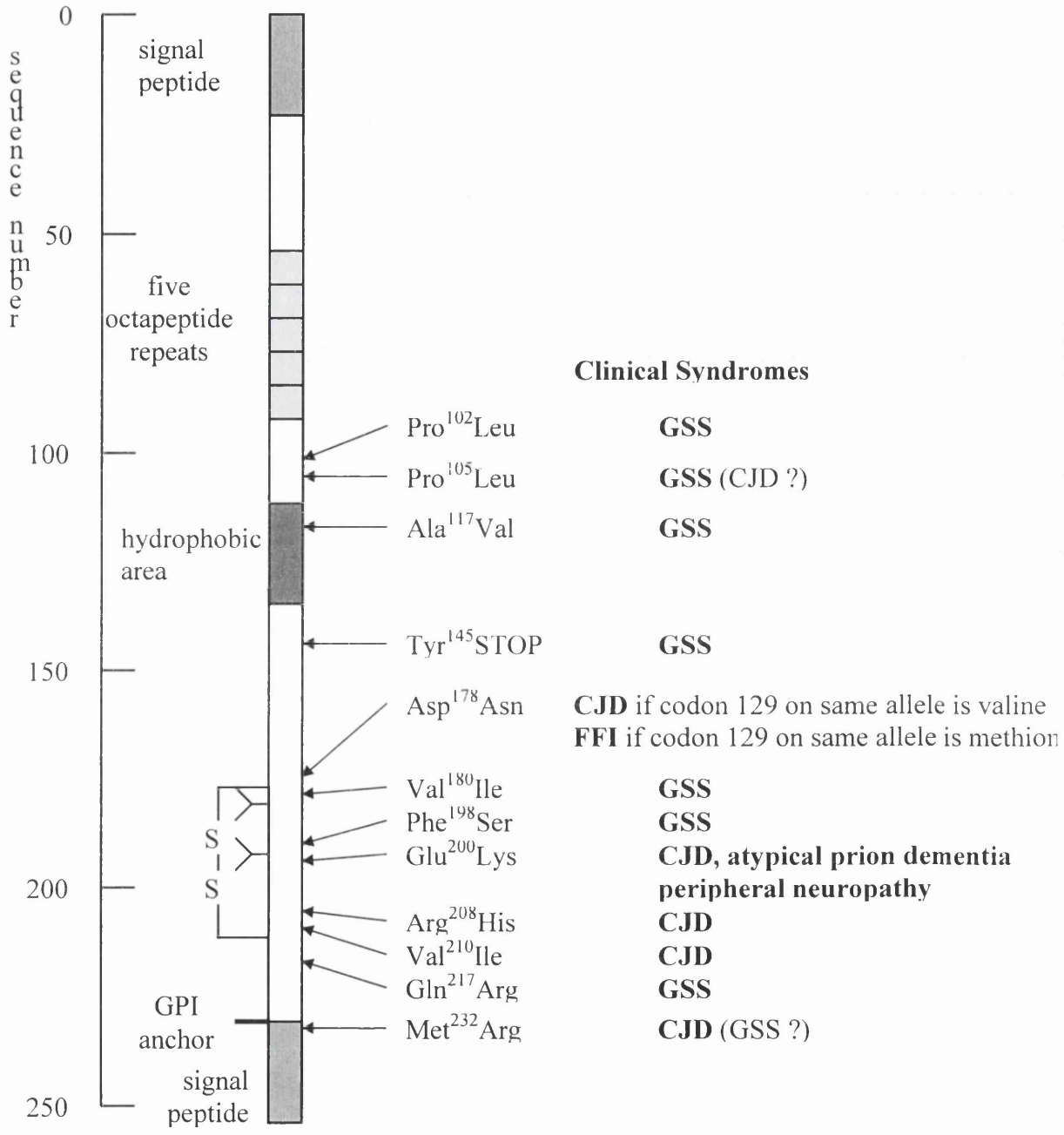


Figure 2 Structure of the 253 amino acid human prion protein illustrating the site of reported amino acid substitutions and the clinical syndrome with which they have been associated. Modified from Collinge and Palmer (1997).

1.3.1.2.3 Insertional Mutations

The 253 amino acid human prion protein and sites of insertion of additional integral numbers of repeat elements within the normal series of tandem octapeptide repeats near the N terminal is shown in Figure 3.

1. PrP 24 bp insertion (one extra repeat) (Laplanche *et al.* 1995)
2. PrP 48 bp insertion (two extra repeats) (Goldfarb *et al.* 1993).
3. PrP 96 bp insertion (four extra repeats) (Laplanche *et al.* 1995; Campbell *et al.* 1996; Goldfarb *et al.* 1991*a*)
4. PrP 120 bp insertion (five extra repeats) (Goldfarb *et al.* 1991*a*)
5. PrP 144 bp insertion (six extra repeats) (Owen *et al.* 1989; Meyer *et al.* 1954; Collinge *et al.* 1990).

Codon 129 determines the age of onset in this mutation. As the mutation occurs on a methionine 129 PrP allele, affected individuals may either be methionine 129 homozygotes or methionine 129/valine 129 heterozygotes. Heterozygous individuals have an age of onset about a decade later than homozygous individuals (Poulter *et al.* 1992).

6. PrP 168 bp insertion (seven extra repeats) (Goldfarb *et al.* 1991*a*)
7. PrP 192 bp insertion (eight extra repeats) (Goldfarb *et al.* 1991*a*)
8. PrP 216 bp insertion (nine extra repeats) (Owen *et al* 1992; Krasemann *et al.* 1995)

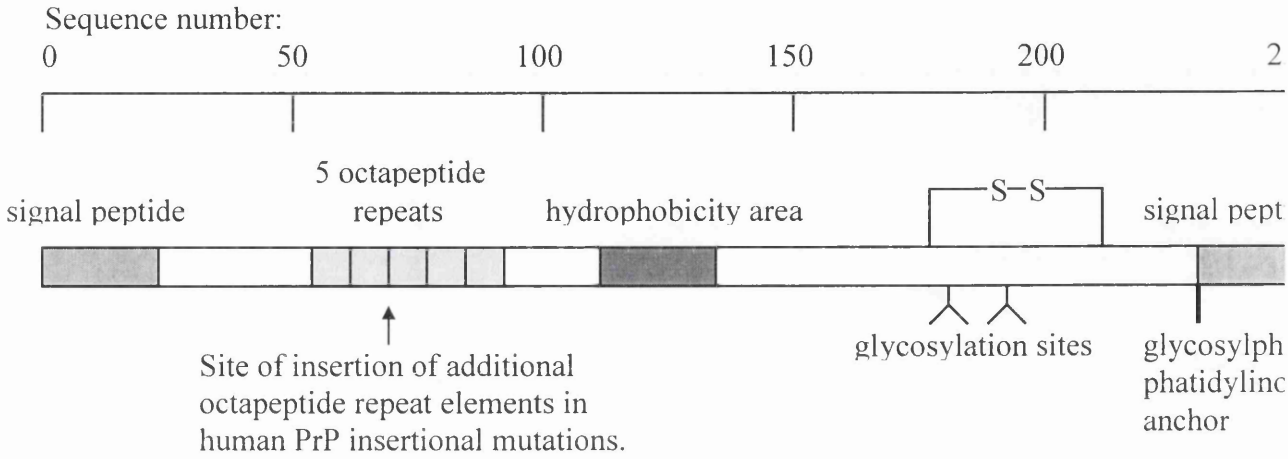


Figure 3 Structure of the 253 amino acid human prion protein demonstrating the site of insertion of additional integral numbers of repeat elements within the normal series of tandem octapeptide repeats near the N terminal. Modified from Collinge and Palmer (1997).

1.3.2 Acquired TSE's

1.3.2.1 Kuru

This disease of the Fore people in the highland rain forests of Papua New Guinea was first described by Gajdusek and Zigas while they were trekking there (Gajdusek and Zigas 1957). The highlands were unknown to the outside world until 1930 and administrative contacts started in the 1950s. There is reason to believe that the disease first appeared in the early part of this century and gradually increased to near epidemic proportions by the mid 1950s, at which stage the annual mortality was approximately 200 (Collinge and Palmer 1997). The disease affected only members of the Fore linguistic group and, to a lesser degree, the surrounding tribes with which they intermarried.

1.3.2.1.1 Clinical presentation

Kuru is the native word for shivering and describes the fine tremor of the head, trunk and limbs which is associated with a gradual onset of ataxia. The disease is progressive, with patients initially displaying prodromal signs including headaches and joint and limb pain, followed by the development of cerebellar signs and finally by hypotonia, hyporeflexia and brain stem signs. Death is usually as a result of bronchopneumonia and respiratory failure.

A feature of the disease is the development of behavioural changes (mood changes and hilarity). Due to the bizarre uncontrollable laughter the disease has been termed 'the laughing death'.

1.3.2.1.2 Epidemiology

The disease predominantly occurred in young adult women and children (over four years of age) of both sexes and this originally led the investigators to suspect either a sex-linked genetic abnormality or an endocrine disorder. Only about 2% of the cases occurred in adult males (Alpers 1987). The epidemiology of Kuru is interesting in that the disease occasionally occurred in clusters, with several members of the same clan developing Kuru within several months of each other.

About ten years after the development of formal administrative contacts the disease had disappeared in children under 10 years of age (Gajdusek *et al.* 1966). This decrease in incidence in the younger age groups continued after the banning of the

ritual cannibalism by the administration, with no new cases reported in children born after 1956 (Collinge and Palmer 1997). The decline in incidence associated with the ban on ritual cannibalism led to further investigation into a possible link between the two. The ritual form of cannibalism practised by the Fore people was performed as a mark of respect to dead relatives. As part of this ritual it was customary for the remaining members of the community to consume the dead relative. The men would eat the meat while leaving the remainder of the body, including the brain to the women and children. The infectious agent has been demonstrated in a high titre in brain tissue, but almost never in muscle tissue (Brown 1980) explaining the higher incidence in children and women. Apart from the actual consumption of the brain tissue, the preparation of the cadaver by the women and children would put them at further risk of exposure. The liquefied brain tissue was scooped by hand into bamboo cylinders for preparation and therefore additionally exposed the women and children to the risk of intradermal (scratching insect bites) and conjunctival (rubbing eyes) exposure to the infectious agent (Collinge and Palmer 1997).

After a lengthy period of unsuccessful transmission studies using rodents, the disease was finally transmitted to a chimpanzee in 1965 (Gajdusek *et al* 1966). Further experimental transmission using the oral route was successful (Gibbs *et al.* 1980). The origin of Kuru in this region was thought to be due to the development of a single sporadic case of CJD in the region in the early part of the century (Collinge and Palmer 1997). The occurrence of clusters of Kuru in members of the same community of different ages can be explained by the participation of the entire extended family in a ritual cannibalistic feast. An important feature to be recognised from the Kuru epidemic is the absence of vertical transmission, in that most of the children born from affected mothers after 1956 and all of those born after 1959 did not develop Kuru.

Currently 6 to 8 cases are occurring per year, all in individuals aged over 40 years, which is consistent with exposure prior to 1956 and indicated an incubation period which can exceed 40 years. The shortest calculated incubation period (based on age of onset of disease) was found to be $4^1/_2$ years. Experimentally, incubation periods of up to eight years have been demonstrated in the rhesus monkey (Gajdusek and Gibbs 1972).

1.3.2.1.3 Pathology

The pathology of Kuru is very similar to that of CJD (Bendheim 1984, Collinge and Palmer 1997). It is characterised by mild to moderate neuronal vacuolation, a marked reactive astrocytosis, corticospinal and spinocerebellar tract degeneration and, in at least half the studied cases, the presence of numerous amyloid plaques. These amyloid plaques are termed 'Kuru plaques' (Masters *et al.* 1981) and tend to be most common in the cerebellum. The similarities between Kuru and CJD prompted the successful experimental transmission of CJD in 1968 by Gibbs *et al.*

1.3.2.2 Iatrogenic CJD

The mechanism behind infection in these cases is direct bodily penetration with contaminated instruments or tissues. Recognised iatrogenic routes of transmission include treatment with human cadaveric pituitary-derived growth hormone (Brown 1990) or gonadotropin (Cochius *et al.* 1992), dura mater (Thadani *et al.* 1988) or corneal grafting (Duffy *et al.* 1974) and the use of inadequately sterilised neurosurgical instruments (Bernoulli *et al.* 1977; Will and Matthew 1982).

Cases of acquired CJD arising through peripheral injection tend to have a longer incubation period and develop a progressive ataxia syndrome. Comparison of these cases with Kuru reveals similarities in both the clinical presentation and the incubation period. Incubation periods of 20 to 30 years between the last participation in ritual cannibalism and the onset of clinical signs have been documented in Kuru (Klitzman *et al.* 1984). This period is similar to incubation periods of up to 20 years between the last hormonal treatment and the development of clinical signs in iatrogenic CJD (Brown 1988). In cases with direct intracerebral or intraocular inoculation of the infective agent by means of contaminated instruments, corneal transplants or dura mater grafts and in experimental intracerebral inoculation in chimpanzees the incubation period is two years or less (Brown 1990). Additionally these patients tend to develop the classic form of CJD with the development of a rapidly progressive dementia.

The infectivity of inadequately sterilised neurosurgical instruments is illustrated by the example where in 1977 two patients developed CJD 16 and 20 months respectively after receiving stereotactic electroencephalographic depth recordings for epilepsy. The same electrodes had been used earlier for stereotactic exploration of a

CJD patient and had been sterilised with 70% alcohol and formaldehyde vapour. One of the electrodes was found to be still infective when implanted into a chimpanzee's cerebral cortex (Bernoulli *et al.* 1977).

In the case of CJD developing secondary to the use of human cadaveric pituitary-derived growth hormone (Brown 1988) there have been about 200 cases reported world-wide. The relative high risk of this group of people (around 10000 people in the USA and 1908 people in the UK have received this treatment) can be explained by the preparation of the vials. Each batch of growth hormone was prepared from pooled pituitary glands and around 2000 pituitary glands were used per batch. In addition the remnants of one batch were sometimes mixed with the next batch. The potential therefore existed for a single pituitary gland from a case of sporadic CJD to contaminate a large number of vials. The cases of pituitary derived gonadotropin have so far been limited to patients treated in Australia (Collinge and Palmer 1997).

Of interest is the apparent genetic susceptibility to iatrogenic CJD demonstrated in the cases of CJD arising from treatment with cadaveric pituitary-derived growth hormone. Studies performed firstly in the UK (Collinge *et al.* 1991) and subsequently in France (Laplanche *et al.* 1994) and the USA (Brown *et al.* 1992*b*) have demonstrated an excess of codon 129 homozygotes and in particular valine homozygotes. When you consider that only 11% of the population are valine homozygotes (and for example 4 of the 7 cases in the UK are valine homozygotes) this may imply that this section of the population are at greater risk of undergoing conversion to the disease related isoform.

1.3.2.3 New Variant (vCJD)

With the re-institution of CJD surveillance in the UK in 1990, as a result of the BSE epidemic, three cases of CJD with unusual clinical and pathological findings were reported in young individuals (Bateman *et al.* 1995; Britton *et al.* 1995; Tabrizi *et al.* 1996). Included in the unusual pathological findings was the presence of Kuru-type plaques. Based on further cases in 1996 vCJD was described (Will *et al.* 1996), the first reported patient developing clinical signs in February of 1994. So far, the disease appears to be restricted to the UK, with only a single case reported in France (Chazot *et al.* 1996), despite similar CJD surveillance programs being instituted in Europe (France, Italy, Germany and the Netherlands). The vCJD is characterised by (Will *et al.* 1996) a specific and apparently unique neuropathological profile, a low age of

onset for CJD, absence of EEG changes typical of CJD, a characteristic and protracted clinical course and the presence of methionine homozygosity at codon 129 of the PrP gene (Collinge *et al* 1996). Molecular analysis of prion strain variations and transmission studies using mice have indicated that the prion strain which is responsible for the vCJD is the same prion strain responsible for BSE (Bruce *et al.* 1997; Collinge *et al.* 1996).

1.3.2.3.1 Clinical presentation

Clinical findings in the first eight cases to die of vCJD in the UK have revealed an age range at death of 19 to 41 years (mean 29 years), while the interval between disease onset and death was 7.5 to 22.5 months (median 12 months). If these findings are compared to first 185 reported cases of sporadic CJD identified since starting the 1990 CJD surveillance program, the average age of onset was found to be 65 years and the median duration of disease 4 months. Only two cases of sporadic CJD with ages of less than 45 years (both 44 years old) are included in these 185 cases (Will *et al.* 1996). Of the first ten cases of vCJD reported in the UK, nine had behavioural changes as an early clinical feature (initially necessitating referral to a psychiatrist) with dysaesthesia as a prominent feature in four cases. Nine patients developed ataxia as an early feature, all patients developed dementia, seven cases developed myoclonus (often late in the clinical course) and none of the cases displayed the characteristic EEG features usually associated with CJD (Will *et al.* 1996).

1.3.2.3.2 Pathology

The neuropathological features (Will *et al.* 1996; Ironside and Bell 1997) of vCJD are consistent and distinct from those of classical CJD with the presence of spongiform changes, neuronal loss and astrocytosis especially evident in the basal ganglia, thalamus and focally in the cerebrum and cerebellum. The presence of the spongiform changes and PrP plaques confirm the presence of CJD. The PrP plaques are the most striking and consistent abnormality in these cases with the plaques being extensively distributed throughout the cerebrum and cerebellum with smaller numbers in the thalamus, basal ganglia and hypothalamus. These plaques resembled Kuru-type plaques with a dense eosinophilic centre and a pale periphery and usually surrounded by a zone of spongiform change. Examination of 175 cases of sporadic CJD did not demonstrate the presence of any PrP plaques. The presence of PrP plaques had only

previously been reported in scrapie as 'florid plaques' and in Kuru as 'Kuru-like plaques' (Collinge and Palmer 1997).

Immunocytochemistry for PrP (Will *et al.* 1996; Ironside and Bell 1997) revealed a pericellular distribution of PrP in the cerebral cortex and the molecular layer of the cerebellum (which suggested deposition around small neurons), plaques and pericellular deposits in the cerebrum and cerebellum (in the absence of confluent spongiform changes in the surrounding neuropil) and an apparent perivascular deposition in the basal ganglia and thalamus with resultant linear tract-like deposits.

1.3.2.3.3 Epidemiology

Analysis of risk factors in nine of the first ten cases (Will *et al.* 1996) revealed no history of potential iatrogenic exposure to CJD through neurosurgery or treatment with human cadaveric derived pituitary hormones. Only one patient had worked as a butcher and one other had visited a BSE-free dairy herd for a week a year from 1976 to 1986. All nine of the cases had eaten beef or beef products in the last ten years, although none had eaten brain and one patient had been a strict vegetarian since 1991.

Recent studies (Bruce *et al.* 1997, Collinge *et al.* 1996) have demonstrated that the prion strains causing vCJD, BSE, FSE and spongiform encephalopathy in two species of exotic ruminants have characteristics which are identical to each other, but which are distinct from the strain characteristics of other forms of CJD. From these studies it was concluded that the PrP strain type causing vCJD, BSE, FSE and the spongiform encephalopathy of exotic ungulates is identical and is derived from a common source, most likely as a result of BSE material entering the food chain. These studies are discussed in more detail in the review of FSE.

1.3.3 Sporadic TSE's

The majority of human cases of TSE are sporadic (with, for example, only around 15% being hereditary) and the exact aetiology of these cases is still poorly understood (Bendheim 1984). The sporadic TSE's are characterised by the absence of any characteristic aetiological marker but the disease can be transmitted, for example, to primates. In the absence of any identifiable aetiological marker it is likely that further as yet unidentified forms of sporadic CJD exist with a more diverse phenotype and in which transmission to primates is more difficult.

1.3.3.1 Sporadic Creutzfeldt-Jakob Disease

Sporadic CJD is the predominant human sporadic spongiform encephalopathy, with the other forms being classified as atypical forms of sporadic CJD.

1.3.3.1.1 Epidemiology

Sporadic CJD occurs at a frequency of approximately 0.5 to 1 per million without evidence of a geographic, familial or other distribution (Brown *et al.* 1987). There does appear to be a genetic predisposition for the development of sporadic CJD, with the majority of classical cases of sporadic CJD being homozygous at codon 129 of the PrP gene for either methionine or valine (Collinge *et al.* 1996). In one study of 22 clearly defined sporadic cases of CJD, only one patient was not homozygous for either methionine or valine (Palmer *et al.* 1991). In sporadic CJD there does not appear to be a coding mutation or evidence of iatrogenic exposure, and the cases occur scattered throughout the population without any identifiable geographic distribution (apart from a frequency related to local population density) or any consistent associations with dietary components or occupational groups.

Of particular interest is the absence of any evidence of case-to-case spread, besides by iatrogenic methods, and the absence of any correlation to the local prevalence of scrapie. As an example of the absence of correlation to the local incidence of scrapie, the incidence of CJD in Australia and New Zealand, where scrapie has not been present for some years, is similar to that of the UK where scrapie is endemic (Collinge and Palmer 1997). Recent monitoring of all suspected cases of CJD in the UK by means of the CJD surveillance unit, and in other EU countries, has led to a slight increase in the number of reported cases of CJD. This increase is thought to be due to increased awareness of the disease rather than an actual increase. Concern about the occurrence of CJD in a number of dairy farmers in the UK being related to occupational exposure to BSE have been proven to be unfounded by the demonstration of a typical pathoclinical picture of sporadic CJD in these cases (Collinge and Palmer 1997).

1.3.3.1.2 Clinical presentation

The clinical onset is between 45 and 75 years, with a peak onset between 60 and 65 years. The clinical presentation is characterised by a rapidly progressive multifocal dementia, usually with myoclonus. Progression occurs over weeks with akinetic

mutism and death often occurring within 2 to 3 months. Approximately 70% of cases will die in less than six months. In approximately a third of cases prodromal signs can be identified, including fatigue, insomnia, depression, weight loss, malaise, headaches and ill-defined painful sensations. Additional neurological signs are frequently present and may include cerebellar ataxia, extrapyramidal signs, pyramidal signs and cortical blindness (Collinge and Palmer 1997).

Ante-mortal diagnosis of CJD is difficult. Prospective epidemiological studies have demonstrated that cases with progressive dementia, a typical EEG and two (or more) of the following signs: akinetic mutism, myoclonus , cortical blindness or pyramidal, cerebellar or extrapyramidal signs almost always are confirmed as cases of CJD on neuropathological examination (Collinge and Palmer 1997). In some cases mild cerebral and cerebellar atrophy may be apparent on MRI and CT scans of the brain.

1.3.3.1.3 Pathology

Neuropathologically sporadic CJD is characterised by the presence of spongiform changes, neuronal loss and an astrocytosis, but PrP amyloid plaques are usually absent. Protease resistant PrP can be demonstrated by immunoblotting of brain homogenates.

1.3.3.2 Atypical forms of Creutzfeldt-Jakob Disease

A variety of atypical forms of CJD have been described and are well recognised (Brown *et al.* 1984; Collinge and Palmer 1997).

- Approximately 10% of CJD cases have a clinical history of longer than two years. These cases may represent the occasional occurrence of CJD in individuals with heterozygosity for PrP polymorphism, especially at codon 129 (Collinge and Palmer 1997). Individuals with heterozygosity for PrP polymorphism appear to have a reduced susceptibility, and in those individuals that do develop CJD the clinical course is much more prolonged.

It appears that in heterozygotes producing two different forms of PrP there is an internal barrier present delaying the onset of the disease. One explanation for this may be due to the disease process between the PrP^{Sc} and one of the constitutional forms of the PrP only occurring at half speed due to the homologous constitutional protein to the PrP^{Sc} only being present at 50% of the total level. The other constitutional protein, which is not homologous to PrP^{Sc}, making up the other 50%

(Collinge and Palmer 1997). Evidence for this theory is provided by mice which are heterozygous for a PrP-null allele (and therefore only produce 50% of the normal levels of PrP), have a very prolonged incubation period and then develop a much more slowly progressive disease than wild type mice (Bueler *et al.* 1993, 1995).

- Ataxic CJD, occurring in about 10% of CJD cases, where individuals present with cerebellar ataxia rather than cognitive impairment (Gomori *et al.* 1973).
- In Heidenhain's variant of CJD cortical blindness is the dominant feature, with marked involvement of the cortical lobes.
- In the panencephalopathic type of CJD there is extensive degeneration of the cerebral white matter in addition to spongiform vacuolation of the grey matter (Gomori *et al.* 1973).
- Amyotrophic forms of CJD with prominent early muscle wasting have been described but most cases are not experimentally transmissible (Salazer *et al.* 1983). These forms are not well understood and may be a late form of motor neurone disease.

1.4 Relationship of PrP to the disease process

Recent extensive research into the PrP protein and the underlying infective agent has been undertaken, partially encouraged by the current BSE epidemic and the potential risk to man. The understanding of the PrP diseases has been greatly advanced, but a large controversy still exists as to the exact nature of the infectious agent. The two main theories that exist are the prion hypothesis and the virion hypothesis. The prion hypothesis proposes that these diseases are caused by a proteinaceous particle devoid of nucleic acid. The virion hypothesis proposes that an agent-specific replicable information molecule (either a nucleotide or polynucleotide) is bound to a protective host protein, PrP (Farquhar *et al.* 1998).

For the debate to be satisfactorily resolved either the production of infectivity will have to be demonstrated through defined conformational changes of PrP, or an informational molecule with strain-specific properties will have to be demonstrated.

1.4.1 Virion hypothesis

The virion hypothesis was proposed due to the failure to explain how the PrP protein alone could specify the different TSE strain characteristics and how these characteristics could be strongly retained. The presence of a small host-independent informational molecule encoding strain-specific characteristics does allow explanation of the different TSE strain characteristics (Farquhar *et al.* 1998). Ionising irradiation studies have demonstrated a small target size for the infectious agent, which would be consistent with the size of the nucleic acid genomes of small viruses (Rohwer, R.G. 1984). Extensive studies have so far demonstrated no evidence of a nucleic acid associated with the TSE's and the virion hypothesis does not explain the transmissibility of PrP^{Sc} formed as a result of a mutation of the PrP gene.

1.4.2 Prion hypothesis

As early as 1967 the idea of a protein-only infectious particle was proposed (Griffiths 1967), but it was only once Prusiner was able to co-purify prion protein with hamster scrapie infectivity that the infectious agent could be described as a proteinaceous infectious particle (Prusiner 1982). The term prion was introduced to distinguish these diseases from viral diseases, the term being derived from the nature of the agent as a *pro*teinaceous *in*fectious particle and a proposed working diagnosis described

prions as 'small proteinaceous infective particles which resist inactivation by processes that modify nucleic acids' (Prusiner 1987). The prion, or protein-only, hypothesis suggests that replication occurs without the requirement of informational input from DNA (Smith and Clarke 1997).

The main argument against the protein only hypothesis is the large number of prion strains that exist. The insoluble nature of the PrP^{Sc} has made analysis of its structure extremely difficult, but it does appear that information contained within the protein conformation of the PrP^{Sc} molecule may explain the strain variations that exist. That conformational information of the PrP^{Sc} may be transferred to other PrP molecules, including PrP molecules of a different primary structure, allows explanation of strain stability and strain modification (Ridley and Baker 1997). Similar examples of the transfer of protein conformations have been demonstrated in the synthesis of p53 and in experimental induction of β-amyloid deposition in the brains of middle aged monkeys on intracerebral injection of β-amyloid containing brain tissue (Baker *et al.* 1994). In p53 synthesis, mutant p53 may drive the wildtype p53 to adopt the conformation of the mutant protein (Milner and Metcalf 1991).

1.4.3 Normal function and dynamics of PrP

PrP^{C} (normal cellular isoform of prion protein) is highly conserved in mammals and may be present in all vertebrates, indicating that it probably has an important function. Certainly PrP^{C} is found in high concentrations in neuronal membranes and lymphoid tissues (Jefferys 1997). The development of at least two strains of PrP-null mice has led to a further understanding of the functions of PrP. The most surprising factor being the absence of severe clinical signs associated with the absence of PrP. This has allowed certain theories about the exact function of PrP to be discounted and demonstration that PrP plays a role in synaptic inhibition. Based on the absence of severe clinical signs associated with PrP-null mice, it has been proposed that PrP^{C} is strongly conserved not because it is essential to the well being of the organism, but rather that mutations of the PrP gene are associated with severe disease. Any deviations from the conserved sequence are therefore likely to be lethal (Ridley and Baker 1997).

PrP^{C} is rapidly synthesised and degraded, in contrast to the slow post-translational synthesis of PrP^{Sc}. The initiation of PrP synthesis is in the endoplasmic reticulum (Caughey and Chesebro 1997) and from there the PrP transits through the Golgi

apparatus. It is proposed that PrP^C is transported in secretory vesicles to the cell surface, where the GPI anchor anchors it to the external surface. In contrast to this, although PrP^{Sc} is transported through the same excretory pathways, it accumulates in cytoplasmic vesicles within the cell, most of the cytoplasmic vesicles being secondary lysosomes. From the external cell surface the PrP^C is degraded inside the cell. It is thought that PrP^{Sc} formation takes place intracellular, the process being supplied by PrP^C found briefly on the external surface of the cell (Prusiner 1997).

1.4.4 Proposed route of infection of PrP^{Sc}

Experimental studies have suggested that route of spread of PrP^{Sc} within the affected host is related to the route of infection. The parenteral routes, especially where direct inoculation of the CNS occurs, are most efficient. In a field situation, infection through the gastrointestinal tract (GIT) is probably more significant. After introduction of the PrP^{Sc} into the stomach the first site of infectivity is the lymphoid system, including the tonsils, thymus, lymph nodes and spleen (Brown 1997; Klein *et al.* 1997). The widespread distribution of target organs suggests that the agent spreads via the blood stream and this has been demonstrated experimentally (Brown 1997). Once infectivity is present in the peripheral lymphoid tissues then infectivity spreads to the CNS. The spread is though to be along peripheral nerves innervating these target tissues.

1.4.5 Proposed disease mechanism of PrP^{Sc}

The actual disease mechanisms in the TSE's are still poorly understood. The disease related isoform of PrP, PrP^{Sc}, is thought to interact with, and cause a conformational change in, the post-translational PrP^C, resulting in the conversion of PrP^C to PrP^{Sc}. The pathogenic mechanism in the TSE's is though to relate either to accumulation of PrP^{Sc} or depletion of functional PrP^C. PrP^{Sc} is resistant to proteolysis and therefore accumulates in affected cells. Certainly *in vitro* experiments have shown that PrP^{Sc} accumulation is toxic to neurons, resulting in apoptosis, will result in astrocyte proliferation and may disturb the metabolism of some neurotransmitters (Caughey and Chesebro 1997). Development of PrP-null mice has demonstrated only minor neurological abnormalities, with predominantly weakened synaptic inhibition, but no evidence of the dramatic clinical signs of the TSE's. This absence of severe clinical signs has added weight to the argument that the pathogenic mechanism is due to

accumulation of the PrP^{Sc} (Jefferys 1997). It has been suggested, however, that the pathogenic mechanism may still be as a result of loss of function of the PrP^{C}. This is as a result of the rapid loss of function in the disease state, while in the PrP-null mice the loss of function is present over the lifetime of the animals, thus allowing adaptive changes to take place (Smith and Clarke 1997).

Although an extensive understanding of the PrP has been developed over the preceding few years, of which some brief points are raised here, a number of questions have still to be answered. The most perplexing of these is the exact nature of the infective agent.

1.5 Spongiform changes in the CNS unrelated to prion diseases

A number of situations exist where spongiform changes occur in the CNS, either as a normal feature or secondary to a variety of non prion-related diseases. These need to be differentiated from the TSE's. The majority of these diseases are leukoencephalopathies and may be hereditary. Examples of these diseases will be discussed.

1.5.1 Vacuolation as a Normal Feature

Large cytoplasmic vacuoles may be seen as a normal feature in scattered neurons in the red nucleus of cattle (Wells and McGill 1992). In 47 of 242 BSE negative bovine brains submitted under the BSE orders in Scotland, vacuolation of the substantia nigra was present (Jeffrey 1992), and in the majority of these cases the cause of the vacuolation was not determined. In sheep occasional vacuolated neurons are reported in the medulla (Zlotnick and Rennie 1958), while similar changes probably occur as a normal feature in pigs, cats (Wells and McGill 1992) and possibly other mammalian species. In older animals especially (but also younger foals) axonal spheroids and vacuoles may frequently be found in the lateral cuneate nucleus of the medulla (Summers *et al.* 1995).

In the sympathetic ganglia of apparently normal horses and dogs it is not unusual to find large neuronal vacuoles which may occupy the majority of the perikaryon and displace the nucleus (Brownlee 1959; Itakura 1960). In mature horses these large vacuoles may contain pale eosinophilic material. In humans an increased incidence of vacuolation of autonomic neurons is seen in certain diseases of the heart, blood vessels, intestines and larynx (Scharf 1958). Additionally, pseudovacuoles may be seen with retraction of the neurons from their surrounding satellite cells.

1.5.2 White Matter Spongiform Degeneration

1.5.2.1 Spongy Degeneration of the Bogaert-Bertrand Type (Canavan's Disease)

This human disease is considered a classic example of spongiform changes in a non-prion-related disease. It was first reported in 1949 by Bertrand and Bogaert (Adornato *et al.* 1972), and is characterised by a spongy degeneration affecting predominantly the white matter and grey matter margins of the brains of affected children. Three patterns are recognised: congenital, infantile and juvenile forms.

Gross lesions are evident in the brain at post mortem, with the brain being heavy, the lateral ventricles dilated and the white matter appearing grey and gelatinous. Histopathology reveals diffuse white matter vacuolation (described as status spongiosis) and pallor (Summers *et al.* 1995). The underlying hereditary defect results in elevated levels of N-acetylaspartic acid in the blood and urine, with depressed levels of tissue aspartoacylase activity (Matalon *et al.* 1988).

1.5.2.2 Hepatic Encephalopathy

In hepatic encephalopathy, and to a lesser extent in renal encephalopathy, neuropathological changes consist of polymicrovacuolation (spongiform changes of the white matter) and Alzheimer type II cells (which are single or small groups of astrocytes with clear, swollen nuclei). A large disparity exists between the severity of clinical signs and the degree of neuropathological changes in the brain.

The polymicrovacuolation associated with hepatic encephalopathy occurs diffusely through the neuroaxis, particularly affecting the white matter (and especially where myelinated fibres are interspersed with grey matter). Particular regions affected include the peripheral corona radiata, internal capsule, thalamus, hypothalamus, cerebellar white matter and peduncles, pons, medulla oblongata and the fasciculus proprius of the spinal cord. The bilaterally symmetrical nature of the changes is consistent with a metabolic disease (Summers *et al.* 1995).

1.5.2.3 Toxicity Associated with Spongy Degeneration of the CNS

1.5.2.3.1 Lupinosis in sheep

Vacuolation of the white matter of the brain stem, in the absence of other changes in the CNS, has been reported in acute and chronic experimental lupinosis and chronic, naturally occurring lupinosis in sheep. The degree and extent of the CNS vacuolation reported was related to the severity of the primary liver lesion (Allen and Nottle 1979).

1.5.2.3.2 Hexachlorophene

A diffuse spongy degeneration affecting the entire CNS has been reported in cats due to accidental ingestion of, or medical use of, hexachlorophene, which has been incorporated into soaps and other products (O'Brien 1996). Other toxins that have

induced a spongy degeneration include triethyltin, isoniazid, Cuprizone and bromethalin (O'Brien 1996).

1.5.2.4 Other causes of white matter spongiform degeneration

Numerous other causes of spongiform degeneration of the white matter have been described. Some of those of significance in light of the recent BSE epidemic and the associated research include: spongy degeneration in British white cattle (O'Brien 1996), maple syrup urine disease of Hereford cattle (Cordy *et al.* 1969), cerebral oedema of Hereford cattle, spongy degeneration in outbred Swiss-Webster mice, spongy degeneration of rats and hereditary ataxia of rabbits.

1.5.3 Grey Matter Spongiform Degeneration

1.5.3.1 Ischaemic Injury

Fine neuronal vacuolation, due to swelling of the endoplasmic reticulum or mitochondria, may be found as a result of an ischaemic incident (Summers *et al.* 1995).

1.5.3.2 Bull Mastiff puppies

A disorder has been described in bullmastiff puppies characterised by neurological abnormalities, including ataxia, proprioceptive deficits, hypermetria, visual deficits and bizarre behaviour. Bilaterally symmetrical vacuolation and gliosis, most marked in the cerebellar nuclei, is apparent on neuropathological examination (Carmichael *et al.* 1983).

1.5.3.3 Saluki puppies

A similar disorder to that in bullmastiff puppies has been described in saluki puppies. The disease is characterised by the presence of a pronounced spongiosis with associated swollen astrocytes in the grey matter of nuclei like the olivery nuclei and cerebellar nuclei (Luttgen and Storts 1987).

1.5.3.4 Malinois Shepherd-cross puppies

A disorder has been described in two malinois shepherd-cross puppies associated with progressive and generalised tremors. Pathological examination revealed a widespread spongiosis with associated astrocytosis of the CNS, particularly affecting the grey

matter. The changes were present in the cerebral cortex, basal nuclei, brain stem, cerebellar nuclei and grey matter of the cervical and lumbar enlargements (Cachin and Vandevelde 1991).

1.5.3.5 Birman Kittens

A progressive disorder characterised by paraparesis and ataxia has been described in young Birman kittens. Pathological examination revealed spongiform changes affecting the neuropil of the cerebral cortex, thalamus, caudal colliculi, oculomotor nuclei and the medulla (Jones *et al.* 1992).

1.5.3.6 Lysosomal Storage Diseases

The accumulation of cytosomes due to an inherited or acquired lysosomal enzyme defect results in cell swelling and a granular to vacuolar appearance of the cytoplasm (Summers *et al.* 1995).

1.5.3.7 Rabies

Vacuolar changes in the neuropil and neuronal cell bodies have been described in experimental and naturally acquired rabies in skunks, foxes and a heifer (Foley and Zachary 1995). In the heifer the spongiform changes affected the neuropil and neuronal cell bodies of especially the thalamus and cerebral cortex. Although no Negri bodies were found in any of the sections the brain was positive for rabies virus antigen using fluorescent antibody testing.

1.5.3.8 Neuronal Vacuolation and Spinocerebellar Degeneration in Young Rottweiler Dogs

Three recent reports (Kortz *et al.* 1997; Andrade-Neto *et al* 1998; van den Ingh *et al* 1998) have documented a progressive disorder in rottweiler puppies characterised by tetraparesis, ataxia and proprioceptive deficits. A prominent feature of the histopathological examination was vacuolation of the neuronal cytoplasm, particularly in the cerebellar nuclei and nuclei of the extrapyramidal system. Similar vacuolation was reported in the spinal ganglia (dorsal root ganglia) and the autonomic ganglia.

1.5.3.9 Neuronal Vacuolation in Racoons

A recent report (Hamir *et al.* 1997) has documented neuronal vacuolation in two apparently clinically normal racoons in the United States (from different geographical

locations). The vacuolar changes in the first racoon were limited to the brain stem, with bilateral, asymmetrical vacuoles (single or multiple) in the neurons of the facial nucleus. In the second case, the changes were similar, but more widespread in the brain stem and cerebrum, but not in the cerebellum. In both cases no inflammatory infiltrate was associated with the vacuolar changes and in both cases the animals were negative for rabies and scrapie associated fibrils.

1.5.3.10 Vacuolation of grey matter neuropil in laboratory animals

In light of the rodent models used to study the prion diseases, other causes of grey matter neuropil vacuolation in these species are potentially significant. Examples include a retroviral infection in mice, the grey tremor mutant mouse (Kinney and Sidman 1986) and the zitter rat (Gomi *et al.* 1990).

1.6 Aims of this thesis

The major aims of the present study are threefold.

1. To present a review of the current literature on feline spongiform encephalopathy. A brief resume of the relevant current literature on other transmissible spongiform encephalopathies is included and their relationship to feline spongiform encephalopathy (where appropriate) is discussed.

2. To present the clinical features of pathologically confirmed cases of FSE, as seen at the University of Glasgow Veterinary School, and to compare these with the published data. The clinical criteria for the presumptive antemortem diagnosis of feline spongiform encephalopathy are considered. Furthermore, a limited epidemiological study is presented, where appropriate details are available from the cases included in this project.

3. To present a detailed study of the pathology of feline spongiform encephalopathy in the cases included in this study. The distribution and nature of changes in the brain and spinal cord are investigated, using classical methods and immunocytochemical techniques. This latter aspect includes an investigation into both the appearance and pattern of distribution of the disease-related isoform of PrP deposition. An attempt is made to correlate the clinical presentation with the observed pathological changes.

2. MATERIALS AND METHODS

2.1 Collection of clinical material

2.1.1 Case selection

All cases of histopathologically confirmed cases of FSE seen at the Faculty of Veterinary Medicine, University of Glasgow, were included in this study, up to and including May 1998. Two of the cases were presented for post mortem examination after investigation at the referring veterinary surgeon, while the other seven cases were presented to the Department of Veterinary Clinical Studies for investigation of an apparent neurological abnormality. Details of the cases are presented in chapter 3.

In the cases presented to the Department of Veterinary Clinical Studies for investigation a detailed history was obtained and a full clinical and neurological examination was performed, although details in the earlier cases were not as complete as in later cases. In one of the two cases presented for post mortem examination a detailed history had been taken and a full clinical and neurological examination had been performed by the referring veterinary surgeon, while in the second case only limited information was available.

2.1.2 Investigation of cases

The signalment was obtained for all cases. Full histories were obtained, where possible, by a retrospective review of the clinical records and hospital database or, in the three cats presented during the course of the study, by detailed questioning of the owners.

A full clinical and neurological examination was performed as detailed by Oliver, Lorenz and Kornegay (1997), including a partial ophthalmologic examination. Further details of the functional tests that were found to be the most useful are presented in chapter 3.

2.1.3 Ancillary investigations

Further diagnostic procedures were performed in the majority of cases in order to rule out other potential causes of the clinical signs. The relevant tests are detailed in the clinical appraisal chapter.

Routine haematological evaluation was performed in five cases and routine biochemical evaluation was performed in six cases. These evaluations were performed to rule out the possibility of systemic disease and as a pre-anaesthetic evaluation of general health. Red blood cell counts, haematocrit, haemoglobin concentration, mean corpuscular volume, mean cell haemoglobin, platelet count and total and differential white blood cell counts were determined. Plasma concentrations of urea, creatinine, sodium, potassium, chloride, calcium, phosphate, glucose, cholesterol, bilirubin, alkaline phosphatase, alanine transferase (ALT), aspartate transferase (AST), creatinine kinase, total protein, albumin and globulin were analysed. Results of these tests were compared to reference ranges of GUVS laboratories.

Blood samples were submitted to the Feline Virus Unit at GUVS for determination of antibodies to feline leukaemia virus (FeLV) in five cases, feline immunodeficiency virus (FIV) in four cases and feline coronavirus (FIP) in four cases. Furthermore the antibody titre to toxoplasmosis was determined using a dye test in three cases.

Thyroid function was evaluated in two cases by determining the plasma thyroxine levels. A bone marrow aspirate was performed in one case. The technique used is described by O'Keefe (1992).

In two cases CSF samples were obtained, in one case from the cerebellomedullary cistern and in the other case from the lumbar cistern. The collection site was clipped and aseptically prepared. The cerebellomedullary cistern sample was collected using a 22 gauge, one-inch hypodermic needle. The technique used is described by Rand (1995). The lumbar cistern sample was collected via the interarcuate space between the sixth and seventh lumbar vertebrae, as described by Wheeler *et al* (1985). A one and a half inch, 22 gauge spinal needle (Monoject Spinal Needle, Sherwood Medical Industries, St. Louis, USA) was used.
The samples were inspected for colour, consistency and turbidity. The cell counts and total protein concentrations were determined and compared to reference values given by Bailey and Higgins (1985).

2.1.4 Collection of control material

Brains were collected from ten control cases, six of which had no abnormalities in the CNS and four of which had non-prion-related disease of the CNS. The spinal cord was collected from six of the control cases, five of which had no abnormalities in the CNS and one with an intracranial astrocytoma. Fixation and processing of the control cases was identical to the affected cases, with the exception that the control material for haematoxylin and eosin (H&E) staining and GFAP immunocytochemistry was not immersed in formic acid prior to processing. The control material for PrP immunocytochemistry was pre-treated with formic acid in a similar was to the material from the affected cases. Details of the signalment, clinical details and available tissue from the control cases are summarised in Table 4. In all the control cases the brain was sectioned in a standard manner, with the positioning of the sections as described in Fraser and Dickinson (1968).

Signalment	Diagnosis	Tissue
9.5 year old neutered female domestic short hair	meningioma, over left ventral brain stem and piriform lobe	brain
8 year old neutered male domestic short hair	astrocytoma of 4th and 5th cervical spinal cord segments	brain
12 year old male neutered domestic short hair	chronic renal failure, polyneuropathy and cerebral meningioma	brain
6 year old female neutered domestic short hair	iliac saddle thrombus	brain
1 year old domestic short hair	normal	brain; spinal cord segments C3, C7 and T3; spinal ganglia
1 year old domestic short hair	normal	brain; spinal cord segments C3, C7 and T2; spinal ganglia
1 year old domestic short hair	normal	brain; spinal cord segments C2, C6 and T3; spinal ganglia
1 year old domestic short hair	normal	brain; spinal cord segments C2, C6 and T2; spinal ganglia
1 year old domestic short hair	normal	brain; spinal cord segments C2, C6 and T4; spinal ganglia
5 year old female neutered domestic long hair cat	intracranial astrocytoma	brain; spinal cord segments from cervical and lumbar enlargements and the thoracic region; spinal ganglia

Table 4 Control cases: signalment, clinical details and tissues processed

2.2 Tissue collection and fixation

2.2.1 Post-mortem examination

Due to the risk to personnel, in any suspected FSE case the post-mortem was performed according to guidelines. Personnel involved in the examination were kept to a minimum and all personnel involved were required to wear protective gowns and gloves. The person responsible for removing the brain and spinal cord additionally wore a protective face visor. Access to the post-mortem area was limited for the duration of the post-mortem examination. All work surfaces were covered with disposable drapes. On completion of the post-mortem all the protective gowns, drapes and gloves were incinerated. Where possible, disposable instruments were used and these were disposed of by incineration. The carcass was also incinerated. Remaining, non-disposable instruments and table surfaces were decontaminated by exposure to sodium hypochlorite (Presept) and the instruments kept separate from the other instruments in the post-mortem room.

2.2.2 Buffered neutral formaldehyde, 4% (BNF)

This fixative was used for tissues destined for routine H&E staining and immunocytochemistry (see appendix for details). The brain was removed entire and immersion-fixed for at least a week prior to sectioning. The spinal cord was removed with the surrounding meningeal layers intact and was suspended in a BNF-filled cylinder, with the end weighted to keep it straight and prevent artefact. A representative sample of the lymphoid tissues, including spleen, peripheral, colonic and bronchial lymph nodes and tonsil, was removed from three of the cases and immersion fixed in 4% BNF.

2.3 Tissue processing

2.3.1 Paraffin processing

Prior to processing, the blocks from the clinical cases were immersed in neat formic acid (96%) for 30 minutes to decrease the infectivity of the tissues and therefore decrease the risk to personnel. The control material for H&E staining and GFAP immunocytochemistry was not immersed in formic acid, although the sections for PrP immunocytochemistry were. It was not thought that this difference in tissue processing would significantly affect the H&E staining or the GFAP

immunocytochemistry. The 24-hour paraffin wax processing cycle was used for blocks from the spinal cord and smaller brain blocks perfused with BNF. The seven day paraffin wax processing cycle was used for larger brain blocks perfused with BNF. The blocks of neural tissue were placed in processing cassettes and loaded in the basket of a Shandon Elliot automatic processor (Histokinette). The tissues were then passed through dehydrating solutions and infiltrated with wax (see Appendix for details of the solutions) before being embedded in paraffin wax at 60°C.

2.3.2 Sections

The paraffin-embedded tissue sections were sectioned on a Biocut 2035 microtome (Leica) at 8μm. The sections were mounted on APES-coated slides and incubated overnight at 60°C.

2.4 Staining Techniques

2.4.1 Haematoxylin and Eosin

Paraffin sections were stained with H&E to assess tissue morphology and preservation (see Appendix for details of the staining method). Once stained the sections were mounted in DPX (BDH).

2.4.2 Haematoxylin

Sections for GFAP and PrP immunostaining were counterstained with haematoxylin to demonstrate nuclear detail (see Appendix for details of the staining method).

2.5 Immunocytochemistry

2.5.1 GFAP immunocytochemistry

Representative sections from the affected and control cases were processed for GFAP immunocytochemistry. The rabbit anti-GFAP antibody was used at a dilution of 1:3000 (DAKO), with a goat anti-rabbit link antibody (1:10, Sigma) and a rabbit (1:40) peroxidase anti-peroxidase (PAP) complex (ICN).

The paraffin wax-embedded sections were hydrated in:

1. xylene 2 min.
2. absolute alcohol 2 min.
3. methylated spirit 2 min.

4. water 2 min.
5. Lugol's iodine 1 min.
6. water 1 min.
7. 5% sodium thiosulphate (hypo) 1 min.
8. water

Endogenous peroxidase activity was quenched by immersing the slides in 3% hydrogen peroxidase (in absolute alcohol) for 30 minutes, followed by a wash in running water for 30 minutes. Incubating in 10% normal goat serum (NGS) in PBS for 2 hours at room temperature blocked non-specific binding. Sections were incubated in the rabbit anti-GFAP antibody in 1% NGS in PBS overnight at 4°C.

Sections were adjusted to room temperature and washed for 30 minutes in PBS (6 changes), followed by the goat anti-rabbit link antibody in 1% NGS at room temperature for 1 hour. Excess antibody was removed by washing in PBS for 30 minutes (6 changes) and the sections incubated in the PAP complex for 30 minutes at room temperature. Excess was removed by washing in PBS for 30 minutes (6 changes). The chromogen was developed in filtered 0.1M phosphate buffer (pH 7.3) containing 0.5mg/ml 3,4,4`,4`,-tetraminobiphenyl hydrochloride (DAB) and 0.003% hydrogen peroxide until the required colour intensity had been achieved (30 sec. to 5 min.). Sections were washed in running water, dehydrated and mounted in DPX (BDH).

2.5.2 PrP immunocytochemistry

Representative sections from the affected and control cases were processed for PrP immunocytochemistry. The monoclonal PrP antibody 3F4 was used and the pre-treatment of the sections included incubation in 96% formic acid for five minutes, then incubation in 4M of guanidine thiocyanate for two hours and then hydrated autoclaving at 121°C for 10 minutes.

The PrP immunocytochemistry was performed at the National Creutzfeldt-Jakob Disease Surveillance Unit in Edinburgh and details of the methodology have been published (Bell *et al* 1997; Goodbrand *et al* 1995).

2.6 Morphometry

2.6.1 Histopathological assessment

Sections from the spinal cord and brain from the affected cases were assessed for the presence of vacuolar changes, both intraneuronal and in the surrounding neuropil. The presence of neuronal loss was determined, by comparison to control cases, and the degree of glial reaction was assessed.

2.6.2 Vacuolar score

Sections from the brains of affected cases, in which the level of the section correlated to standard levels described by Fraser and Dickinson (1968) were assessed for the degree of vacuolation in nine defined areas. The limits of these nine areas were defined by Fraser and Dickinson (1968). The degree of vacuolation in these defined areas was ascribed a subjective numeric score ranging from zero to five, according to the degree of vacuolation grossly visible (Fraser and Dickinson 1968).

The numeric score was converted to a graphically depicted lesion profile (Fraser and Dickinson 1973), which allows comparison with the lesion profiles published for TSE's in other species, including that described in bovine spongiform encephalopathy (Wells *et al* 1992)

2.6.3 Astrocytic response

Assessment of the astrocytic response was performed by comparison of glial numbers and appearance of affected cases and control cases on H&E sections. The distribution and intensity of GFAP immunocytochemistry of affected and control cases was compared. In both methods the comparison was purely subjective and no attempt at exact quantification was made.

2.6.4 PrP distribution

The appearance and distribution of the PrP^{Sc} deposition, by means of PrP immunocytochemistry, was compared in affected cases and control cases. This was performed at all brain and spinal cord levels available.

2.7 Image recording

Photographs of histological sections were taken on an Olympus Vanox-S. Suitable images were scanned from transparencies using a Coolscan II (Nikon) and the images printed on an Epson Stylus Color 800.

3. A CLINICAL APPRAISAL OF FELINE SPONGIFORM ENCEPHALOPATHY

3.1 Introduction

The clinical signs and epidemiology associated with FSE have been reported. These descriptions have been limited to a single large series of cases (Pearson *et al.* 1993), supplemented by a number of individual case reports (Bratberg *et al.* 1995; Leggett *et al.* 1990; Synge and Waters 1991). Details of the published clinical presentation of FSE are summarised in chapter 1. The present study allows comparison with the single published large series of cases (Pearson *et al.* 1993) and contributes to the limited published database available on FSE.

3.2 Aims

One major aim of the present study was to present the clinical appearance of pathologically-confirmed cases of FSE seen at Glasgow Veterinary School and compare these to the published findings, thereby improving the clinical knowledge of FSE. Attempting to define clinical criteria for presumptive ante-mortal diagnosis of FSE was of particular interest. A limited epidemiological discussion is presented on cases where appropriate details are available.

3.3 Materials and Methods

3.3.1 Selection of cases

The cases were sourced from a wide distribution over northern England and Scotland, details of the geographic distribution are presented in the discussion on the epidemiology. Two of the cases were presented to the Glasgow University Veterinary School for post mortem examination after telephonic discussion with the referring veterinary surgeon. The other seven cases were presented to the Department of Clinical Studies for investigation. Only cases where the diagnosis of FSE was confirmed on post mortem examination are included in this study. Details of the cases included in this study are presented in Table 5.

	Case number	Age at euthanasia	Breed	Sex
1	XN1241	9 years	Domestic Short Hair	Male Neutered
2	114446	8 years	Domestic Short Hair	Female Neutered
3	117393	6 years	Domestic Short Hair	Female Neutered
4	118260	9 years	Domestic Short Hair	Male Neutered
5	123398	5 years	Domestic Short Hair	Female Neutered
6	126399	7 years	Siamese Blue-point	Female Neutered
7	129453	3 years	Domestic Short Hair	Female Neutered
8	131333	9.4 years	Domestic Short Hair	Female
9	134380	6 years	Domestic Short Hair	Male Neutered

Table 5 Clinical details of the FSE cases (domestic cats) included in this study.

3.3.2 Investigation of cases

The signalment was obtained for all cases. Full histories were obtained, where possible, by a retrospective review of the clinical records and hospital database or, in the four cases presented during the course of the study, by detailed questioning of the owners. Recorded details in some of the retrospectively identified cases were incomplete.

A full clinical and neurological examination was performed as detailed by Oliver *et al* (1997) (for details see chapter 2), including a fundic examination. The following functional tests were found to be the most useful in the FSE cases:

- Allowing the cat to walk unimpeded around a consulting room, having first given the cat chance to become accustomed to the room. The cat's posture was evaluated while the cat was walking around the room and by getting it to stand still on the examination table.
- The cat's interaction with the environment was assessed, as was the cat's level of consciousness and response to visual, auditory and tactile stimuli.
- Weakness was evaluated by hopping and wheelbarrow, hemi-stand, hemi-walk and extensor postural thrust testing. Due to the timidity and/or aggression displayed as part of the clinical presentation these tests were either performed only partially or impossible to perform in a number of the cases.
- Proprioception, or the animal's awareness of the position of its limbs in space, was evaluated by paw position testing and further sensory function was studied by tactile placing and visual placing tests. Again this was not possible in all cases due to the aggression and/or timidity which was a feature of FSE.
- The spinal reflexes evaluated included, in the hind limbs: the patellar reflex, pedal reflex and assessment of muscle tone; in the forelimbs: the extensor carpi radialis reflex, pedal reflex and muscle tone; and along the thoraco-lumbar spine the panniculus reflex. All limbs were assessed for the presence of muscle atrophy.
- A full cranial nerve examination was performed, including assessing the sympathetic supply to the cranial region.

In one of the two cases presented for post mortem examination a detailed history and full clinical and neurological examination were available from the referring veterinary surgeon, while in the second case only limited information was available.

A variety of laboratory investigations were performed, including complete blood count, biochemistry, virus and toxoplasmosis status determination, cerebrospinal fluid (CSF) analysis, bone marrow aspirates and serum thyroxine determinations. The results were compared to the normal laboratory values.

3.3.3 Therapies

The TSE's are progressive and invariably fatal neurodegenerative disorders for which there are no recognised therapies. Due to the absence of a definitive ante-mortem test to confirm the presence of FSE, a number of cases in this series where tried on a variety of drugs, as treatment for other potential differential diagnoses. Details of the therapies administered in the various cases are presented in Table 6.

3.3.4 Epidemiology

Due to the strong circumstantial evidence linking the outbreak of FSE to contamination of cat feed with BSE material, detailed dietary information was obtained from each of the affected cases to assess if any risk factor could be identified. Available dietary information of the cases is presented in Table 9. The geographic distribution of the cases was examined, to determine if any regional differences could be identified, taking into account the normal area serviced by the veterinary school.

	Case	Therapies
1	XN1241	Details not available
2	114446	Treatment # on day 1, 3 and 7 with: • Betamethasone injectable (Betsolan, Mallinckrodt Veterinary) • Vitamin B_{12} injectable (Anivit B_{12}250, Animalcare) • Amoxycillin injectable (Amoxypen LA, Intervet)
3	117393	Details not available
4	118260	Treatment # on day 1 and 2 with: antibiotic injection Treatment # on day 4 and 7 with: antibiotic and steroid injections
5	123398	Details not available
6	126399	Treatment # on separate dates with: • Steroid injection and diazepam tablets (Valium) • Potentiated sulphonamide injectable (Zaquilan 20%, Mallinckrodt Veterinary) and antibiotic tablets
7	129453	Treatment # with: • Canaural ear drops and antibiotic tablets
8	131333	Treatment # with: • Dexamethasone injectable (Voren 14, Boehringer Ingleheim) • Prednisolone 5mg tablets (Prednicare): one tablet daily for five days Treatment with: • Prednisolone 5mg tablets (Prednicare)
9	134380	Treatment # with: • Clavulanate potentiated amoxycillin tablets (Synulox 50mg tablets, Pfizer) • Steroid and Ovarid tablets

Table 6 Details of therapies administered (day numbers are days from the start of treatment; the symbol # implies that the treatment was given at the referring veterinary surgery, available details are provided).

3.4 Results

3.4.1 Number of cases in the study

Nine cases of FSE were identified in domestic cats in this series. Details of the cases are presented in Table 5. Five cases were female neutered, one was an entire female and the remaining three cases were male neutered. Eight of the cases were domestic short hair cats and the remaining case a blue-point Siamese.

3.4.2 History

All of the cases presented with a combination of behavioural changes and ataxia. In all cases the onset of the behavioural changes preceded or coincided with the onset of the ataxia.

The altered behaviour and ataxia were progressive in all of the cases and varied in onset from 2 weeks to nine months prior to presentation. Not all of the behavioural changes were evident in all cases, and various combinations were present. Only limited details were available in some of the cases.

The behavioural changes reported by the owners are summarised in Table 7. All of the cases were characterised by the presence of hyperaesthesia. The hyperaesthesia was present in response to auditory stimuli in all cases, but in a number of cases it was additionally noticed in response to tactile and visual stimuli.

In one case the owners reported that the cat had started urinating and defecating inappropriately in the house. In another two cases, the owners remarked that the cats had altered their toilet habits. In one case the cat started using its litter tray where it had never used it before (this case also developed extreme reluctance to go outside) and a further case had difficulty using the litter tray because of the moderate ataxia which had developed by that stage.

The altered behaviour in six of the cases was characterised by marked timidity, with the cats hiding under furniture and appearing apprehensive if people approached. Three of these cases demonstrating timidity also displayed aggression, one towards other cats in the house and one towards strangers. Five of the cats displaying timidity appeared reluctant to venture outside or into open spaces and in some of the cases the owners remarked that cats which had previously appeared outgoing and independent did not now leave the house voluntarily.

Two cats were reported to be sleeping in unusual places and not where they usually slept. One of these cases started sleeping on the banister of the stairs, while the other case started sleeping on the back of chairs. In both cases, due to the development of the ataxia, the owners remarked that they would frequently fall off.

In two cases the owners remarked that the cats were not interacting normally with the other cats in the household, manifesting either as the affected cat ignoring normal cats in the household, or vice-versa.

Decreased grooming was a feature of three cases, although in one of these cases the owners ascribed this to the marked ataxia and therefore a physical inability to both maintain its balance and groom. In only one case was there evidence of pruritis, which was based on the presence of a positive nibble reaction when the cat was scratched. A noticeable feature in five of the cases was the presence of hypersalivation.

One case appeared dull and depressed according to the owners. In one case the cat had difficulty eating, which was ascribed to the presence of the ataxia and intention tremor.

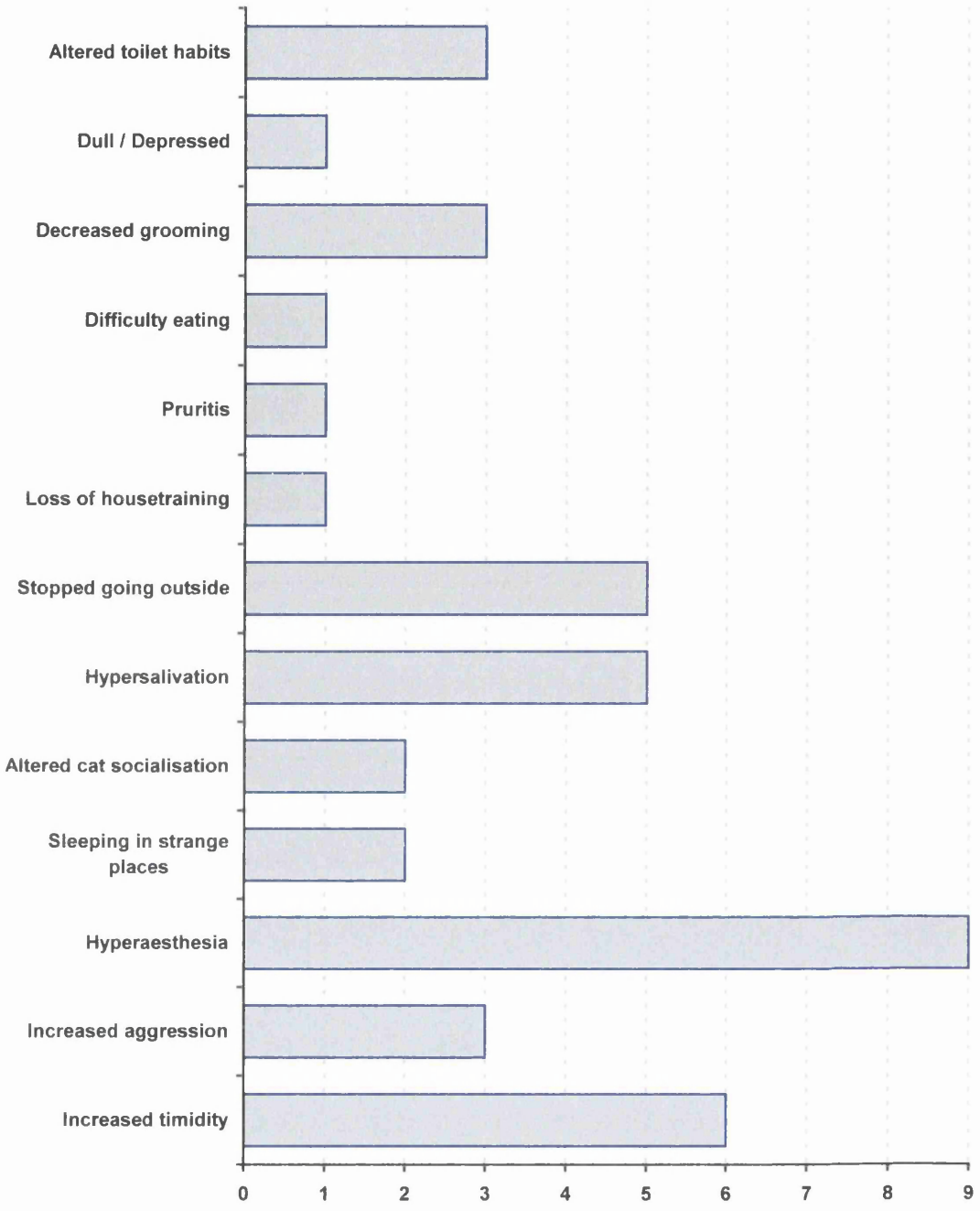


Table 7 Reported altered behaviour, and relative frequency at which they occurred (due to limited records some aspects may be under-reported).

3.4.3 Physical examination

Apart from the abnormalities discussed under the history (including hypersalivation, saliva staining of the face and decreased grooming) the only significant abnormalities detected on the physical examination are discussed under the neurological examination (including ataxia and intermittent pupillary dilation).

In three cases, routine ophthalmologic examination (including a fundic examination) did not reveal any abnormalities. No details are available as to whether the remaining cases had an ophthalmologic examination.

3.4.4 Neurological examination

The neurological findings were characterised by a multifocal lesion distribution, dominated by a cerebellar-based ataxia and tremor. Serial neurological examinations demonstrated a progression of signs. The findings of the neurological examination are summarised in Table 8.

Ataxia was present in all cases and in 6 of the 9 cases the hind limbs appeared more severely affected than the forelimbs. In two cases there were only limited details available of the neurological examination. Many of the other features may, therefore, be under-represented.

A tremor was frequently present and included mild involvement of the head and tail at rest and the presence of an intention tremor. In addition a marked truncal sway was frequently present, becoming more noticeable with effort. Hypermetria was evident in three cases, a wide based stance in three cases, a crouched posture in three cases and an inability to jump up or down onto surfaces in four cases.

The falling and circling to the left in one case was interpreted as vestibular disease (due either to damage to the vestibular nuclei or nerve, or to loss of inhibition of the right vestibular nuclei).

As evidence of either brain stem or cerebral involvement, four cases had mild sensory deficits, including conscious proprioceptive deficits (CPD's), in the hind limbs.

A feature of the clinical picture in 6 of the 7 cases where details of a complete neurological examination were available, was an intermittent pupillary dilation, in the presence of normal pupillary light reflexes.

3.4.5 Further investigations

CSF analysis was successfully performed in two cases. In one cat the protein level, on a lumbar puncture, was elevated at 1150mg/l (normal <450mg/l), while it was within normal limits in the other case. The CSF cell count was within normal limits in both cases.

In the five cases where serology was performed for FeLV, and the four cases where serology was performed for FIV, there was a negative result. In the four cases where an FIP titre was performed, the titre was zero. The Toxoplasmosis titre was determined by means of a dye test in three cases, in two cats it was considered negative, while in a third cat the titre was elevated at 30 IU/l.

Routine biochemistry was performed in six cases, and in five cases routine haematology was performed. Although occasional anomalies were present, no consistently abnormal values were identified affecting a majority of the cases. The bone marrow aspirate performed in one case did not reveal any abnormalities. The thyroxine determination performed in two cases was within normal limits.

3.4.6 Therapies

In none of the cases where treatment was attempted was there any documented improvement on assessment by the owner, the referring veterinary or myself. Progression of the clinical signs was evident in cases eight, nine and ten, during the period of treatment. Details of the therapies attempted are presented in Table 6.

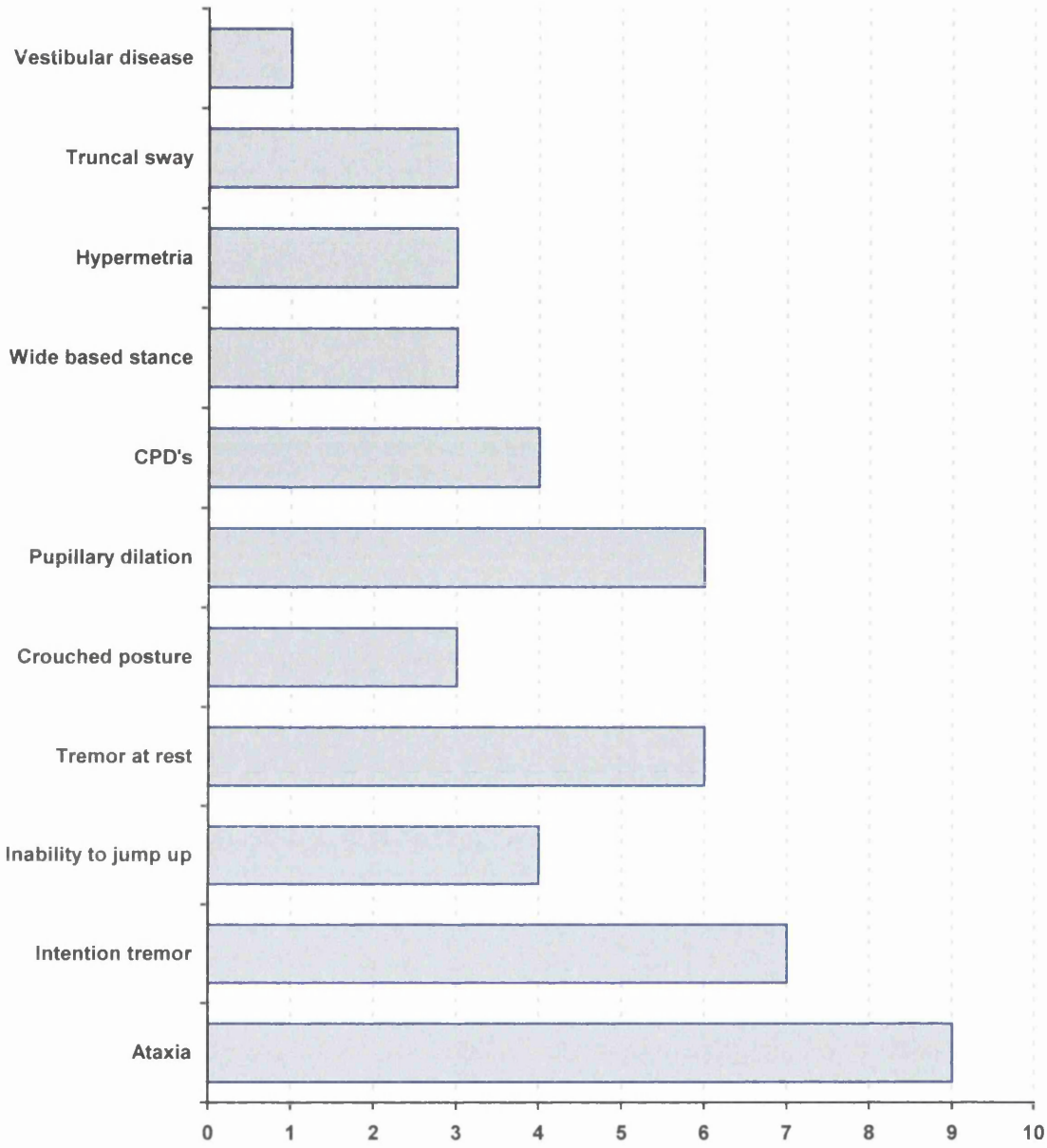


Table 8 Findings on neurological examination, excluding features in Table 7.

3.4.7 Epidemiology

3.4.7.1 Dietary information

In the majority of the cases the diet consisted of commercially available tinned cat food. A variety of the widely available brand types were represented. In addition a number of cases were fed additional table scraps and milk, although in all cases these additional components did not constitute the majority of the diet. Two cases were never given milk as a part of the diet, in an additional case the cat was given milk only when a kitten and not subsequently. The available dietary information is presented in Table 9.

3.4.7.2 Geographic distribution

Details of the geographic origin of eight of the cases are available. The majority (6 cases) originated from the region of Scotland immediately serviced by Glasgow Veterinary School (including Prestwick, Hamilton, Ayr, Uddingston, Lanark and Larkhall), while two of the cases originate from the midlands and north England (Leicester and Leeds).

	Case	Diet
1	XN1241	Not available
2	114446	Tinned commercial cat food Occasional dry cat food, tuna and chicken Never given milk
3	117393	Not available
4	118260	Tinned commercial cat food (predominantly fish flavoured) Occasional dry cat food, cheese, meat and table scraps Never given milk
5	123398	Not available
6	126399	Tinned commercial cat food Occasional milk and table scraps
7	129453	Tinned commercial cat food
8	131333	Tinned commercial cat food
9	134380	Tinned commercial cat food Very occasional ham and cheese to give pills Milk as kitten, but not subsequently

Table 9 Available dietary information.

3.5 Discussion

Although the number of cases in this series is limited to nine, the total number of pathologically confirmed cases of FSE to the 1st of January 1998 numbered only 84 worldwide, with 81 of those occurring in the UK. In relation to the total number of confirmed and the limited number of published cases of FSE (Bratberg *et al.* 1995; Leggett *et al.* 1990; Pearson *et al.* 1993; Synge and Waters 1991), a number of aspects, based on the clinical material presented in this study, are apparent.

The history and neurological findings in this series correlates well with published clinical details for FSE. The onset of the behavioural changes precedes or coincides with the onset of the ataxia, which is in contrast to that reported by Gruffydd-Jones (1997), but correlates with that reported by Pearson *et al.* (1993).

Some of the aspects evident from the history, signalment and clinical investigations include:

1. No evidence of a sex or breed predilection could be identified, in contrast to the male predominance in a previous series of FSE cases (Pearson *et al.* 1993).
2. No identifiable geographic clustering of cases could be demonstrated.
3. Routine clinical investigation (besides the findings apparent on the history and the neurological examination), including blood tests and serology, did not identify any clinical criteria that would support a diagnosis of FSE.
4. CSF analysis did not appear useful in identifying any criteria supporting the diagnosis of FSE. CSF analysis may, however, be of use to rule out other differential diagnoses, but an abnormal CSF analysis does not necessarily preclude a potential diagnosis of FSE. Addition consideration should be given to the potential zoonotic risk, when performing the collection and analysis of CSF in suspected cases.
5. No demonstrable improvement was apparent in any of the cases where treatment was attempted. In the absence of a specific ante-mortem test for FSE, a treatment regime designed to rule out other potential causes of the clinical signs would be justified.
6. Circumstantial evidence suggests that the origin of FSE is related to contamination of cat feed with BSE infected material (Bruce *et al.* 1997, Collinge *et al.* 1996). In

the seven cases in this series where detailed dietary information was available, the predominant dietary component was commercially available tinned cat food.

Based on the clinical features of the cases in this series, a set of clinical criteria were identified which, if they are present in a case, would allow a presumptive ante-mortem diagnosis of FSE.

1. All cases presented with progressive behavioural changes (details of which, and the frequency at which they occurred, are presented in Table 7) which preceded, or coincided with, the development of progressive ataxia.
2. Hyperaesthesia, either to auditory, tactile or visual stimuli was present in all cases.
3. Ataxia was present in all cases. The nature of the ataxia suggested a predominantly cerebellar location.
4. Hypersalivation was evident in five of the cases. Limited clinical details in some of the earlier cases and the fact that this feature was identified as being related to FSE only after a number of cases had already been described, suggests that this feature may have been underreported in the earlier cases and may therefore occur at a higher frequency.
5. Intermittent pupillary dilation with intact photo-motor reflexes was evident in six of the cases but for the same reasons as listed in point 4 may have been underreported.

The clinical presentation allows some prediction as to the site of the pathology that would be expected. The history and findings on the neurological examination are suggestive of a widespread and bilaterally symmetrical lesion within the CNS. In all cases, the altered behaviour and neurological deficits slowly progressed which would be consistent with a diffuse and progressive intracranial lesion. The presence of the ataxia, with variable combinations of: hypermetria, truncal sway, crouched posture, tremor at rest, inability to jump up and intention tremor are all consistent with a diffuse cerebellar lesion. The altered behaviour in the absence of seizures and with no evidence of a central blindness suggests a diffuse, but relatively mild, forebrain lesion.

For a more detailed correlation of the clinical presentation with the observed pathological changes see chapter four.

4. A Pathological Appraisal of Feline Spongiform Encephalopathy

4.1 Introduction and Aims

The pathology associated with FSE has been reported. These descriptions have been limited to a single large series of cases (Pearson *et al.* 1993), supplemented by a number of individual case reports (Bratberg *et al.* 1995; Leggett *et al.* 1990; Zanusso *et al* 1998).

Published pathological reports have predominantly concentrated on the spongiform changes evident in the grey matter. These spongiform changes have been described as widely dispersed in the CNS and consisting of discrete, round vacuoles in the neuropil with an increased prominence in the medial geniculate body, thalamus, corpus striatum, cerebellum and deeper cerebral cortex. Vacuolation of the perikarya of individual neurons has also been reported as part of the spongiform change, predominantly in the raphe nucleus, dorsal nucleus of the vagus nerve, red nucleus and vestibular nuclei (Pearson *et al.* 1993). A widespread glial reaction has been reported, as have changes in the white matter associated with axonal degeneration.

Immunostaining for the presence of the disease-related isoform of PrP in feline spongiform encephalopathy has been reported in only two domestic cats (Bratberg *et al.* 1995; Zanusso *et al* 1998) and in a puma (Willoughby *et al.* 1992). In one of the cats (Bratberg *et al.* 1995) there was strong immunolabelling for PrP in the grey matter neuropil of the head of the caudate nucleus, putamen and the cerebral cortex, and was similar to previous cases seen by one of the authors. In the second case report (Zanusso *et al* 1998) the authors claim to have identified a new variant of FSE with the presence of type-1 PrP^{Sc}, rather than the expected BSE-associated type-4 PrP^{Sc}.

In the puma PrP immunolabelling was reported in the medulla and cervical spinal cord. A more detailed review of the literature on the pathology of FSE and related spongiform encephalopathies is included in the introductory chapter (chapter one).

One of the aims of this study was to present a detailed description of the pathology of feline spongiform encephalopathy in the cases included in this study. The distribution and nature of changes in the brain and spinal cord were investigated, using classical methods and immunocytochemical techniques. This latter aspect includes an investigation into both the appearance and pattern of distribution of the disease-

related isoform of PrP deposition. An attempt is made to correlate the clinical presentation with the observed pathological changes.

4.2 Materials and Methods

4.2.1 Animals

Nine cats with a clinical diagnosis of FSE are included in this study. Of the nine cats, pathological material was available from eight of the cases. In the remaining case only descriptions of the original pathological report are available. Neural tissues from six of the cats were obtained after a retrospective review of cases presented to the pathology department at the University of Glasgow Veterinary School. Three further cats were obtained during the course of the study as cases admitted for investigation to the neurology service at the University of Glasgow Veterinary School. These cases where subsequently confirmed as feline spongiform encephalopathy on post mortem examination. Details of the control cases used in the classical histopathological methods and immunocytochemical techniques are presented in Table 4 of chapter 2.
Post mortem examination was performed in all of the cats shortly after euthanasia with intravenous injection of pentobarbitone sodium (Euthatal, Rhone Merieux Ltd.). In all cases the brain was removed and in those cases where the spinal cord was removed the integrity of the dural sheath was maintained where possible. The spinal ganglia were dissected out and removed with the spinal cord. The brain was fixed in 4% BNF (see appendix for fixatives). The spinal cord was suspended in a cylinder in 4% BNF and weighted to maintain it in straight alignment and avoid any bending which would have distorted the structure.
Details of the methodology of, and the precautions taken during the post mortem examination are presented in chapter 2.

Details of the tissues that were available and used in this study are presented in Table 10. As part of the study was performed as a retrospective review, full representative tissues were not available from all cases.

	Case no.	Available pathological material (individual tissue blocks)
1	XN1241	Medulla oblongata (level of rostral aspect of the dorsal nucleus of the vagal nerve) Medulla oblongata and cerebellum (level of the descending radix of the facial nerve) Midbrain (level of the commisure of the inferior colliculi) Thalamus and cerebral hemispheres (level of the infundibulum)
2	114446	Medulla oblongata (level of nucleus of accessory nerve) Medulla oblongata (level of radix of motor nerve of the vagus) Cerebellum (level of radix of motor nerve of the vagus) Midbrain (level of the commisure of the inferior colliculi) Cerebral hemispheres (level of septal nuclei)
3	117393	None available
4	118260	Medulla oblongata and cerebellum (level of vagal nerve) Thalamus and cerebral hemispheres (level of the pineal gland)
5	123398	Spinal cord – lumbar enlargement Spinal cord – thoracic segments Spinal cord – cervical segments Medulla oblongata (level of rostral aspect of the dorsal nucleus of the vagal nerve) Medulla oblongata and cerebellum (level of the genu of the facial nerve) Thalamus and cerebral hemispheres (level of the pineal gland) Cerebral hemispheres (level of septal nuclei)
6	126399	Spinal cord – thoracic segments Medulla oblongata and cerebellum (level of radix of hypoglossal nerve) Midbrain and cerebral hemispheres (level of the commisure of the inferior colliculi) Thalamus and cerebral hemispheres (level of the premamillary nuclei) Cerebral hemispheres (level of septal nuclei)

7	129453	Medullas oblongata (level of nucleus of accessory nerve) Medulla oblongata (level of radix of hypoglossal nerve) Cerebellum (level of radix of hypoglossal nerve) Medulla oblongata and cerebellum (level of the descending radix of the facial nerve) Thalamus (level of the premamillary nuclei)
8	131333	Spinal cord – sacral segments Spinal cord – sacral segments Spinal cord – lumbar enlargement Medulla oblongata and cerebellum (level of the medial vestibular nucleus) Cerebral hemispheres (level of septal nuclei) Cerebral hemispheres (level of the rostral limit of the caudate nucleus)
9	134380	Medulla oblongata and cerebellum (level of the glossopharangeal nerve) Midbrain and cerebral hemispheres (level of the commisure of the inferior colliculi) Thalamus and cerebral hemispheres (level of the pineal gland) Cerebral hemispheres (level of septal nuclei)

Table 10 Details of the tissues available from each of the pathologically-confirmed cases of feline spongiform encephalopathy used in this study.

4.2.2 Processing of tissue for paraffin wax sections

The brain was cut into transverse slices. In the FSE cases, prior to processing, the brain slices were immersed in a formic acid solution to decrease the potential infectivity of the tissues. In all the FSE cases the brain was processed in a Shandon Elliot 24-hour automatic tissue processor (Histokinette) as described in chapter 2. The smaller control brain slices (brain stem, cerebellum and occipital cortex) were processed in a Shandon Elliot 24-hour automatic processor. The larger control brain slices (thalamus and cerebral hemispheres) were processed in a Shandon Elliot seven day automatic tissue processor (Histokinette) allowing them to be sectioned whole, as described in chapter 2.

In those cases where the spinal cord was removed, one-cm lengths were removed from representative levels. Prior to processing, the spinal cord slices from the affected cases were immersed in a formic acid solution to decrease the potential infectivity of the tissues. The spinal cord slices were then processed in a Shandon Elliot 24-hour automatic tissue processor (Histokinette).

Paraffin-embedded tissue sections were cut on a Biocut 2035 microtome (Leica) and mounted onto Apes-coated microscope slides (for details see chapter 2). Paraffin wax sections were routinely stained with H&E (for details see chapter 2).

4.2.3 Morphometry

Details of the methods employed in the histopathological assessment, vacuolar score, astrocytic quantification and PrP distribution are described in chapter 2.

4.3 Results

4.3.1 Histopathological assessment of the brain and spinal cord

In all of the cases the described changes were very similar, and included the presence of grey matter neuropil vacuolation, intra-neuronal vacuolation, a reactive astrocytosis and Wallerian-type degeneration of selective white matter tracts.

In addition, in three cases evidence of an inflammatory process was identified by the finding of occasional mononuclear cell perivascular cuffs. In one case only a single perivascular cuff in the deep cerebellar white matter was identified, while in the other two cases occasional scattered small and medium sized perivascular cuffs were present in the brain stem and cerebellum.

4.3.1.1 Grey matter neuropil vacuolation

Vacuolation of the grey matter neuropil was present throughout the CNS. However, certain regions of the brain consistently appeared to be more severely affected. In the cerebellum the neuropil vacuolation appeared predominantly located in the granule cell layer, with occasional neuropil vacuolation present in the molecular layer (Figure 4). The pattern of neuropil vacuolation of the brain stem tended to mirror the distribution of the location of the grey matter itself with no particular area spared or severely affected. The only exception to this was the marked vacuolation of the cochlear nuclei. Throughout the brainstem to the level of the midbrain scattered occasional neuropil vacuolation was present. At the level of the thalamus marked neuropil vacuolation was present in the thalamic nuclei, hypothalamus and medial geniculate nucleus, with the surrounding nuclei, including the lateral geniculate nucleus, appearing to be less severely affected.

Particularly striking in the cerebral hemispheres was the band of neuropil vacuolation affecting the deeper cerebral layers (Figure 5). This was especially evident at the base of the cerebral sulci. The degree of neuropil vacuolation of the cerebral hemispheres was relatively mild. This was in stark contrast to the severe neuropil vacuolation of the basal nuclei (including the caudate nucleus, globus pallidus and the putamen) and the septal nuclei, with the neuropil vacuolation in certain regions of these nuclei becoming confluent. In the caudate nucleus the neuropil vacuolation appeared to become more prominent as the junction between the caudate nucleus and the internal capsule was approached. In the septal nuclei the neuropil vacuolation increased towards the ventral half of the nucleus.

In contrast to the deeper layers of the cerebral hemispheres and the basal nuclei, the hippocampus appeared to only have occasional evidence of neuropil vacuolation (Figure 6). On comparison of the hippocampus in affected cases to that in control cases, the density and thickness of the layer of double pyramidal cells appeared

decreased, indicating the possibility of neuronal loss in this region. Quantification studies were not, however, performed.

4.3.1.2 Neuronal vacuolation

Vacuolation of individual neurones appeared to follow a strongly conserved pattern, with certain nuclei never being affected, some nuclei being affected on an occasional basis or at a low level and some nuclei always being moderately or severely affected. Occasional small vacuolated neurones could be identified in the band of grey matter neuropil vacuolation in the deep cerebral layers and affecting occasional small thalamic neurones. The neuronal vacuolation was most noticeable in the sections through the brain stem.

The brain stem nuclei consistently affected at a high level included the dorsal nucleus of the vagal nerve and the dorsal and ventral raphe nuclei. The dorsal nucleus of the vagal nerve, which mainly comprised medium to small neurones, was identified in five of the cases. From 27% to approximately 50% of the neurones in this nucleus tended to be vacuolated, with the vacuoles being small to medium sized. The majority of affected neurones had single vacuoles with only the occasional neurone having two vacuoles (Figure 7).

The ventral raphe nucleus was identified in five cases, with additionally the dorsal raphe nucleus being identified in three of these cases. In all of the identified raphe nuclei approximately half of the neurones present in the nucleus were vacuolated. The vacuoles tended to be medium sized, with the occasional large vacuole. The incidence of multiple vacuoles in these neurones was higher than the dorsal nucleus of the vagal nerve, with some neurones having up to three or four vacuoles.

The hypoglossal nucleus, reticular formation and vestibular nuclei all were affected by occasional neuronal vacuolation. In the case of the hypoglossal nucleus this feature was identified in three of the six cases where the section level included the hypoglossal nucleus. In these three cases the vacuoles tended to be small and ranged from only a single neurone being affected, to three neurones being affected in the most severe case. Sections at the level of the reticular formation were available in eight cases. In six of these cases neuronal vacuolation was identified affecting the reticular formation. This was characterised by the presence of very large occasional vacuoles (up to four affected neurones in the most severely affected case) affecting

the nucleus reticularis giganto and in one case each, single neurones with large vacuoles in the lateral reticular nucleus and the ventral reticular nucleus.

Vacuolation of the vestibular nuclei neurones was present in the majority of cases (in five of the six cases where the level of the section allowed evaluation of the vestibular nuclei). The neuronal vacuolation of the vestibular nuclei was characterised by the presence of medium to large sized vacuoles occurring singularly in affected neurones, either in the lateral or the medial vestibular nuclei. This neuronal vacuolation of the vestibular nuclei was present at a low level, with the most severely affected case having four vacuolated neurones.

In one case approximately 25% of the neurones in the nucleus periventricularis praeopticus contained medium to small sized vacuoles, with the majority of the vacuoles occurring singularly. In a second case intra-neuronal vacuolation was identified in the nucleus periventricularis hypothalami, again occurring as single small to medium sized vacuoles at a moderate frequency.

4.3.1.3 Wallerian-type degeneration of white matter tracts

Wallerian-type degeneration of the white matter tracts was present throughout the CNS, but again certain regions appeared to be consistently affected to a greater degree. In the spinal cord the most severe changes were present in the lateral and ventral funiculi, particularly in the dorsal aspect of the ventral funiculus at the junction with grey matter. The dorsal funiculus, although still affected, had only occasional evidence of degenerating axons.

In the brain the pyramids, cerebral peduncles and internal capsule in the region of the corpus striatum were particularly severely affected, with the caudal cerebellar peduncles, rubrospinal tracts and in some cases the corpus callosum affected to a moderate degree.

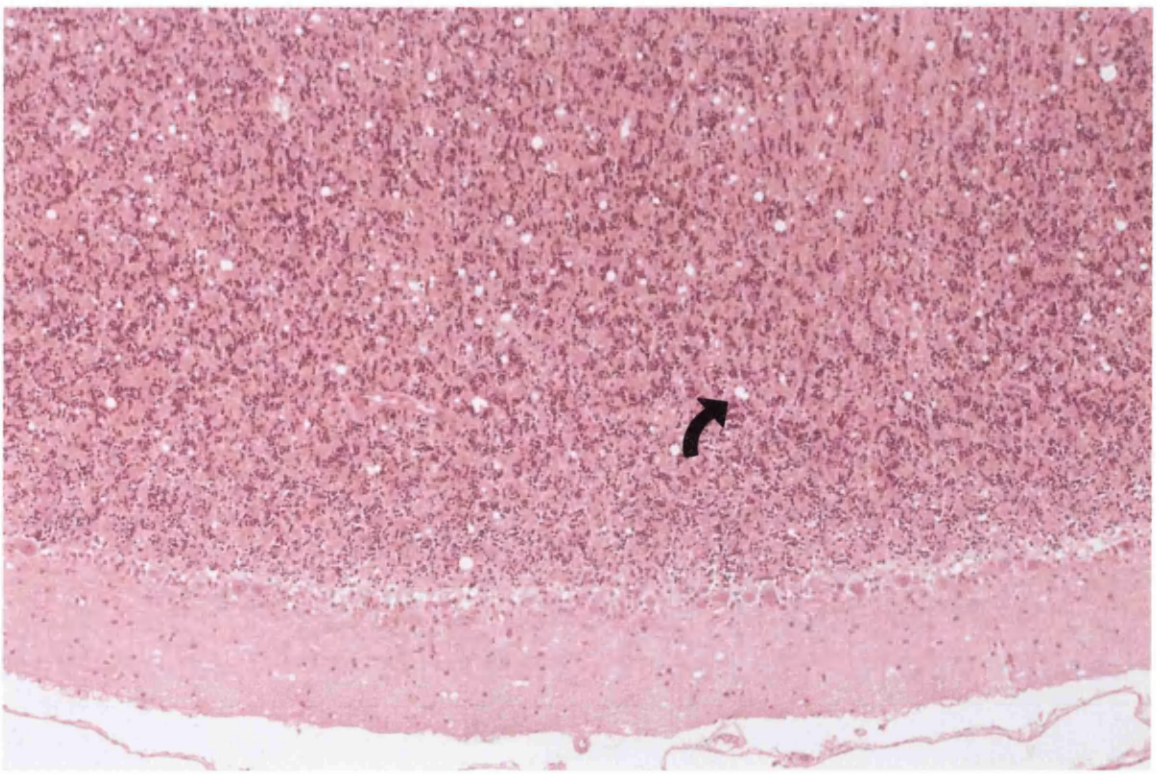


Figure 4 Transverse section through the molecular, Purkinje cell and granule cell layers of the cerebellum of an affected cat. The neuropil vacuolation is predominantly located in the granule cell layer (arrow) of the cerebellum. (H&E, 35x magnification)

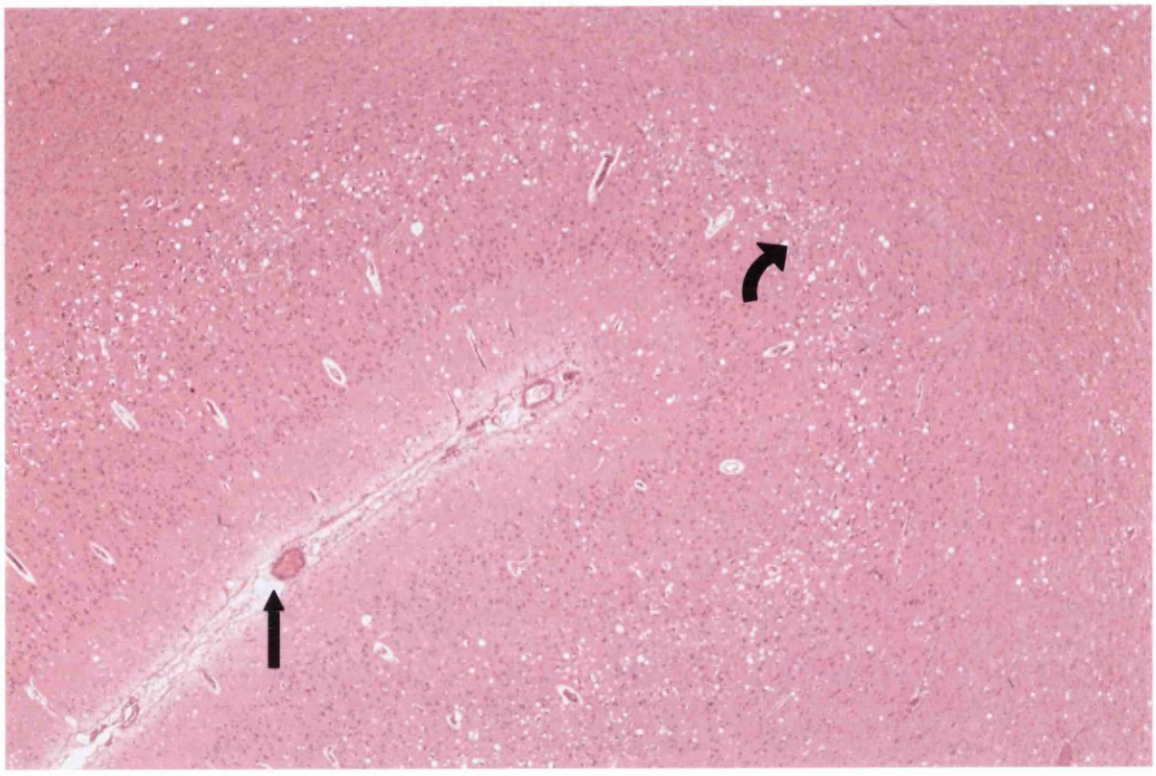


Figure 5 Transverse section through the base of a cerebral hemisphere sulcus (straight arrow) in an affected cat, demonstrating a band of neuropil vacuolation in the deeper layers of the cerebral cortex (curved arrow). (H&E, 30x magnification)

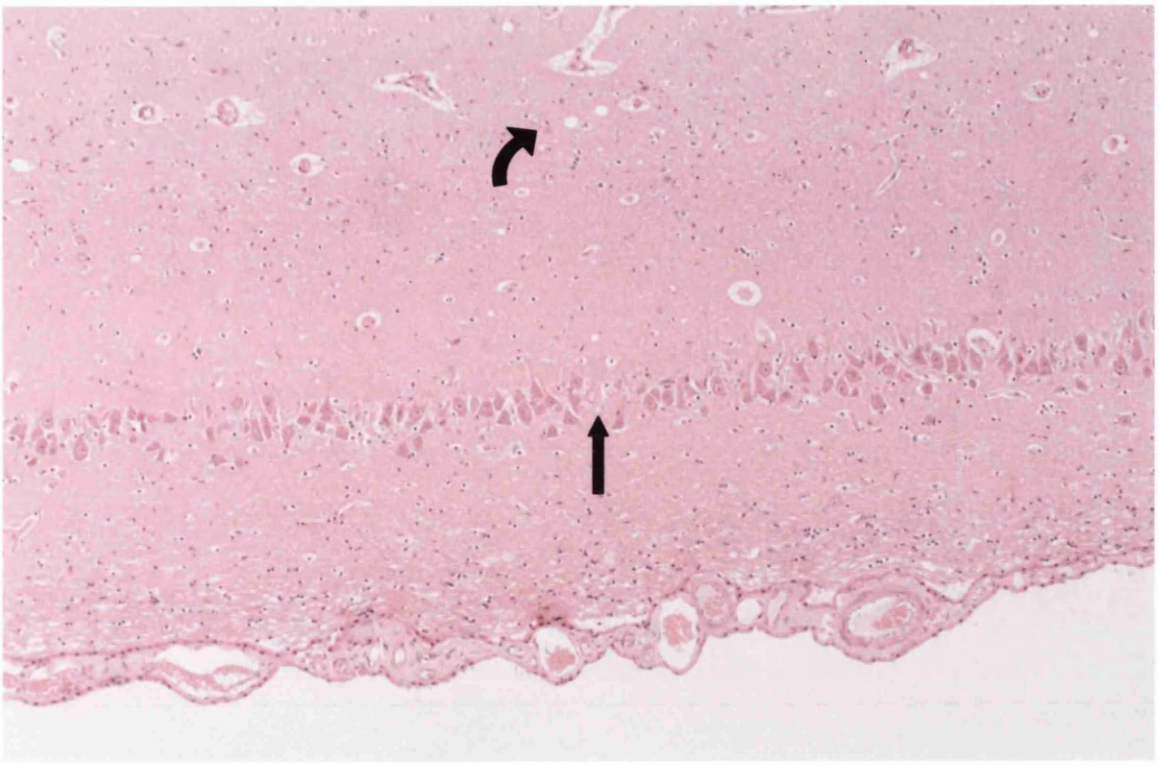


Figure 6 Only occasional neuropil vacuolation (curved arrow) is evident in the hippocampus of affected cats, although areas of neuronal loss may be present in the layer of double pyramidal cells (straight arrow). (H&E, 45x magnification)

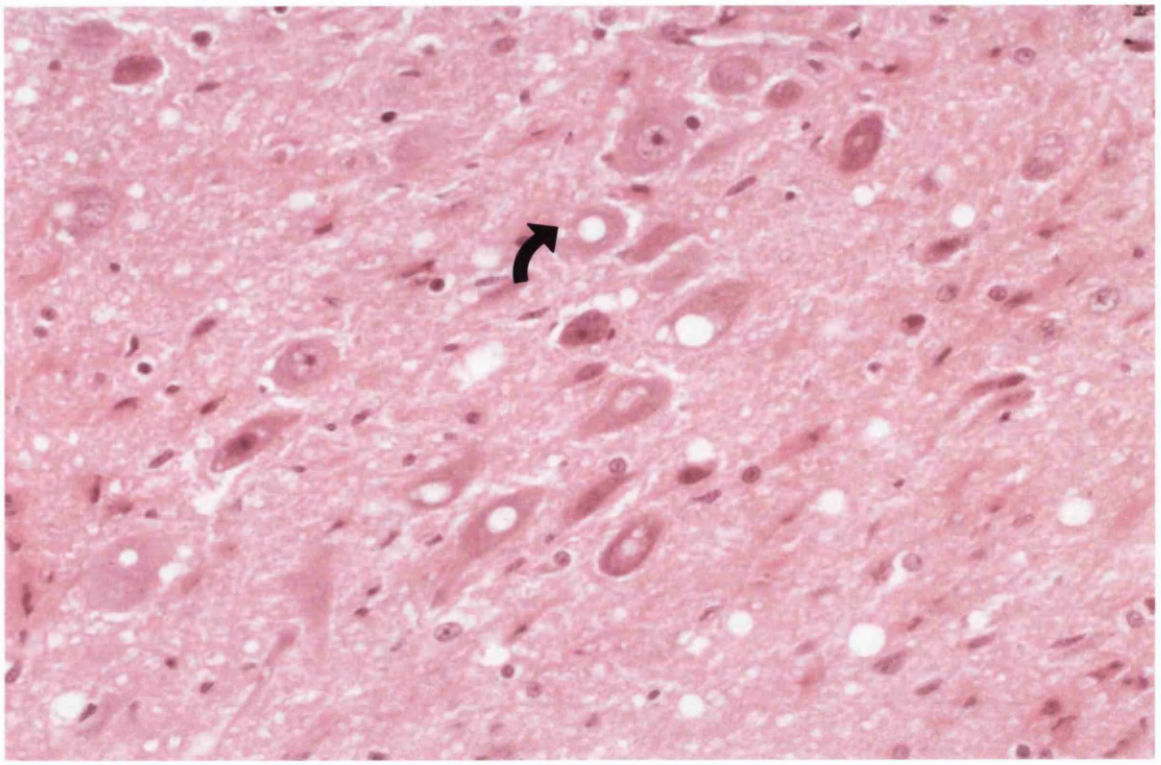


Figure 7 Vacuolation of the perikaryon of individual neurons (curved arrow) in the dorsal nucleus of the vagus nerve was a consistently identified feature in affected cats. (H&E, 285x magnification)

4.3.1.4 Astrocytic response

Widespread evidence of a reactive astrocytosis was evident in all levels of the brain examined, as compared to the neurologically normal control cases. The presence of a reactive astrocytosis could be demonstrated by a subjective increase in the number of astrocytes, by an apparent increase in the astrocytic cytoplasm on H&E sections and by an increased prominence of astrocytic cell processes on GFAP immunocytochemistry.

The reactive astrocytosis was particularly evident in the cerebellum, where in addition to an increase in the numbers of Bergmann glia, an increase in both astrocytic cell bodies and processes was evident. These changes were particularly evident on comparison of GFAP immunocytochemistry in control cases (Figure 8) to affected cases (Figure 9). The increase in astrocytic cell bodies was particularly evident in the granule cell layer and white matter tracts of the cerebellum, while astrocytic processes were evident in the molecular of the affected cases on comparison to with the controls.

In addition to the presence of a reactive astrocytosis, an increase in the numbers of microglia was evident on comparison of the affected cases to controls. Exact numerical quantification was not performed.

In the spinal cord a reactive astrocytosis was also evident, with the degree appearing to correlate with the severity of the neuropil vacuolation and in areas clusters of astrocytes were identified.

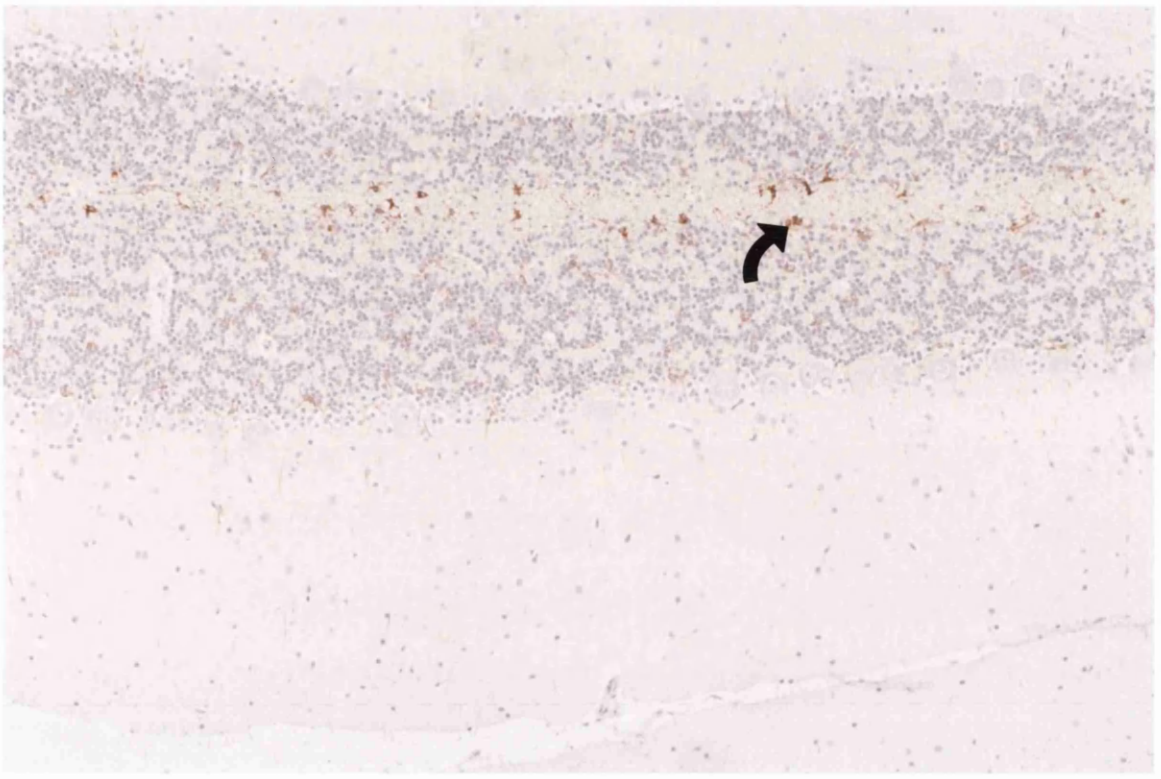


Figure 8 Transverse section through the cerebellum of a control case demonstrating the normal number of astrocytic cell bodies (curved arrow) and processes. The subarachnoid space, molecular layer, row of Purkinje cells, granule cell layer and white matter tracts can be identified. (GFAP peroxidase-antiperoxidase, 55x magnification)

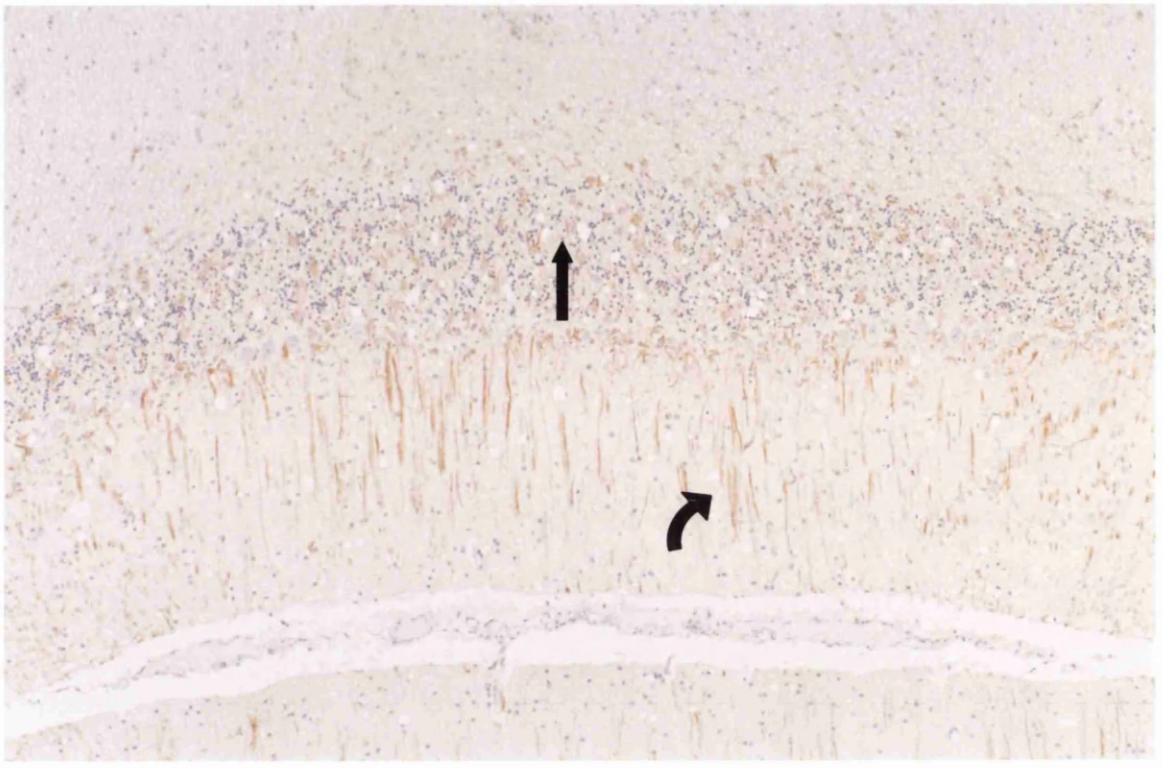
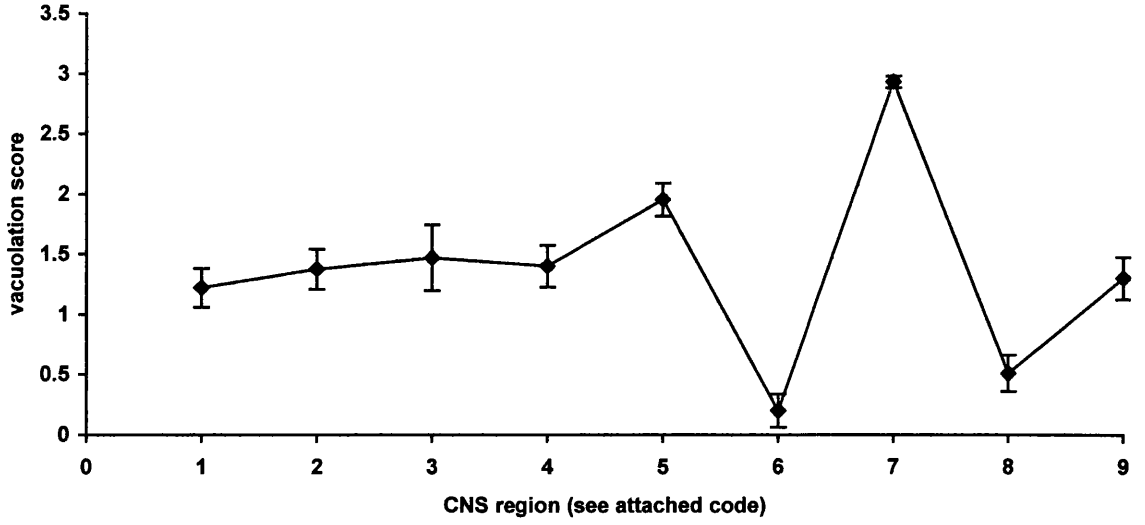


Figure 9 Transverse section through the cerebellum of an affected cat demonstrating an increase in number of both astrocytic cell bodies (straight arrow) and processes (curved arrow). (GFAP peroxidase-antiperoxidase, 40x magnification)

4.3.2 Vacuolar score

The lesion profile in this series of cases revealed a strongly conserved pattern of neuropil vacuolation. Due to limited availability of suitable pathological material in certain of the cases in this series, some of the vacuolar scores are based on observations in a reduced number of cases. Care must therefore be taken not to over interpret the results presented here. Details of the number of cases included in determining the average vacuolar score for each defined region are listed below. Consistent with observations that have already been made earlier in this study, certain regions appeared to be consistently more, or less severely, affected. The more severely affected regions include the thalamic nuclei, the hypothalamus and the septal nuclei. Particularly the hippocampus appeared to have only occasional neuropil vacuolation. The graphical presentation of the results as a visual profile is shown in Figure 10.

CNS regions:	**Cases included**
1. Dorsal medulla	8
2. Cerebellar cortex	8
3. Midline tectum	4
4. Hypothalamus	3
5. Thalamus	6
6. Hippocampus	7
7. Septal nuclei	4
8. Posterior, midline cerebral cortex	7
9. Anterior, midline cerebral cortex	5



CNS regions:	Number:
1. Dorsal medulla	8
2. Cerebellar cortex	8
3. Midline tectum	4
4. Hypothalamus	3
5. Thalamus	6
6. Hippocampus	7
7. Septal nuclei	4
8. Posterior, midline cerebral cortex	7
9. Anterior, midline cerebral cortex	5

Figure 10 Graphical representation of the vacuolar score of nine defined brain regions (lesion profile) in the eight FSE cases in this study where suitable tissue sections were available (sample statistics are mean ± standard error).

4.3.3 PrP Immunocytochemistry of the brain and spinal cord of cats with FSE

PrP immunocytochemistry revealed a very similar pattern of PrP deposition between all of the cases. This pattern was characterised not only by the intensity of the deposition, but also by the characteristics of the deposited material (for example in a granular pattern, linear pattern or a more homogenous pattern). The PrP deposition tended to occur in the grey matter neuropil, occasionally in a punctate pattern on the cell surface, thought to represent synaptic deposition of the PrP, with an absence of positive staining in the nuclei of individual cells.

In all control cases no evidence of positive staining for the disease-related PrP isoform was evident (details of the cases and tissue levels included are presented in Table 4 of chapter 2). These included both the neurologically normal control cases and the control cases with a non-prion-related neurological disease.

In the cerebellum of the affected cases there was a marked variation between the degree of PrP deposition in the different regions of the cerebellum. The granule cell layer appeared to be the region with the most intense PrP deposition, with the deposition tending to occur in a granular pattern. The molecular layer had a more homogenous and slightly lighter pattern of PrP deposition, while the Purkinje cell layer appeared to have only very mild PrP deposition. This resulted in the Purkinje cell layer appearing as a band of decreased PrP deposition between the molecular and granule cell layers. In the white matter tracts of the cerebellum there was a paucity of PrP deposition (Figure 11). In those cases including a section through the cerebellar nuclei, these nuclei appeared to have an slight increase in the degree of PrP deposition when compared to surrounding regions.

In the brain stem of the affected cases, up to the level of the thalamus, the pattern of PrP deposition tended to mimic the distribution of the grey matter neuropil. No demonstrable PrP deposition was present in the white matter tracts. The PrP deposition tended to occur in a predominantly granular pattern, with certain regions of grey matter neuropil and nuclei appearing to have more intense PrP deposition. The regions of grey matter neuropil with a more intense pattern of PrP deposition (with a characteristic granular pattern) included the dorsal nucleus of the vagus, the nucleus ambiguus, the nucleus gracilis and the nucleus of the spinal tract of the trigeminal

nerve. The brain stem nuclei with a more intense pattern of neuronal PrP deposition included the dorsal nucleus of the vagal nerve, the raphe nuclei, the lateral and ventral reticular nuclei, the cuneate nucleus, the olivary nuclei, the medial and lateral vestibular nuclei and to a lesser extent the hypoglossal nucleus. Similar to the marked neuropil vacuolation of the cochlear nucleus, this region demonstrated intense deposition of PrP in a granular pattern.

The granular pattern of PrP deposition was maintained as far rostral as the level of the thalamus, where the thalamic nuclei demonstrated heavy deposition of PrP. The deposition of PrP in individual thalamic neurones appeared particularly marked (Figure 12).

In the cerebral hemispheres the pattern of PrP deposition was particularly striking. In particular the basal nuclei (including the caudate nucleus, globus pallidus and putamen) and septal nuclei displayed intense PrP deposition. In the caudate nucleus a light homogenous pattern of PrP deposition was superimposed with a coarse granular pattern, with an additional linear pattern in regions. The linear pattern probably represents deposition of PrP along the processes of individual neurones. The degree of PrP deposition in the caudate nucleus tended to increase towards the junction of the caudate nucleus with the medially situated internal capsule (Figure 13).
The microscopic appearance was very similar in the globus pallidus and putamen to that in the caudate nucleus, while in the septal nuclei the superimposed granular pattern appeared more delicate and tended to become more intense towards the medial aspects of the septal nuclei.

In the cortices of the cerebral hemispheres the pattern of PrP deposition occurred in a linear band at the level of the deep cerebral layers, corresponding to the band of neuropil vacuolation (Figure 14). A second, less intense and narrower band of PrP deposition was present immediately beneath the surface of the cerebral hemispheres.

Within the spinal cord of those cases where relevant tissue samples were available the pattern of PrP deposition corresponded to the shape of the spinal cord grey matter. A band of more intense PrP deposition consistently occurred at the level of the second, and possibly extending down to the third, spinal cord lamina (Figure 15). Marked

deposition of PrP was evident in the large ventral horn neurones and the affected neurones tended to display a granular pattern of PrP deposition, thought to represent synaptic deposition of PrP (Figure 16). In the grey matter surrounding the central canal, variable PrP deposition was evident with some cases displaying minimal deposition in this region, while other cases had an average level of deposition. No PrP deposition was evident in the white matter tracts, although some PrP staining was evident in the ventral funiculus along cell process extending from the spinal cord grey matter, primarily in a horizontal pattern.

Figure 11 Characteristic patterns and degrees of PrP immunolabelling are evident in the cerebellum of affected cats. The heaviest deposition of PrP occurs in the granule cell layer with a granular pattern (straight arrow). The Purkinje cell layer (curved arrow) appears as a lightly labelled zone between the granule cell layer and the lighter, more homogeneously labelled molecular layer (open arrow). (PrP immunocytochemistry plus haematoxylin staining, 30x magnification).

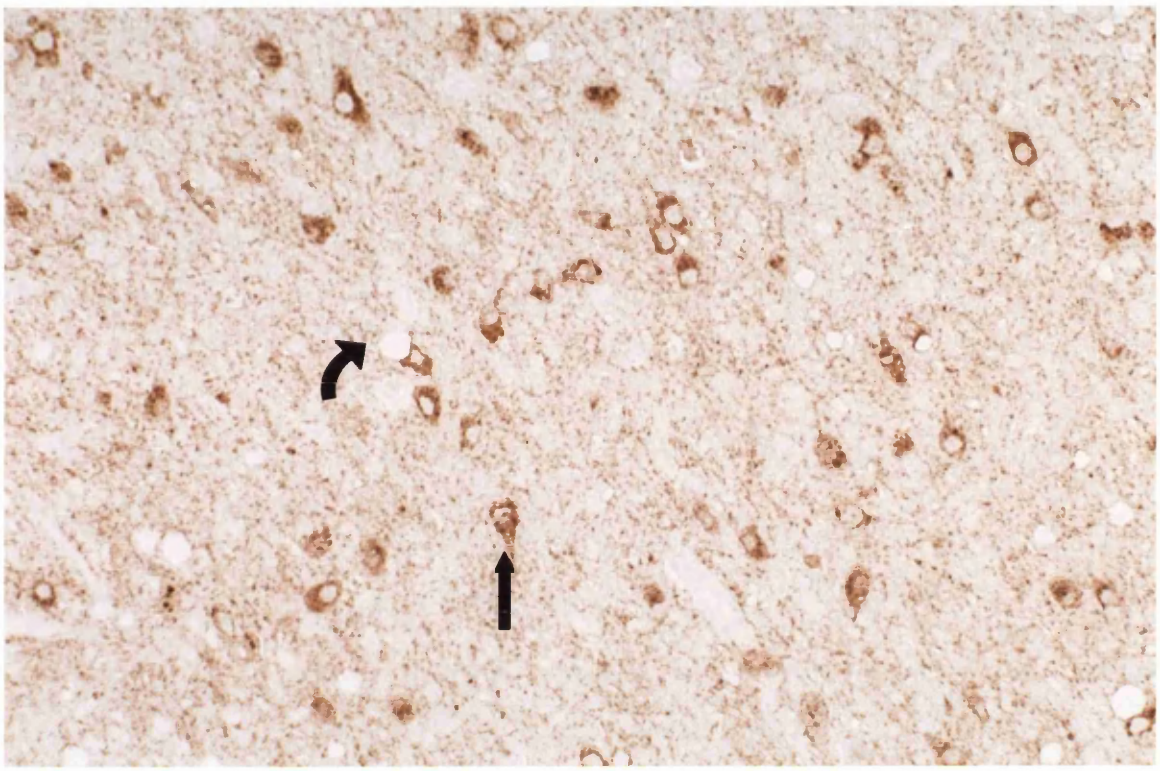


Figure 12 The pattern of PrP deposition in the thalamus of affected cats tended to occur in a granular or punctuate pattern, particularly affecting the neuronal perikaryon (straight arrow). Some vacuolation of the perikarya of individual neurons is evident (curved arrow) (the absence of immunolabelling of the nuclei should not be confused with vacuoles). (PrP immunocytochemistry plus haematoxylin staining, 105x magnification).

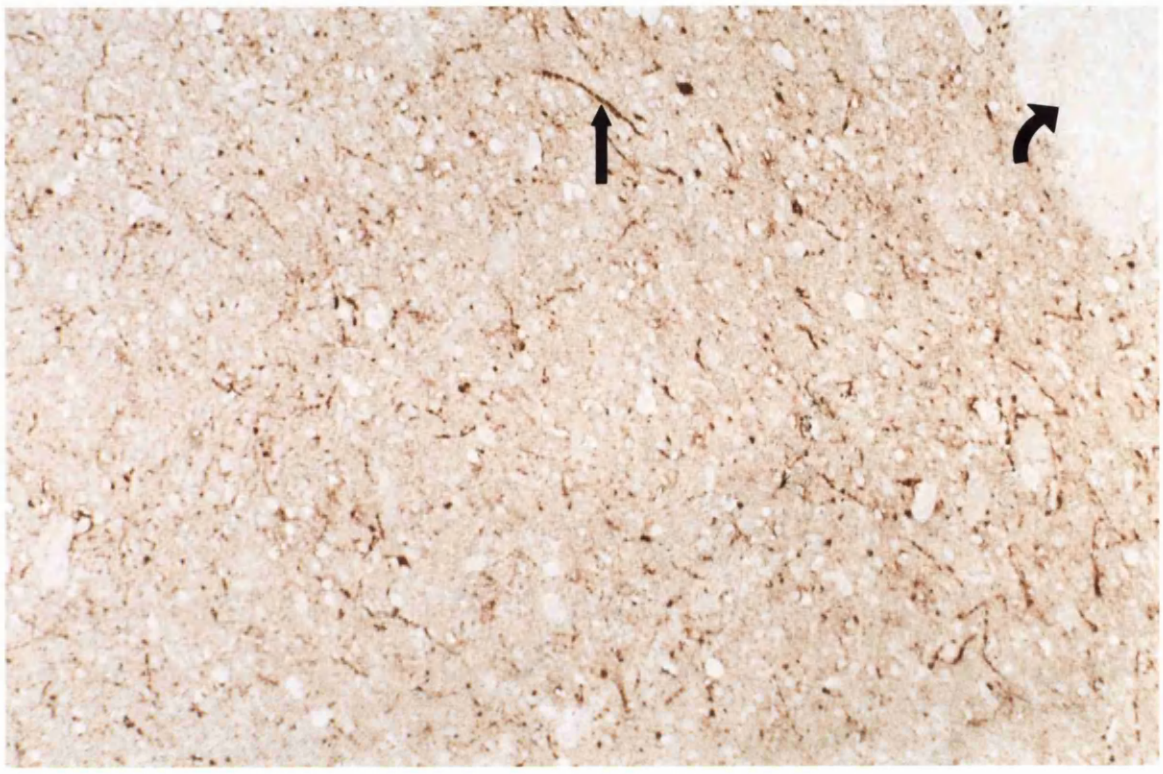


Figure 13 Transverse section through the head of the caudate nucleus demonstrating the characteristic linear PrP deposits (straight arrow) in an affected cat. The white matter tracts are visible at the top right of the picture (curved arrow). The linear PrP deposits are similar to those seen in vCJD. (PrP immunocytochemistry plus haematoxylin staining, 90x magnification)

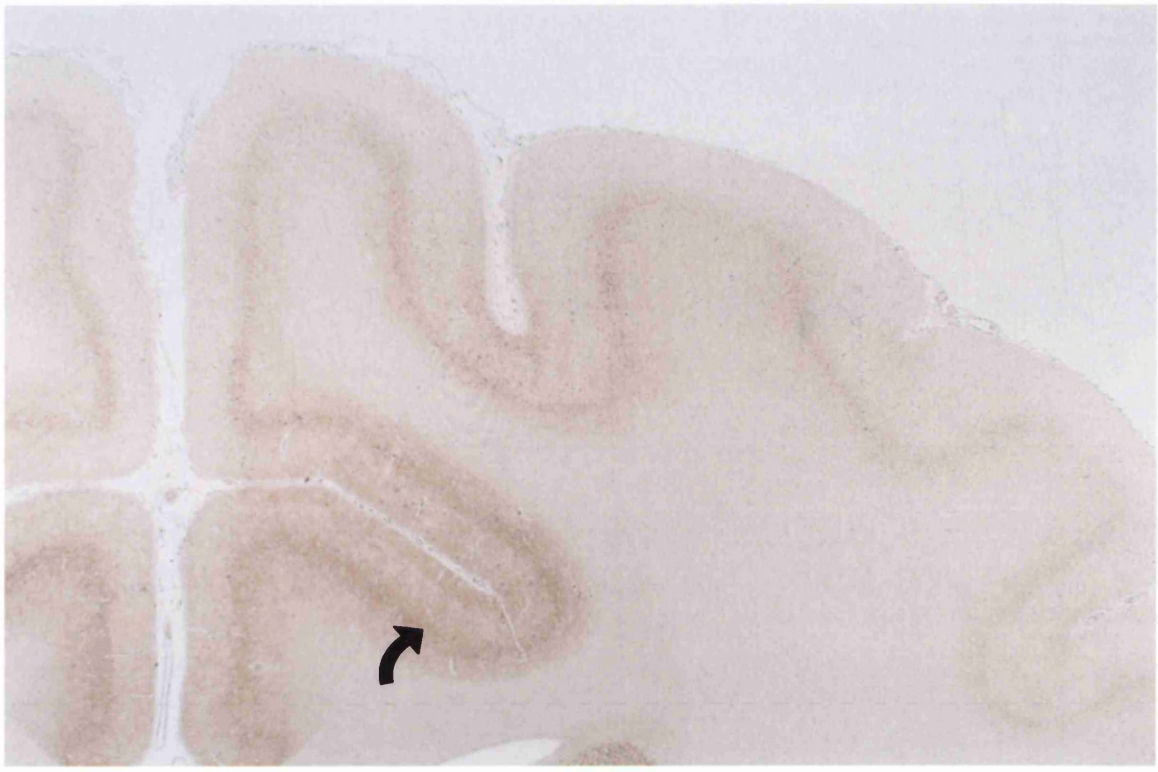


Figure 14 Transverse section through the dorsal and rostral aspect of the cerebral hemispheres demonstrating the PrP immunolabelling occurring predominantly in the deeper layers of the cerebral cortex (curved arrow). (PrP immunocytochemistry plus haematoxylin staining, 15x magnification)

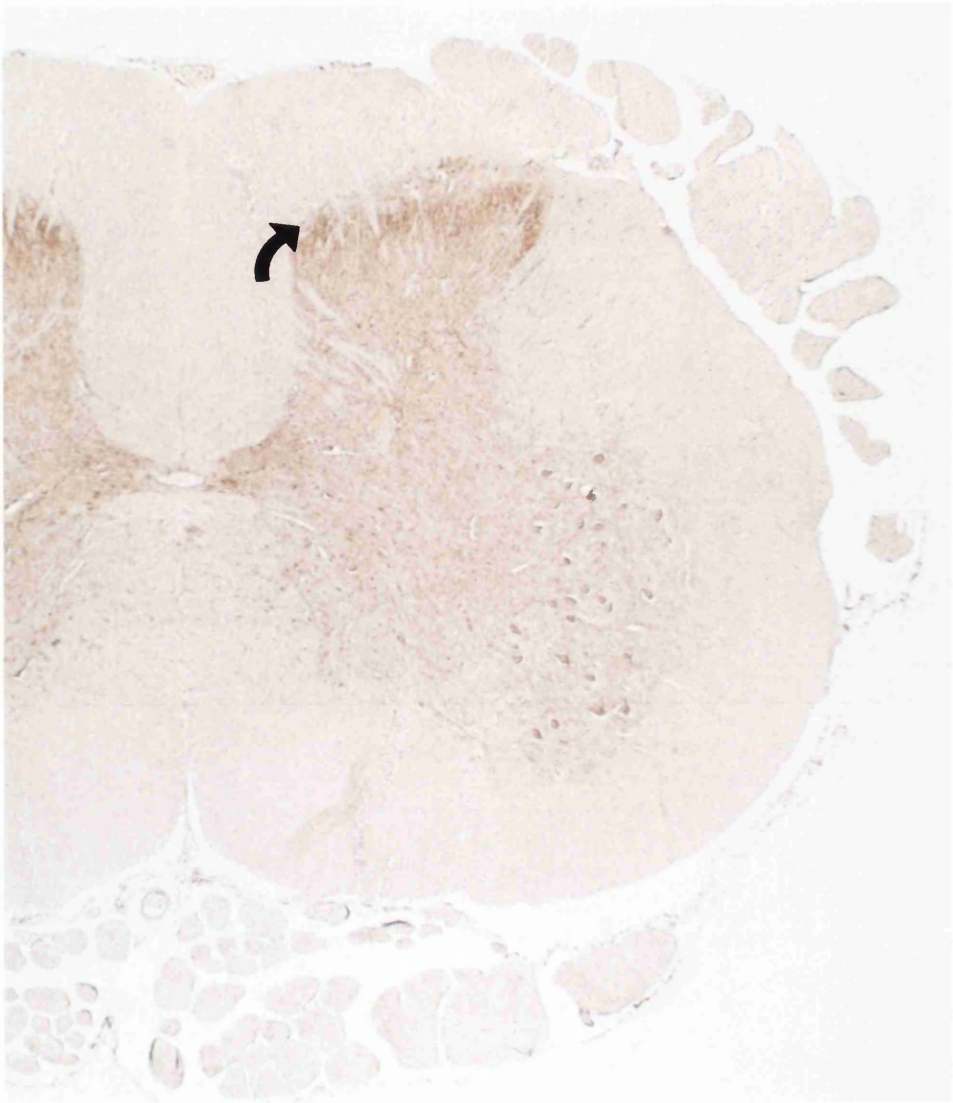


Figure 15 Transverse section through the lumbar spinal cord of an affected cat demonstrating PrP immunolabelling of the grey matter neuropil. The immunolabelling of the second and third grey matter laminae is particularly evident (curved arrow). (PrP immunocytochemistry plus haematoxylin staining, 22x magnification)

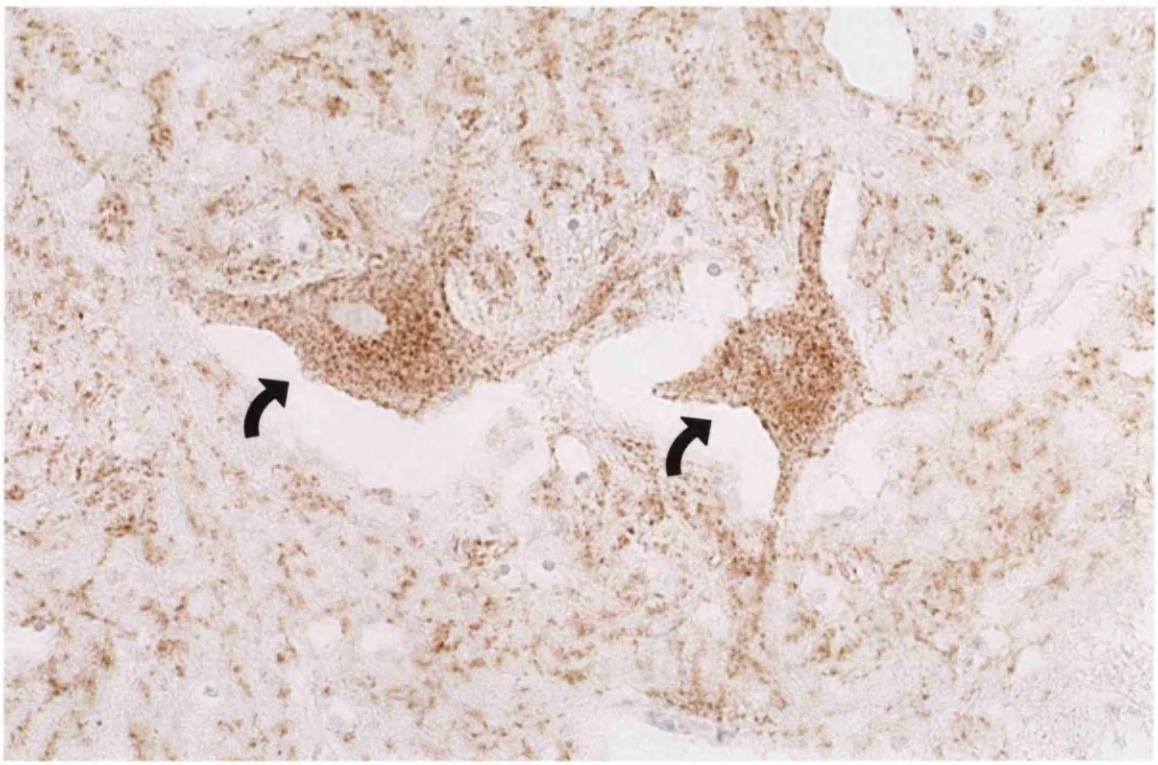


Figure 16 Transverse section through the lumbar spinal cord of an affected cat demonstrating the punctuate (synaptic) pattern of PrP immunolabelling of the ventral horn cells. (PrP immunocytochemistry plus haematoxylin staining, 240x magnification).

4.4 Discussion

The nature and distribution of the pathological changes reveals a very similar pattern in all the cases in this series. The significant changes are confined to the central nervous system (CNS) and include the classical changes to be expected in a transmissible spongiform encephalopathy. The changes include vacuolation of grey matter neuropil, vacuolation of individual neurones, a reactive astrocytosis and Wallerian-type degeneration of selected white matter tracts. Of note is the absence of any demonstrable amyloid plaques, which have been identified in certain other transmissible spongiform encephalopathies, including vCJD. The pathological changes in this series of FSE cases correlate well with the limited published details available (Pearson et al. 1993; Wyatt *et al.* 1991).

In three of the cases mononuclear cell perivascular cuffs are present in the brain stem and cerebellum. In one of these cases only a single perivascular cuff is present, while in the remaining two a low number of scattered perivascular cuffs are present. Perivascular cuffs have been identified in reported cases of FSE (Pearson et al. 1993) and their significance is uncertain. They may represent an incidental inflammatory process superimposed on the feline spongiform encephalopathy or an inflammatory component may exist as part of the FSE disease process. Inflammatory CNS disease due to an underlying infectious agent is a not uncommon diagnosis in the domestic cat. As these perivascular cuffs are only present in three cases, and the virus (FIP, FeLV and FIV) and Toxoplasma status in these three cases is not known, it is probable that these perivascular cuffs do represent an incidental superimposed inflammatory disease process. The finding of perivascular cuffs does therefore not preclude a diagnosis of FSE and care must be taken when evaluating feline post mortem samples not to overlook subtle spongiform encephalopathy lesions in the presence of mononuclear perivascular cuffs.

A set of criteria have been identified in this series of cases allowing characterisation of the expected nature and distribution of the pathological features in FSE cases arising in conjunction with the BSE epidemic. In the event of a future outbreak of a spongiform encephalopathy in domestic cats the pathological criteria described in this

study would allow comparison with those of the new outbreak in order to ascertain whether the same strain of infectious agent is responsible for both outbreaks.

The identified pathological criteria in this series of cases includes:

1. Widespread grey matter neuropil vacuolation within the CNS, with certain regions appearing to be more severely affected. These regions include the cerebral basal nuclei (putamen, globus pallidus and caudate nucleus), the septal nuclei, thalamic nuclei, medial geniculate nucleus, cerebellar granule cell layer, deep cerebral layers and to a lesser extent the spinal cord grey matter. In particular, the cerebral basal nuclei and the septal nuclei appear to have the greatest intensity of vacuolation. In contrast, some regions of the CNS grey matter appeared to have only occasional neuropil vacuolation, including the hippocampus and cerebellar molecular layer.
2. Vacuolation of individual neurones in this series of cases appears to have a very strongly preserved pattern with a predictable distribution. Nuclei that are particularly affected include the dorsal nucleus of the vagal nerve and the raphe nuclei (dorsal and ventral), in which the majority of the neurones are vacuolated. In other regions individual neurons appear vacuolated at a much lower frequency, although still very predictable in pattern. These nuclei include the vestibular nuclei, reticular formation, hypoglossal nucleus, small neurons of the cerebral cortex and occasional spinal cord grey matter neurones.
3. In all of the cases a widespread reactive astrocytosis in the CNS can be demonstrated. This is most striking in the cerebellum where, in addition to an increase in the prominence of astrocytic cytoplasm and numbers of astrocytic nuclei (including Bergmann glia) on H&E sections, a dramatic increase in astrocytic processes in the molecular layer on GFAP immunocytochemistry is evident.
4. Although Wallerian-type degeneration of the white matter tracts appears widespread, a number of white matter tracts are selectively affected. These include the pyramids, the cerebral peduncles, the caudal cerebellar peduncle, the internal capsule in the region of the corpus striatum and to a lesser extent the ventral and lateral funiculi of the spinal cord.
5. Characteristic patterns of pathological PrP accumulation are present in the CNS grey matter on PrP immunocytochemistry. In the cerebellum, the Purkinje cell

layer appears as a light staining band between the granule cell layer (with the most intense PrP deposition in a granular pattern) and the more homogeneously staining molecular layer. In the brain stem and mid-brain the PrP deposition occurs in a granular pattern, accentuating certain regions, particularly the thalamic nuclei. The most striking PrP deposition is present in the basal nuclei and septal nuclei of the cerebral hemispheres with a predominantly granular pattern, becoming linear in regions, with PrP deposition along cell processes. In the cerebral hemispheres the PrP deposition mirrors the band of vacuolation affecting the deeper cerebral layers.

The pathological abnormalities in these cases allow an explanation of some of the described clinical abnormalities. In all of the cases the pathological lesion are characterised by a widespread and bilaterally symmetrical distribution within the CNS. This is consistent with the multifocal and symmetrical localisation of the neurological abnormalities.

In particular, the presence of a symmetrical ataxia, with variable combinations of: hypermetria, truncal sway, crouched posture, tremor at rest, inability to jump up and an intention tremor are all consistent with the presence of the diffuse and symmetrical cerebellar lesion.

Anisocoria characterised by pupillary dilation contralateral to an asymmetrical cerebellar lesion, with intact pupillary light reflexes, has been reported as an occasional finding (DeLahunta 1983). The diffuse cerebellar lesion in this series of cases may therefore explain the presence of bilateral pupillary dilation with intact pupillary light reflexes. The precise mechanism is unclear (if the hypothesis is correct) and may relate to a loss of cerebellar modulation of the inhibitory prosencephalic pathways acting on the oculomotor general visceral efferent neurones.

The diffuse vacuolation affecting the deeper cerebral layers may allow explanation of the altered behaviour, with the lesion being insufficiently severe to result in seizures or decreased vision. Either the diffuse cerebral lesion or the selective vacuolation and PrP deposition of the thalamic nuclei may explain the conscious proprioceptive deficits found in a number of the cases.

The marked neuropil vacuolation of the basal and septal nuclei of the cerebral hemispheres is particularly evident in this series of cases. The functions of these nuclei are not fully understood, but experimental ablation of the caudate nucleus in the cat has been performed. In the experimental cases, removal of the caudate nuclei resulted in no obvious motor disturbance but behavioural abnormalities were noted, including the presence of hyperaesthesia (Villablanca *et al.* 1976). In the series of cases in this study the hyperaesthesia and some of the behavioural abnormalities can therefore be explained by the severe lesions in the caudate nucleus.

The presence of hypersalivation in a number of cases in this series is difficult to explain. One possibility may be facilitation of parasympathetic efferents from either the facial or glossopharyngeal nuclei due to a loss of higher centre inhibition of these nuclei. No obvious clinical signs can be ascribed to the consistent and severe neuronal vacuolation that is present in the dorsal nucleus of the vagus and the raphe nuclei. No inability to swallow food was identified in this series of cases and it is therefore unlikely that the clinical feature of "hypersalivation" is actually a consequence of dysphagia.

5. DISCUSSION

It is recognised that different strains of infective agent must be present to explain the patterns of disease among the described transmissible spongiform encephalopathies. A single strain of infective agent results in a strongly conserved pattern of disease in a specified host-type. Differentiation between the strains can be achieved by demonstrating the different disease characteristics they cause in both naturally occurring cases and in experimentally infected animals. In particular, the clinical presentation, incubation period and characteristic neuropathology (including the lesion profile of spongiform changes and distribution of PrP^{Sc} within the CNS) allow differentiation between the strain types.

Consistent with this strain hypothesis, all except one (Zanusso *et al.* 1998) of the reported cases of Feline Spongiform Encephalopathy (FSE) have a similar clinical presentation and neuropathology, suggesting that they have arisen from a common source. Based on the reported different clinical presentation and neuropathology, the case from Italy (Zanusso *et al.* 1998) is hypothesised to have arisen from a different strain of the infective agent, more consistent with that resulting in sporadic CJD.
The first case of FSE was reported in 1990 by Wyatt *et al.*, just over three years after Bovine Spongiform Encephalopathy (BSE) was first identified. Strong circumstantial evidence exists to suggest a link between the strain of infective agent responsible for FSE and BSE. This link is likely to be through a common origin, or from cats being exposed to either BSE infected tissues or a product of BSE infected tissues. Certainly the temporal and geographic occurrence of FSE suggests an origin from BSE rather than scrapie (Bradley 1997). The weight of evidence suggests that the source of infection in cats is likely to be related to the feeding of BSE-contaminated feed.

Supportive of the strain hypothesis, a number of studies have demonstrated a link between the agent causing BSE, new variant CJD, FSE and the TSE of exotic ungulates in the UK. This agent appears to be distinct from that causing iatrogenic and sporadic CJD (Collinge *et al.* 1996).
Transmission of FSE to mice (Fraser *et al.* 1994) has demonstrated similar incubation periods, lesion profile and percentage of affected mice as that of BSE but distinct from that of scrapie. The authors concluded that FSE and BSE are likely to have arisen from a similar source.

Western blots of PrP^{Sc} after proteolytic cleavage, in the sporadic and iatrogenic forms of CJD, allow differentiation between the type-1, type-2 and type-3 forms by evaluation of differing band sizes. Type-4 CJD, or new variant CJD, while having similar band sizes to type-3 CJD, can be clearly distinguished from the other three forms of CJD by a characteristic pattern of band intensities (Collinge *et al.* 1996; Aguzzi and Weissmann 1996). Similar western blot analysis of BSE, naturally occurring FSE and experimental BSE in a macaque revealed an almost identical pattern of band intensities, which was distinct from that of type-1, type-2 and type-3 CJD. From this it was concluded that new variant CJD, BSE and FSE are all due to infection from a common source, with the same strain of PrP^{Sc}.

A study by Bruce *et al.* (1997) demonstrated striking similarities in the lesion profile for RIII mice after transmission of new variant CJD from three cases, BSE from four cattle, FSE from two cats and a spongiform encephalopathy from a nyala and a kudu. This lesion profile was distinct from that generated by transmission into panels of these mice of scrapie from six sheep and sporadic CJD from two cases. As a further part of the same study, which is still on-going at the time of my study, a strong correlation has been demonstrated between the incubation periods in four strains of mice after transmission of new variant CJD from three sources, BSE from eight sources and FSE from two sources. This correlation is again distinct from that obtained by transmission of scrapie from six sources into the same panel of mice. The authors concluded that it is likely that the same agent strain is responsible for new variant CJD, BSE, FSE and the TSE in captive exotic ungulates in the UK.

Prior to my study only limited published clinical and pathological descriptions of FSE were available. Certain criteria have been identified in my study allowing the characterisation of the expected clinical and pathological profile of FSE cases occurring in conjunction with the BSE outbreak. These criteria are discussed in greater detail in the relevant chapters and allow the characterisation of the BSE-related strain of infective agent in cats. Should a future outbreak of a spongiform encephalopathy occur in domestic cats the identified clinical and pathological profile could then be compared to the criteria identified in this study in order to determine whether a different strain of infective agent was responsible for the new outbreak.

The clinical and pathological features in the different cats included in my study appear constant, suggesting that the same strain of infectious agent was responsible for all of the cases. Additionally the clinical and pathological features in this series of cases bear a strong resemblance to that described for vCJD, with in particular the initial altered behaviour followed by ataxia, and the marked pathological abnormalities with linear PrP deposits in the basal nuclei, being similar. The absence of demonstrable amyloid plaques in the FSE cases is a notable difference.

The FSE outbreak has resulted in a better understanding of TSE's with the opportunity to study freshly collected pathological material derived form a natural source. The cat is a useful model to allow predictions on the future disease prevalence in the human population. In both the cat and man the disease appears to have been derived from a common source, both populations are likely to have been exposed through ingestion of the infective agent and both man and the cat are monogastrids. The occurrence of a TSE in lemurs fed on bovine derived protein in a French zoo does, however, suggest that lemurs may be a better animal model in which to study TSE's as they have a closer genetic relationship to man.

Further research into FSE would allow further understanding of the disease process. To support evidence to suggest that PrP infectivity can be identified in the lymphoid tissues, analysis on samples taken from some of the cases in my study are ongoing. This includes PrP immunostaining on lymphoid tissue and Western blots on fresh frozen lymphoid and brain tissue.

The absence of an identified spongiform encephalopathy in the domestic dog suggests there may be a genetic difference in susceptibility between the cat and dog. Both the domestic dog and cat have been exposed to similar environmental and dietary risk factors. Sequencing of both the canine and feline PrP gene to allow comparison, and particularly codon 129, would be of interest. Furthermore, this would allow the importance of codon 129 in determining the genetic susceptibility to the BSE-related strain of infective agent in the domestic cat to be determined. This may allow a more accurate prediction of susceptibility of the human population to the BSE-related infective agent, with regard to PRNP codon 129 genotype.

6. APPENDIX

6.1 Tissue fixation and processing

6.1.1 Buffered neutral formaldehyde, 4% (BNF)

To prepare 1 litre of fixative:

40% formaldehyde*	100ml
tap water	900ml
sodium dihydrogen orthophosphate	4g
dipotassium hydrogen orthophosphate	8g

*40g paraformaldehyde in 100ml water, heat to between 60°C and 70°C, add1M sodium hydroxide drop by drop until the precipitate has cleared. Cool to 4°C.

6.1.2 Paraffin wax processing

Solutions used in the Shandon Elliot 24-hour automatic tissue processor (Histokinette) to prepare BNF perfused blocks from the spinal cord, brain and lymphoid tissues for paraffin embedding:

1)	70% methylated spirits / 5% phenol	2 hr	room temperature
2)	90% methylated spirits / 5% phenol	2 hr	room temperature
3)	methylated spirits	2 hr	room temperature
4)	ethanol / 5% phenol	2 hr	room temperature
5)	ethanol / 5% phenol	1 hr	room temperature
6)	ethanol / 5% phenol	1 hr	room temperature
7)	1% celloidin in methyl benzoate[1]	4 hr	room temperature
8)	xylene	1 hr	room temperature
9)	xylene	1 hr	room temperature
10)	xylene	1 hr	room temperature
11)	paraffin wax	4-6 hr	60°C
12)	paraffin wax	7 hr	60°C

[1]Necloloidine (Merck) solution for microscopy was considered to be 100% celloidin (1ml added per 100ml methyl benzoate)

Solutions used in the Shandon Elliot 7-day automatic tissue processor (Histokinette) to prepare BNF perfused blocks from the brain, primarily larger blocks from control cases, for paraffin embedding:

1)	70% methylated spirits/5% phenol	6 hr	room temperature
2)	methylated spirits	10 hr	room temperature
3)	absolute alcohol/5% phenol	6 hr	room temperature
4)	absolute alcohol/5% phenol	6 hr	room temperature
5)	absolute alcohol/amyl acetate	4 hr	room temperature
6)	amyl acetate I	10 hr	room temperature
7)	amyl acetate II	10 hr	room temperature
8)	1% celloidin in methyl benzoate[1]	20 hr	room temperature
9)	1% celloidin in methyl benzoate[1]	20 hr	room temperature
10)	xylene	4 hr	room temperature
11)	paraffin wax	14 hr	60°C
12)	paraffin wax	10-20 hr	60°C

[1]Necloloidine (Merck) solution for microscopy was considered to be 100% celloidin (1ml added per 100ml methyl benzoate)

6.2 Staining protocols and stains

6.2.1 Haematoxylin and eosin

Sections were passed through the following solutions:

1)	histoclear	2 min
2)	absolute alcohol	2 min
3)	methylated spirit	2 min
4)	water	2 min
5)	Lugol's iodide*	1 min
6)	water	1 min
7)	5% sodium thiosulphate	1 min
8)	water	2 min
9)	Mayer's haematoxylin*	10 min
10)	1% acid alcohol*	3 dips
11)	water	2 min
12)	Scot's tap water substitute*	1 min
13)	water	2 min
14)	methylated spirit	10 sec

15)	saturated aqueous eosin	2 min
16)	methylated spirit	10 sec
17)	absolute alcohol	2 min
18)	histoclear	2 min
19)	xylene	1 min

* for details see Staining solutions

6.2.2 Haematoxylin

Immunocytochemistry sections were counter stained as appropriate:

1)	running water	2 min
2)	Mayer's haematoxylin*	50 sec
3)	water	wash off excess haematoxylin
4)	Scot's tap water substitute*	30 sec

* for details see Staining solutions

6.2.3 Staining solutions

6.2.3.1 Lugol's Iodine

1g	Iodine
2g	Potassium iodide

Made up to 100ml with distilled water

6.2.3.2 Mayer's haematoxylin

1g	haematoxylin
10g	potassium alum
0.2g	sodium iodate

in 1 litre of distilled water, bring to boiling point and allow to cool overnight, then add:

1g	citric acid
50g	chloral hydrate

6.2.3.3 1% Acid Alcohol

1% HCl in methylated spirit

6.2.3.4 Scot's tap water substitute

3.5g	sodium bicarbonate
20g	magnesium sulphate

in 1 litre of distilled water

7. ABBREVIATIONS

A	Adenine or Adenosine
Ala	Alanine
ALT	Alanine transferase
APES	3-aminopropyltriethoxy-silane
Arg	Arginine
Asn	Asparagine
Asp	Aspartic acid
AST	Aspartate transferase
BNF	Buffered neutral formaldehyde
bp	Base pair
BSE	Bovine spongiform encephalopathy
°C	Degrees centigrade
C	Cytosine
C2	Second cervical spinal cord segment
C3	Third cervical spinal cord segment
C6	Sixth cervical spinal cord segment
C7	Seventh cervical spinal cord segment
CJD	Creutzfeldt-Jakob disease
cm	Centimetre
CNS	Central nervous system
CSF	Cerebrospinal fluid
CT	Computed tomography
DNA	Deoxyribonucleic acid
DRG	Dorsal root ganglion
EEG	Electroencephalogram
EU	European Union
F	Female
FeLV	Feline leukaemia virus
FFI	Fatal familial insomnia
FIP	Feline infection peritonitis
FIV	Feline immunodeficiency virus
FN	Female neutered
FSE	Feline spongiform encephalopathy
g	Gram

G	Guanine or Guanosine
GFAP	Glial fibrillary acidic protein
GIT	Gastrointestinal tract
Gln	Glutamine
Glu	Glutamic Acid
Gly	Glycine
GPI	Glycosylphosphatidylinositol anchor
GSS	Gerstmann-Sträussler-Scheinker disease
GSSD	Gerstmann-Sträussler-Scheinker disease
GUVS	Glasgow University Veterinary School
H&E	Haematoxylin and eosin
HCl	Hydrochloric acid
His	Histidine
hr	Hour
Ile	Isoleucine
IU	International unit
kb	Kilobase
kg	Kilogram
L	Litre
LA	Long-acting
Leu	Leucine
Lys	Lysine
M	Molar
Met	Methionine
mg	Milligram
min	Minute
ml	Millilitre
MN	Male neutered
MRI	Magnetic resonance imaging
mRNA	Messenger ribonucleic acid
NGS	Normal goat serum
p53	Protein which has been associated with many tumours
PAP	Peroxidase anti-peroxidase
PBS	Phosphate buffered saline

Phe	Phenylalanine
PNS	Peripheral nervous system
PRNP	Human prion protein gene
Pro	Proline
PrP	Prion protein
PrP^{C}	Normal cellular isoform of prion protein
PrP-null	Genetically modified strain of animal which fail to produce PrP
PrP^{Sc}	Protease-resistant disease-related isoform of prion protein
RBC	Red blood cell
sec	Second
Ser	Serine
sip	Scrapie incubation period
S-S	Disulphide bond
T	Thymine or Thymidine
T2	Second thoracic spinal cord segment
T3	Third thoracic spinal cord segment
T4	Fourth thoracic spinal cord segment
TME	Transmissible mink encephalopathy
TSE	Transmissible spongiform encephalopathy
TSE's	Transmissible spongiform encephalopathies
Tyr	Tyrosine
μm	Micrometer
UK	United Kingdom
USA	United States of America
Val	Valine
vCJD	New variant CJD

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