

CATS - CURRENT AND PROPOSED RESEARCH

SEAC 12/4

1. At its meeting on 28 November 1991, the Committee asked for details of work proposed or in hand with cats. Some of the information that follows was obtained in confidence from the AFRC and Dr G Pearson of Bristol Veterinary School. Members are asked not to discuss this information outside the Committee.

STUDIES IN PROGRESS

2(i) Bristol Veterinary School, Dr G Pearson; NPU, H Fraser (funded by MAFF).

Attempted transmission of FSE from cat brain to mice by i/p i/c inoculation.

Three cat brain homogenates have been inoculated. All three have transmitted. One was formalin-fixed.

(ii) CVL

Imported cats (and dogs) with neurological signs and all indigenous species submitted for rabies diagnosis were to have been examined histopathologically for FSE but there have been problems of releasing tissue from the rabies high security building. These have now been solved but no cases have yet been examined.

(iii) CVL

Since 4 July 1990 all cat (and hound) brains received at CVL from the Veterinary Investigation Service or Bristol Veterinary School are examined for SAF. Of the 67 suspect cats looked at up to 1 December 1991, 18 were positive for SAF.

(iv) CVL

When a case of FSE is confirmed a questionnaire prepared by Mr Wilesmith is completed and returned. Fruitful analytical epidemiological studies of FSE are not possible until about 50 cases have been confirmed.

(v) MAFF

Cases of spongiform encephalopathy have recently been diagnosed in a Puma and Cheetah (in Australia). Investigations are in progress.

PROPOSED RESEARCH

3. Professor Keen, Head of the Bristol Veterinary School,

wrote a letter (SEAC 11/4) to Mr K C Taylor on 5 November 1991 with details of three areas of FSE research. The Committee suggested that the proposals should be put to the AFRC. Similar proposals had however been submitted to the AFRC in 1990 and 1991 and turned down.

There is hearsay evidence that Bristol Veterinary School (Dr K Morgan) unsuccessfully approached the PFMA for funding of an epidemiology project on FSE. Nevertheless the PFMA may be interested in funding or collaborating in a case control study of FSE.

OTHER PROPOSALS

4. Examination of archival material in Veterinary Schools has been conducted and reported by Liverpool and Bristol. Some less complete studies have been conducted in other schools at home and abroad. Formal proposals have been put forward by Bristol. We are not aware of others.

STUDIES ABROAD

5. Studies abroad on FSE are unlikely as disease has not been reported outside the UK. The only possibility is that Dr Gibbs from NIH may be interested in cat brain for transmission studies but we are not aware of any approach for material.

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Feline Spongiform Encephalopathy: Fibril and PrP studies

Attached is a paper on the above which has been submitted for publication and may be of interest.

17/02/92

Title: Feline Spongiform Encephalopathy: Fibril and PrP studies

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Summary

The brains from 18 cats were examined for the presence of the fibrils and modified PrP protein which are molecular diagnostic markers for scrapie-like diseases. Thirteen cats were referred with clinical neurological signs potentially indicative of feline spongiform encephalopathy (FSE). Of these, five had histopathological changes of FSE; five had other central nervous system lesions, and in three the brain was normal. The remaining five cats, referred with no clinical neurological signs, were selected as controls. Fibrils and modified PrP protein were found in the brains of all five cats with FSE and in one cat with neurological signs, but without histopathological changes in the central nervous system. Fibrils were present in the absence of modified PrP in the brains of two cats; one with neurological signs and a histologically confirmed meningioma, the other in a cat with no neurological signs and a normal brain.

Introduction

In May 1990, a scrapie-like spongiform encephalopathy was recognised in a domestic cat (Wyatt and others 1990). Further cases were subsequently reported (Leggett and others 1990, Wyatt and others 1991) and to date (February 1992) a total of 24 cats with this disease have been diagnosed histopathologically in the United Kingdom (MAFF, unpublished data). The name feline spongiform encephalopathy (FSE) has been proposed (Pearson and others 1991).

The clinical signs and pathological changes of this feline encephalopathy resemble those produced in other species by the transmissible pathogens of scrapie, bovine spongiform encephalopathy (BSE) and related disorders (Kimberlin 1990). Infection by these agents leads to a non inflammatory, vacuolar degeneration of the central nervous system, and the accumulation within it of an abnormal isoform (PrP^{SC}) (Chesebro and others 1985, Oesch and others 1985) of a host coded membrane glycoprotein (PrP) (Bolton and others 1982, Hope and others 1986). Detergent extraction of PrP^{SC} , followed by protease treatment, results in a protease resistant core protein PrP27-30 (Prusiner and others 1982) which may be demonstrated by negative stain electron microscopy in the form of abnormal fibrils called scrapie-associated fibrils (SAF) (Merz and others 1981). The fibrils and PrP^{SC} are therefore molecular markers for these diseases (Farquhar and others 1989, McKinley and others 1983, Diringer and others 1983). Detection of fibrils and PrP^{SC} from affected brains has been used to confirm that BSE is a scrapie-like disease (Hope and others 1988, Scott and others 1990).

We have reported briefly the presence of fibrils in cats with histopathologically confirmed spongiform encephalopathy (Pearson and others 1991).

This paper further characterises this novel feline encephalopathy by reporting the results of fibril and PrP^{Sc} studies of the brains of cats with ~~clinical~~ neurological signs in which a ~~potential~~ spongiform encephalopathy was included in the differential diagnosis.

Materials and Methods

Animals: A total of 18 cats were examined. Twelve cats with neurological signs, in which ~~potential~~ spongiform encephalopathy was considered in the differential diagnosis, were referred to the University of Bristol Veterinary School for clinical investigation. One cat with neurological signs was euthanased and submitted for necropsy to Winchester Veterinary Investigation centre following consultation with the referring veterinary surgeon. Five cats with a similar age range referred to the clinic, but without clinical neurological signs, were selected as a control group.

Pathological studies: A full post mortem examination was carried out on all the cats as soon after death/euthanasia as possible. For histopathological examination samples of brain and spinal cord were collected from 11/13 cats with neurological signs (cases 1-8, 10-12). The brain was collected from the remaining 2 cats with neurological signs and from 4 cats without neurological signs. In addition, a variety of other tissues including heart, lung, liver, kidney and spleen were collected. The tissues were fixed in 10% neutral buffered formalin, processed routinely and

embedded in paraplast. Sections were cut at 8 μ m and stained with haematoxylin and eosin.

Fibril studies: From all cats a 1g sample of each of the right and left frontal lobes of the cerebrum was collected into a new sterile glass container and frozen at -70°C . One sample from each cat was used for fibril studies, the other for protein electrophoresis and immunological analysis. Samples were number coded and each examination was performed blind. They were thawed, homogenised in 10% (w/v) *N*-lauroylsarcosine and processed using a modification of the method of Hilmert and Diringer (1984) (Scott and others, in press). The material was stained on formvar/carbon grids with 2% (w/v) potassium phospho-tungstate, pH 6.6 and examined in a Philips EM 410LS electron microscope at 80 kV and x31,000 magnification. Each sample grid was examined for a minimum of 20 minutes and assessed for the presence of fibrils.

Biochemical studies: Fractions of cerebral cortex were prepared with or without the use of proteinase K (+PK and -PK respectively) and analysed for PrP^{SC} by SDS-PAGE and protein staining using standard methods (Hope and others, 1988). Between 0.1 and 0.01g equivalents of cat brain per gel lane were used. Following electrotransfer onto nitrocellulose, PrP-related proteins were identified immunochemically using a rabbit antiserum raised against mouse PrP^{SC} (final dilution 1 in 1000) and a gold-conjugated goat anti-rabbit immunoglobulin/silver enhancement system (Janssen) (Farquhar and others, 1989).

Results

Clinical and Pathological findings: The age, breed, sex, and histopathological diagnoses of the 18 cats are shown in Table 1. Five of the cats with nervous signs (cases 1-5), three of which (cases 2,4, and 5) have been reported previously (Wyatt and others 1991), had clinical signs and histopathological evidence of spongiform encephalopathy. The remaining eight cats had a variety of neurological signs. These included ataxia (cases 6-10); epileptiform seizures (case 11); altered behaviour and hyperaesthesia (case 12); ataxia, episodes of syncope and status epilepticus (case 13). The histopathological findings in the central nervous system of these cats are shown in Table 1. In case 11, lymphocytic cholangitis was present but, in the absence of evidence of hepatic encephalopathy or other lesions in the brain, no association between these hepatic changes and the nervous signs was established.

Nervous signs were not recorded in the 5 cats (cases 14-18) with conditions unrelated to the nervous system. Histopathological changes were not found in the brains examined from four of these cases (Table 1).

Fibril studies

Fibrils similar to those described in other spongiform encephalopathies caused by unconventional agents were seen in 8 cats (Table 1), including all of the cases with histopathological evidence of spongiform encephalopathy (Fig.1a) and 3 in which no spongiform changes were found (Fig 1b & c). In case 18 only occasional fibrils were detected.

Biochemical analysis for PrP^{SC}

PrP-related proteins with the biochemical and immunological characteristics of PrP^{SC} were observed by SDS-PAGE, silver staining and/or immunoblotting of SAF-like fractions from the brains of six of eighteen cats (Table 1). They were found in all five cats diagnosed by clinical signs and histopathology to have a spongiform encephalopathy (cases 1-5), and in a two year-old neutered female cat (case 9) (Fig.2). Typically, as in BSE-affected cows and scrapie-affected sheep (Hope and others, 1988), PrP in SAF-like fractions from cat brain had a molecular weight range of 21-35 kDa when purified without the use of proteinase K (Fig.2, lanes 3 & 5), while a major PrP fragment of molecular weight 27-30 kDa (PrP₂₇₋₃₀) survived treatment with this enzyme (Fig.2, lanes 4 & 6). In the range of sample weight used for this analysis no PrP-related proteins were detected in SAF-like fractions from the remaining twelve cat brains (Fig.2, lanes 1 & 2), including two (cases 13 and 18) in which fibrils were seen by electron microscopy.

Discussion

In the five cats in this study with a spongiform encephalopathy, fibrils were observed by electron microscopy and their major protein, PrP^{SC}, was identified by SDS-PAGE and Western blot. The fibrils were similar to those described in sheep with scrapie (Rubenstein and others 1987, Gibson and others 1987, Scott and others 1987, Dawson and others 1987), cattle with bovine spongiform encephalopathy (Wells and others 1987, Hope and others 1988, Scott and others 1990) and humans with Creutzfeldt-Jakob disease (Merz and others 1984).

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In sheep with scrapie, fibrils can be readily detected in several areas of the brain, including cerebral cortex (Stack and others 1991). By contrast, the frequency with which fibrils were detected in cattle with BSE, depended on the region of the brain sampled; the highest yield being obtained from medulla, midbrain, thalamus and basal nuclei where vacuolar changes are present (Scott and others 1990). This correlation between PrP^{Sc} accumulation and vacuolar pathology is also well established in laboratory animal models of scrapie (Bruce and others 1989). Because of the widespread distribution of changes in FSE (Wyatt and others 1991) and the requirement, in the present study, not to compromise the histopathological examination of the brain, the frontal region of the cerebrum was therefore selected for fibril and PrP^{Sc} examinations. However, studies of the sensitivity of fibril detection in different parts of the brain in cats with FSE are required to determine whether detection can be made as readily in other regions as in the frontal cerebral cortex.

It is of interest that fibrils were detected in the brains of 3 cats (cases 9, 13 & 18) without histopathological evidence of spongiform encephalopathy, and that in only one of them, (case 9), a Western blot for modified PrP was positive. There are precedents for the occurrence of abnormal PrP in the organs of animals incubating scrapie prior to clinical signs and/or spongiform encephalopathy. Ikegami and others (1991) reported that modified PrP protein was found in the brain, spleen and lymph node of one sheep experimentally inoculated with scrapie but killed before the presence of clinical signs or histopathological changes; and in lymphoid and/or CNS tissues from 3/17

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clinically normal sheep. In experimental scrapie of mice, PrP pathology has been shown to predate vacuolar degeneration (Bruce and others 1989). The detection of fibrils and/or modified PrP in these three cases is difficult to explain, and considerably more data are required from the cat population throughout the UK before further interpretations can be made.

In conclusion, fibril detection and the demonstration of PrP^{Sc} in FSE provide further confirmation that this disease is a member of the neurodegenerative diseases caused by unconventional pathogens.

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Table 1. Age, sex, breed, and histological findings in the central nervous system of cats with and without nervous signs; fibril and modified PrP findings in the brain .

Cat No.	Age	Sex	Breed	Histological changes (CNS)	Fibrils	PrP ^{Sc}
Cats with nervous signs:						
1E	4y	MN	DSH	Spongiform enceph.	+	+
2E	5y	MN	DSH	" "	+	+
3E	5y	MN	DLH	" "	+	+
4E	8y	MN	DSH	" "	+	+
5E	12y	FN	DSH	" "	+	+
6E	11m	M	Persian	Wall. degn., SC	-	-
7E	1y	F	Balinese	Dermoid cyst, SC	-	-
8E	2y	MN	DSH	Wall. degn., Brain/SC	-	-
9E	2y	FN	DSH	None	+	+
10D	6y	FN	DSH	Meningitis, Brain/SC	-	-
11E	2y	M	DSH	None	-	-
12E	7y	MN	DSH	None	-	-
13D	9y	MN	DSH	Meningioma, Brain	+	-
Cats without nervous signs:						
14E	9w	F	Siamese	None	-	-
15D	6m	FN	DSH	None	-	-
16D	1y	FN	DSH	None	-	-
17E	5y	MN	DSH	Not examined	-	-
18E	13y	MN	DSH	None	+	-

Legend

CNS, Central Nervous System
 DSH, Domestic shorthair; DLH, Domestic longhair
 PrP^{Sc}, PrP protein with relative MW of 33-35K Daltons
 SC, spinal cord ; Wall degn., Wallerian degeneration ;
 enceph., encephalopathy; D, died; E, euthanasia
 w, weeks; m, months; y, years
 M, male; F, female; N, neutered
 +, present; -, absent

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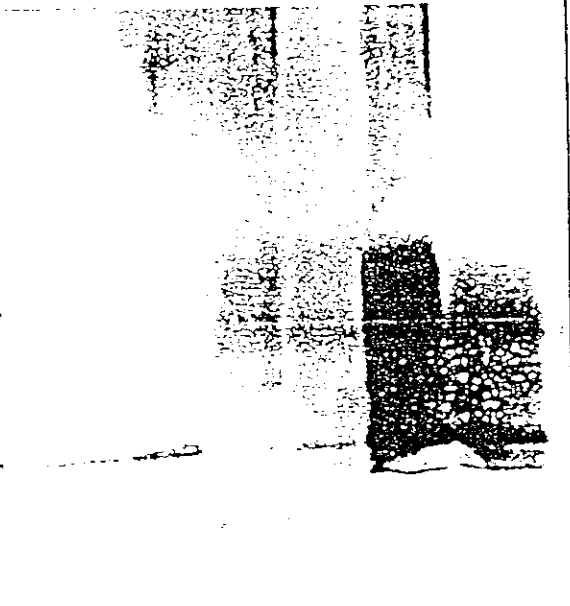
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LEGEND TO FIGURES

- Figure 1. Electron micrographs showing negatively stained fibrils obtained from the brains of cats: a) cat 2; b) cat 9; and c) cat 18. Magnification x 120,000. (See Table 1 for details).
- Figure 2. Immunoblot of fibril proteins from the brains of cats with clinical neurological signs. Lanes 1 & 2, cat 8 - no fibrils isolated and no histopathological evidence of spongiform encephalopathy; Lanes 3 & 4, cat 9 - positive for fibrils, with no histopathological evidence of spongiform encephalopathy; Lanes 5 & 6, cat 1 - positive for fibrils, with histopathological changes of spongiform encephalopathy. Lanes 1,3, & 5 without, and 2,4, & 6 with proteinase-K treatment. Numbers on left hand side indicate apparent molecular mass in KDa.