

Perceptions of unconventional slow virus
diseases of animals in the USA

G A H Wells

REPORT OF A VISIT TO
THE USA
APRIL-MAY 1989

Objectives

1. To discuss surveillance and research of transmissible spongiform encephalopathies of animals in the USA within the context of the occurrence of the BSE epidemic in the UK and to identify potential areas of collaborative studies between MAFF and USDA.
2. To obtain a first hand understanding of the naturally occurring transmissible spongiform encephalopathies of domesticated and wild mammals in the USA.
3. To provide information on BSE.

Itinerary and Principal Contacts

Dates	Place	Contact
24.4.89	USDA Headquarters Washington DC	Dr A B Thiermann
25-26.4.89	USDA ARS Plum Island Animal Disease Center PO Box 848 Greenport NY 11944	Dr R Breeze
27-28.4.89	Dept. of Pathology New York State College of Veterinary Medicine Cornell University Ithaca NY 14853-6401	Dr B Summers
1-3.5.89	Dept Veterinary Science University of Wisconsin Madison 1655 Linden Drive Madison Wisconsin 53706	Dr R F Marsh
5.5.89	NIH Rocky Mountain Laboratory Hamilton Montana	Dr B Chesebro
6-7.5.89	908 South 3rd St Hamilton Montana 59840	Dr W J Hadlow
10-11.5.89	College of Veterinary Medicine and Biomedical Sciences Dept of Pathology Colorado State University Fort Collins Colorado 80523	Dr D H Gould
12.5.89	Wyoming Game and Fish Dept Sybille Wildlife Research & Conservation Education Unit Wyoming	Dr E S Williams Dr S Young

Summary

BSE has not been reported in the USA and it is thought unlikely that such a disease is currently presenting clinically. Disease surveillance systems would readily detect an epidemic of the magnitude experienced in the UK but conceivably would not have detected low instance sporadic occurrence.

Concerns in the USA regarding BSE are two-fold; Firstly, the apparent ease with which scrapie has passed into a new species on a national scale poses a fundamental problem for all countries with endemic natural sheep scrapie. Secondly, the sheep scrapie origin of TME has never been proven. The recent experimental demonstration in cattle of a neuropathology indistinguishable from that of BSE when inoculated with a specific isolate of TME and the association of the disease origin of that isolate with feeding to mink of cattle tissues, but not sheep tissues, raises also the possibility of a cattle origin of TME. There is concern too that in both TME and CWD the absence of evidence of the origins of infection could be explained by the occurrence of unconventional viral agent subclinical or carrier disease states in domestic and/or wild species.

Pathogenesis of unconventional viral agent diseases presenting naturally in ruminant species cannot be assumed to mimic mechanisms determined in experimental models.

In the context of concern regarding BSE the current Federal scrapie programme, which until very recently was being scrutinised with a view to abandonment, is now undergoing urgent reassessment with a view to strengthen the control policy. The concept of a scrapie flock certification plan has been suggested. This would be voluntary and industry orientated.

Information on BSE, especially the results of epidemiological studies, were received with great interest. Requests were made from USDA, University Veterinary Colleges and State Diagnostic Laboratories for copies of the video tape of clinical BSE for extension activities. Illustrative material for foreign disease teaching programmes was also requested.

Visit to USDA Headquarters, Washington DC 24.4.89

USDA Headquarters comprises two large blocks, a north building and a south building, linked by a footbridge and a tunnel. They are situated just to the south of the East Mall which extends from the Washington Monument to Capitol Hill. There is a staff of 3000.

1. A seminar on BSE covering clinical (with videotape), pathological, transmission and epidemiological aspects was presented to an invited audience representing various federal interests. The seminar, with discussion, extended to 1½ hours. Present were:-

<u>Name</u>	<u>Organization</u>
Alex Thiermann	USDA-APHIS, International Programs
Roger Breeze	USDA-ARS, Plum Island
Delmar Cassidy	USDA-APHI-S&T Ames, IA
Chester Gibson	USDA-APHIS-VS Hyattsville MD
Al Strating	USDA-APHIS-S&T Washington DC
Paul Rodgers	American Sheep Industry Assoc. 200 Clayton St Denver, CO 80204
Bill Hadlow	Hamilton, Montana
Mark Robinson	USDA-ARS, Pullman WA
Dick Marsh	University of Wisconsin - Madison
John Gorham	USDA-ARS, Pullman, Washington
Michael F Barile	Centre for Biology, FDI, Bethesda 2089
Gary M Weber	USDA-Extension 3334 S.Bldg 447-2677
Patricia I Koch	Congressman Jim Leach, 1514 Longworth W,DC 20510
Leigh A Sawyer	CBER, DV, FDA Bethesda, MD 20892
Paul Albrecht	CBER, Dir. Virology, FDA

Much of the discussion was predictable and dominated by those representing public health interests. Paul Albrecht indicated later that FDA could be interested in inoculation of Chimpanzees with BSE. The study, probably involving three animals would be carried out at NIH.

2. Meeting with USDA, BSE Task Force

This group comprises Alex Thierman (USDA-APHIS, International Programs) (Chairman), Roger Breeze (USDA-ARS Director, Plum Island), Bill Hadlow (Neuropathologist - retired, formerly of NIH Rocky Mountain Laboratory, Hamilton, Montana), John Gorham and Mark Robinson (USDA-ARS, Pullman, Washington) and Dick Marsh (Dept. Vet. Science, Univ Wisconsin - Madison). The objectives of the group are to assess the implications of the occurrence of BSE for US cattle particularly the risk of BSE occurrence in the US in relation to endemic scrapie agent. The purpose of my invitation to this meeting was to discuss aspects of research which are of common interest and to identify a tentative USDA research programme including any potential collaborative projects. The discussions were informal and there was no agenda. The general opinion of those present was that BSE, as an overt disease phenomenon, could exist in the USA but if it did it was very rare. The need for improved and specific surveillance methods to detect it was recognised. Clinical similarities between BSE and rabies suggested one means of sampling the cattle population which might increase the probability of detection of BSE. It was clear that the bovine rabies negative rate would vary greatly between States but initially this should be determined and as it would inevitably be high relative to positive cases, some differential diagnosis carried out.

1. Identify scrapie infected flocks through the US scrapie eradication/control programme.
2. Focus on areas of concentrations of the sheep population.
3. Investigate rendering operations where scrapie is prevalent.

It is clear that USDA have little information and no regulatory responsibility for rendering plants in the US. Such regulations are apparently the remit of the Food Safety Inspection Service (FSIS) and the Food and Drug Administration (FDA). However, the rendering industry in the US has never been so successful as in the UK because the cost of corn (maize) has never forced much use of animal protein.

Nevertheless, there was a need to know if the USA was vulnerable to a BSE epidemic via the rendering process. Information on scrapie incidence should also be sought.

Marsh stated that in view of his experience with repeated unsuccessful attempts to infect mink with scrapie by the oral route and the 1985 outbreak of TME (Brecke, Stetsonville) in which sheep offal could not be inculcated as a source of infection, the sheep scrapie origin of TME is in doubt. Despite testing many isolates no sheep source of agent given orally to mink has produced TME, whereas given by the intracerebral route sheep scrapie agent produces TME with an incubation of 1-2 years. He considered the need for further research to address the question, is TME a disease of cattle origin? Hadlow thought that some of the Icelandic experiences with pastures apparently remaining contaminated with scrapie for years, though kept free of sheep, might be explained by an alternative source of agent.

The work of Wilbur Clarke at Mission, Texas was discussed briefly. The results of this study in which 10 calves were inoculated with scrapie has not been published and perhaps would not be published. USDA are sensitive regarding publicity of the results of the study which remain far from conclusive. Apparently only 3 of the inoculated animals developed neurological signs. The neuropathology of the affected cattle has not been examined in any depth but Hadlow has the material. Marsh indicated the requirement to obtain the fresh brain material from this study in order to perform PrP extractions.

Because of the successful transmission of the Brecke (Stetsonville) isolate of TME to cattle and the subsequent passage history in mink it was generally considered important that comparisons be made with BSE isolates in mink. Is BSE like scrapie in mink? Is BSE like the Brecke isolate of TME?

Very little was said about CWD but some present considered that its occurrence may indicate a sylvatic origin of agent. It was also agreed that the role of possible subclinical infection in the epidemiology of transmissible spongiform encephalopathies could well be important but was unknown. Marsh remarked on the possibility that BSE was due to an extremely thermostable strain of agent. His experience in the past with one particular Wisconsin isolate of TME (Hayward strain) suggested that i/c biopsy needles could not be effectively "scrapie sterilised", even employing an experimental autoclave system capable of 60 psi and 300°C+ for 5 hours. This experience led him to the policy that in scrapie or TME transmission studies re-use of instruments or glassware that had contained agent was an unacceptable protocol.

The following research programme was proposed:-

1. Experimental BSE in mink

All BSE isolates will be considered by USDA to contain exotic pathogen and thus this work would require to be done on Plum Island Animal Disease Centre.

A single isolate of BSE (from BSE 1-4 or a new isolate) would be inoculated i/c or i/p using groups of 15 with appropriate control groups. Twenty ml of a 1/20 crude unspun homogenate of BSE brain would be required.

The mink breeding cycle determines that the starting date for this study should be 1st April 1990. Mink would be housed in cages within open sided sheds in a wire compound outside laboratory or animal quarantine facilities but on Plum Island.

Parallel mouse studies would be desirable and these could be done on Plum Island but might be undertaken in collaboration with NPU. Principal workers: Marsh and Wells ± NPU.

2. Experimental scrapie and TME in cattle

This series of studies was proposed by Mark Robinson and would be carried out at Pullman, Washington.

Holstein (or possibly a beef breed) calves would receive sheep scrapie, Brecke (Stetsonville) TME isolate or the Idaho TME isolate. Each calf would be inoculated by a single route, i/c, s/c or oral.

In parallel infectivity studies would be set up in which sequential kills at 6 month intervals provided extraneural tissues for passage/bioassay in mink. This study would primarily answer the question: can cattle tissues sustain replication of agent?

Principal workers: Gorham, Robinson and Marsh.

3. Examination of experimental bovine scrapie brain for PrP

The frozen CNS material from the Hourigan and Clarke study carried out at Mission, Texas would be examined by Marsh for PrP. If found mouse inoculations should proceed.

Thiermann indicated to Gorham and Robinson that the work proposed at Pullman (clearly the largest part of the programme) could well receive a high priority but they should anticipate that the work might have to be done within their existing ARS budget (Dejà vu!).

Visit to New York State College of Veterinary Medicine,

Cornell University - 27/28.4.89

A seminar on BSE was given to faculty and students. Material was contributed for a slide seminar for pathology residents, and their case presentations were discussed.

Over dinner with Don Schafer (Director, Cornell Bovine Health Centre, Don Lein (Director NY State Diagnostic Laboratory) and Ed Debovi (Senior Virologist, NY State Diagnostic Laboratory) there were discussions on BSE. NY state does not have a rabies problem and therefore do not see suspected bovine rabies material. Nevertheless it was agreed that surveillance of cattle brains could be increased and the possible occurrence of (red nucleus) vacuolation as an incidental feature investigated.

1-3.5.89

The University is celebrating its Centenary this year. The Department of Veterinary Science has been established seventy years and currently comprises 13 faculty members and 98 graduate students. The Veterinary School (Dept of Veterinary Medicine) is separate, was established 10 years ago and currently has 78 faculty.

After presenting a seminar on BSE it was arranged that we lunch with Dr G R Hartsough. A brief but necessarily incomplete history of TME was recounted by Hartsough:-

1947 1st outbreak in Wisconsin occurred in October in mink born the previous May. Pathological material from this incident was sent to AFIP Washington DC but no diagnosis was established. One hundred females that were transferred from the farm to Minnesota also became affected but their kits remained free of disease.

1952 2nd outbreak in Wisconsin. Only 5-10% affected (ie markedly reduced incidence from original outbreak).

1963 'Hayward' outbreak in Wisconsin. This was discovered in May in females nursing kits. In the following December (pelting season) 10 males were sold off and these subsequently became affected. Hartsough recognised that in this outbreak the clinical signs were similar to those of sheep scrapie and at this time Hadlow was consulted. He concurred that clinically it was "just like Scrapie in mink" and confirmed the nature of the encephalopathy.

1963 Outbreak in Blackfoot, Idaho.

1985 The Stetsonville outbreak (farmer's name: Brecke). In addition to the downer cows and horses Brecke's mink received a cereal supplement. Hartsough's view was that this would contain bone meal and would be from a commercial source. If this were so and it was contaminated with a TME agent why were there no other ranches affected?

Many mink ranches now feed a commercial pelleted diet. Brecke was equipped to process large carcasses using a crusher/mixer which could accommodate a whole cow!

Idaho is the only US state other than Wisconsin in which primary outbreaks of TME have been recorded.

The largest mink farms in the USA are in Wisconsin, Utah and Minnesota but several other states, including Georgia have smaller units.

Finland, Norway and Denmark are major producers of mink and feed only fish and poultry proteins. Denmark has a mink population of 14 million.

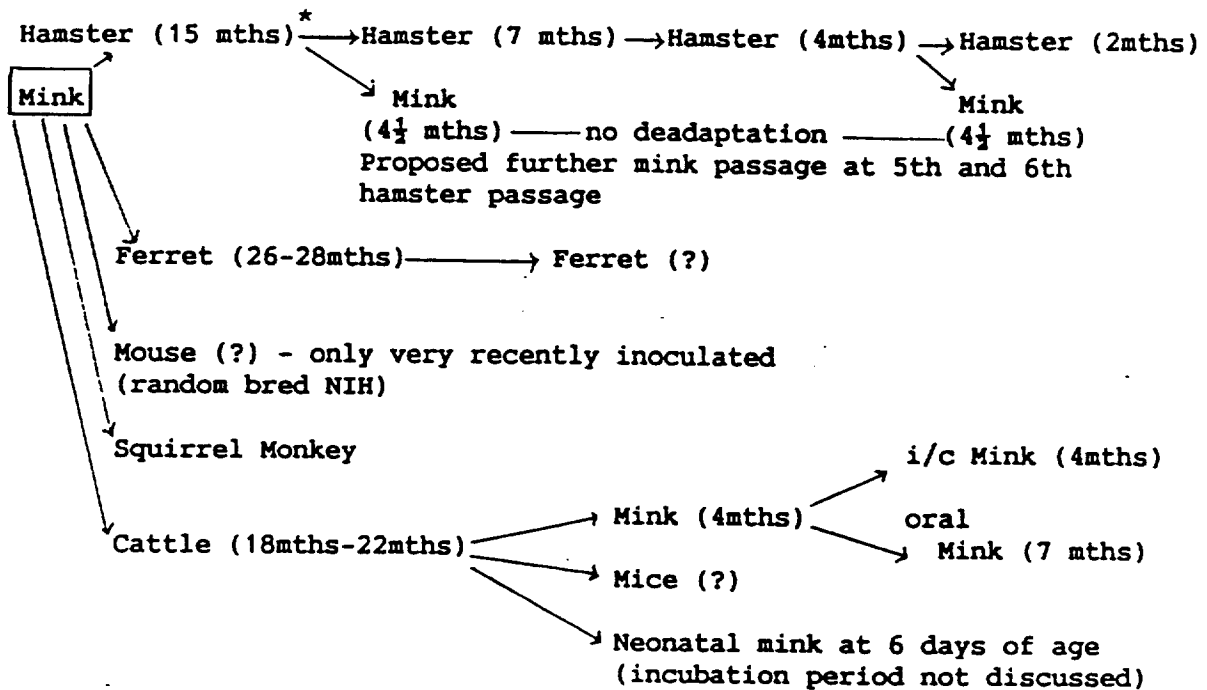
Utah mink ranches feed out of a large cooperative mixer-of poultry and fish sources (but no beef sources) mainly originating in California.

Dead mink go for rendering but are used only in poultry feed.

Scrapie was first recognised in Wisconsin in the 1970's. A state indemnity is paid but, unlike in Michigan, where payment is based on full value of flocks, only partial value is paid.

Recently, USDA has invited comment from experts in the field on the proposals to abandon the national scrapie control programme with the result that there is now a move toward reconsideration. An adjudicating council has been formed to decide future policy, especially against the background of knowledge of BSE. [A draft document: Report of the Scientific Advisory Subcommittee to the Scrapie Task Force of the Sheep Industry Development Program, was passed to me by Dick Marsh for comment].

Transmissions from the Brecke (Stetsonville) TME outbreak to date are:-
(All i/c unless stated otherwise).



* () = incubation period

A visit was made (2.5.89) to the Universities experimental animal resource facility, specifically the Mink Unit which presently houses 50 breeding females. In an adjacent building clinical stages of scrapie (263k) in hamsters were observed. A clinical evaluation system for hamster scrapie was described and demonstrated.

A commercial mink ranch was visited. This was Johny Werth's, Capitol Fur Farm comprising 1400 breeding females. The feed is bought in from a commercial supplier in the form of frozen packs of "poultry", "fish", "dried egg" or "tripe". A commercial mink cereal supplement is used and contains "animal meat meal" which was said to contain material mainly from poultry or fish origin but occasionally from beef sources. The partially thawed packs were tipped into an augur mixer which has a fully loaded capacity of 6000lb and this would feed approximately 15000 mink per day.

In the fall at pelting time the skinned carcasses of the mink are placed in large barrels which are left in the open to freeze. When full, a renderer collects "for use in poultry feeds".

Sections from the brains of the two Brecke TME inoculated cattle were examined and Marsh provided all the blocks from the 2nd steer for study at CVL and comparison with BSE. In general the vacuolar changes were more severe than in most cases of BSE but very similar in distribution. Unfortunately material taken for histopathology from those animals omitted representation of most of the brain stem. PrP has been extracted from both steer brains. About 25g of cerebral cortex is required to obtain 100µg PrP. (The yield would almost certainly be greater from brain stem)

The Central Animal Health Laboratory (the State Diagnostic Laboratory) Wisconsin Department of Agriculture - Trade and Consumer Protection, was visited and a seminar presented on BSE. This laboratory is responsible for diagnosis of only a very small proportion of the bovine suspected rabies cases, most go to the State Hygiene Laboratory. It was thought that only 5-10% of bovine suspect rabies case proved positive. In Wisconsin bovine rabies usually occurred as the 'dumb' form with somnolence as the major feature but occasionally also occurrence of coarse bellowing. Clinical

duration was only a few days. Confusion with BSE on the basis of what Department staff had seen on the videotape was, they considered, unlikely. The question of red nucleus neuronal vacuolation as an incidental finding in bovine brains was raised with Lynne Siegfried, Director and Peter Schmidt a recently appointed veterinary pathologist with an interest in neuropathology but they did not know if it occurred in submissions to the laboratory. It was apparent that routine coronal slicing of brains was not followed and that the midbrain was seldom specifically examined. It was agreed that in conjunction with proposed surveillance for BSE this question would be addressed.

With regard to Scrapie in Wisconsin, there were no clinical cases to date this year.

Visit with Dr W J Hadlow and the National Institute of Allergy
and Infectious Diseases, Rocky Mountain Laboratories,
Hamilton, Montana

Hadlow's views on gaps in our knowledge of natural sheep scrapie were summarised as follows:-

1. How is virus shed? What is the explanation of contagion?

Information is lacking on infectivity of faeces, placenta, nasal secretion and leukocytes (buffy coat). With regard to the latter there is a need to establish the role of buffy coat in pathogenesis (Hadlow maintains that viraemia is a probable route of CNS infection, ie. other mechanisms shown to occur experimentally should not be regarded as exclusive). A sequential study of buffy coat infectivity through the disease course is needed. Hadlow has never been successful in isolating agent from sheep placenta using mouse assays.

It was considered that a sequential study of a heavily infected flock could provide much of this information. Explanations should also be sought as to the period of susceptibility to infection. In sheep Hadlow found that susceptibility was greatest up to 4-6 months. What are the changes in the host which reduce susceptibility after this period?

2. Does subclinical infection, inapparent disease or a carrier state exist in the transmissible spongiform encephalopathies? If so what roles do such states play in epidemiology? In the studies that Hadlow was involved with at Mission, Texas, where an infected flock of Suffolks was maintained, no evidence of a covert disease status was found. Evidence for this statement came from 15 ewes and rams all from high risk blood lines but which themselves survived to 5-7 years

of age. Eleven of their dams died of scrapie. All fifteen were killed and terminal ileum, mesenteric lymph nodes, colon, retropharyngeal lymph node and spleen were assayed in mice with negative results.

There is however, insufficient data on these disease states and Hadlow was concerned that there could exist reservoirs of infection, firstly in other farm animals (cf the Icelandic scrapie experience with introduction of scrapie free sheep to pastures kept free of sheep for years) and secondly the possibility of a sylvatic reservoir.

The rationale for continuing research work on scrapie and BSE is not based on the overt phenomena but on the significance of understanding the species barrier and the reason for new host involvement.

At the Rocky Mountain Laboratories discussions took place with Bruce Chesebro, Rick Race, Byron Caughey, Marcus and Stephanie Czub and John Portis. The scrapie oriented work here is directed toward an understanding of the agent biology in mouse neuroblastoma cell clones. Subcloning has produced lines of cells which can be infected 100% and agent has been retained through 40-50 passages. The system has facilitated a homogeneous source of infectious agent and study of PrP modifications in relation to infection. [Further information on this work is available on request].

Czub and Portis are working on a murine retrovirus which produces a spongiform encephalopathy morphologically resembling those related to the unconventional agents, and originally described by Murray Gardiner but not associated with PrP modifications. The similarity with the unconventional agent diseases also extends to an inability to

demonstrate virus particles on EM, but large amounts of integrated DNA and RNA are present. Preliminary experiments have shown that the virus envelope protein is essential to disease expression. The envelope gene was introduced into an erythroleukaemia virus. Chimeric disease was produced with identical changes to that of the original encephalopathy. The incubation period was also markedly shortened from a wild type incubation range of 4 months - 1 year to a uniform kill of mice in 24 days. Clearly an understanding of the mechanism by which the envelope protein effects vacuolation may have a relevance to the pathogenesis of vacuolation in the unconventional agent encephalopathies.

In Montana the State Diagnostic Laboratory is situated to the east of Hamilton at Bozeman. The work is mainly with cattle diseases. Rabies diagnoses are mainly in skunk. Bill Quinn is the pathologist and Bill Hadlow agreed to acquaint him with the BSE situation and proposed surveillance measures. Wilbur Clarke (reference the Mission, Texas scrapie transmission to cattle study) is now the State Veterinarian for Montana based at Helena.

I was given confidential access to sections from the Clarke scrapie-cattle transmission experiment. Details of the experimental design were as supplied previously by Dr Wrathall (copy of relevant information appended). Only 3 animals (2 inoculated with 2nd pass Suffolk scrapie and 1 inoculated with Angora goat passaged scrapie) showed clinical signs. Clinical signs were characterised by weakness, "a stilted hindlimb gait", disorientation, ataxia and, terminally, lateral recumbency. The two cattle from which I examined material were inoculated at 8 months of age and developed signs 36 months pi (goat scrapie inoculum) and 49 months pi (one of the Suffolk scrapie inoculated) respectively. This latter animal was killed at 58 months

of age and so the clinical duration was only 1 month. The neuropathology was somewhat different from BSE or the Stetsonville TME in cattle. Vacuolar changes were minimal, to the extent that detection required careful searching. Conversely astrocyte hypertrophy was a widespread and prominent feature. The material requires detailed neuropathological assessment but whether or not this will be done remains in question.

Visits to Colorado State University, College of Veterinary Medicine and the Wyoming Game and Fish Department, Sybille Wildlife Research and Conservation Education Unit.

The main objective here was to obtain some understanding of CWD. A visit was made to the University of Wyoming Game and Fish Department, Sybille Wildlife Research and Conservation Education Unit where most of the cases of CWD have occurred. The Sybille Wildlife facility is situated some 50 miles northeast of Laramie, Wyoming through the Laramie Mountains. Here most of the hoofed big game species of North America; Mule Deer (*Odocoileus hemionus*), Whitetail Deer (*Odocoileus virginianus*), Elk (*Cervis canadensis*) Mountain Goat (*Oreamnos americana*), Bighorn Sheep (*Ovis canadensis*) and Pronghorn (*Antilocapra americana*) and some other wildlife species are kept in small numbers for experimental use in the investigation of wildlife diseases.

A colony of the blackfooted ferret (*Mustela nigripes*) has been established because of its imminent extinction. At present there are only 35 but it is proposed to breed up to 200 and then, probably in 1991, re-introduce them into the wild in a nation wide operation. Blackfooted ferret diet is mainly Prairie Dog (*Cynoms spp.*) and it is thought that the elimination of this species from large areas by poisoning campaigns in the past has been responsible for the precipitous ferret decline.

The buildings and pens at the facility are entirely of wooden/log construction with heavy duty wire mesh fences. Pen floors are bare earth. A long race connecting many different areas within the facility enables movement of deer and antelope between pens when necessary. There is provision for holding deer of different sizes in a custom built crush for bleeding and treatments.

The educational role of the unit includes school visits to provide instruction in the work of the department and to promote conservation. I was accompanied on this visit by Stuart Young and Beth Williams. On arrival I was introduced to Hughie Dawson who has managed the facility for some 20 years.

CWD occurred principally in two locations, this one at Sybille and in a similar facility at Fort Collins, Colorado, some 120 miles southwest. It was estimated that in total probably 60-70 cases of CWD have occurred.

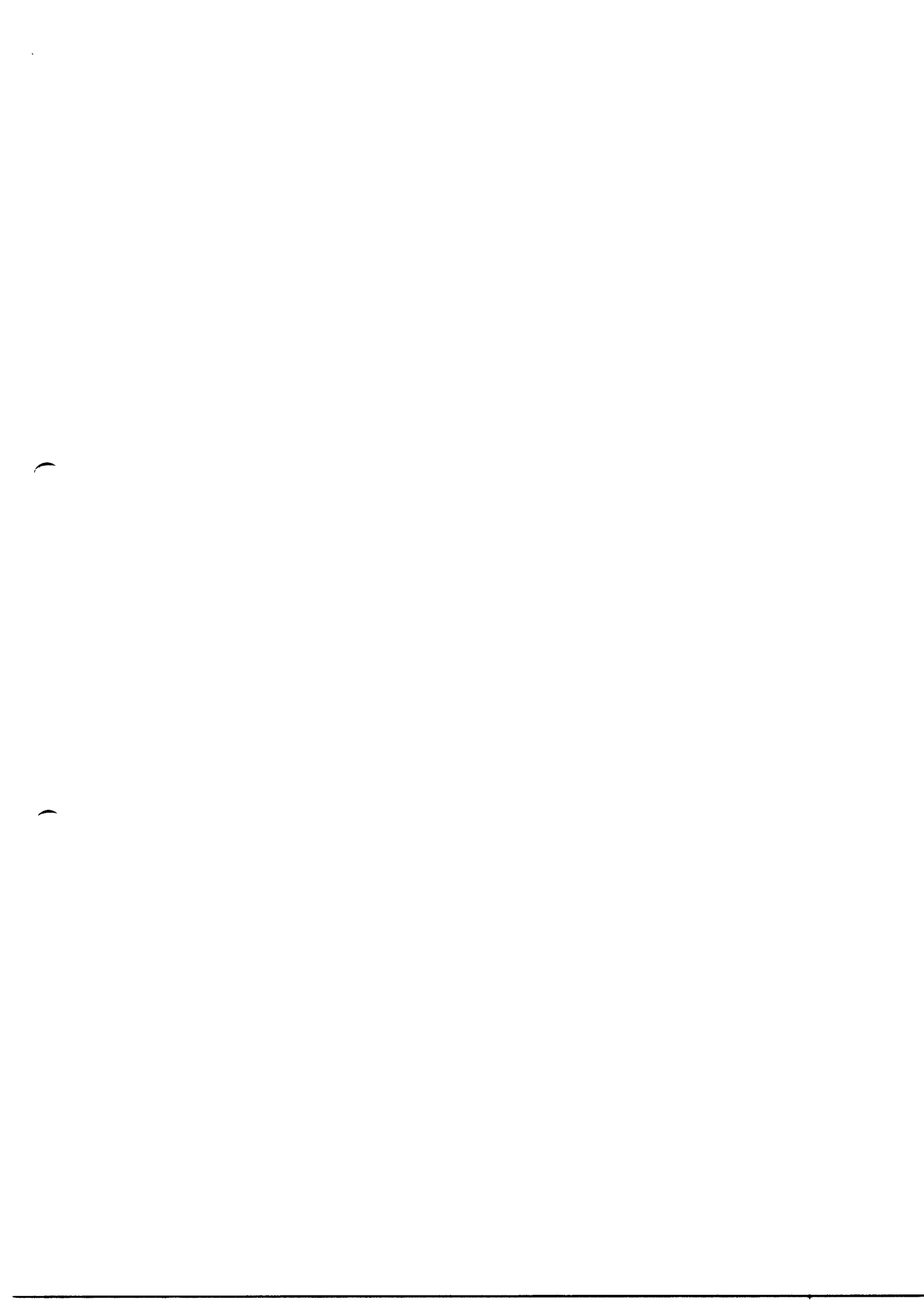
It was difficult to gain a clear account of incidence and temporal sequence of events (- this presumably is data awaiting publication - see below) but during the period 1981-84, 10-15 cases occurred at the Sybille facility. Recollections as to the relative total numbers of cases at each facility were confusing. Beth Williams recalled that more cases had occurred in the Colorado facility.

The morbidity amongst mule deer in the facilities ie. those of the natural potentially exposed group has been about 90% with 100% mortality. The age distribution of affected deer was very similar to that in BSE. The clinical duration of cases was 6-8 weeks. Mortality in CWD cases was greatest in winter months which can be very cold.

When the problem was fully appreciated both the Sybille and the Colorado facilities were depopulated. All cervids were culled but Pronghorn, Bighorn Sheep and Mountain goat, where present simultaneously in the facility, were retained. There have been no cases of CWD in these non cervid species.

A few cases continue to occur at Sybille, the last was 4 months ago.

An account of the occurrence of CWD at the Colorado facility was obtained from Terry Spraker, Diagnostic Laboratory, CSU College of Veterinary Medicine, Fort Collins. He examined tissues from cases of CWD at the Colorado facility some time prior to Beth Williams's involvement and examination of brains which resulted in the initial diagnosis. The deer holding facilities in Colorado comprise the Colorado Division of Wildlife Research Pen, established 10 years ago and some older deer pens at the Foot Hills Campus of CSU, close to Fort Collins. Originally there were just 1-2 cases CWD/year and a total of 24 over several years. In contrast to Beth Williams recollection Terry Spraker thought more cases had occurred at Sybille than in Colorado. The cull at the Colorado facility involved 20-30 clinically normal deer. Early lesions in dorsal nucleus of the vagus and olfactory cortex were found in (some) of these deer. At the time of the cull here Pronghorn was the only other hoofed species present. Bighorn Sheep and Mountain Goat were introduced only one year after the cull and occupied ground where CWD had occurred. Immediately after depopulation the ground was ploughed and disinfection was carried out using 1% NaOH. The buildings/pens were not changed. There has been no recurrence of disease at the Colorado facility since the cull.



Transmission Studies

Mule deer transmissions of CWD were by intracerebral inoculation and compared with natural cases ~~first passage (by this route)~~ resulted in a more rapidly progressive clinical disease with repeated episodes of syncope ending in coma. One control animal became affected, it is believed through contamination of inoculum (?saline). Further CWD transmissions were carried out by Dick Marsh into ferret, mink and squirrel monkey. Transmission occurred in all of these species with the shortest incubation period in the ferret.

Mouse and hamster transmissions were attempted at Wyoming State Diagnostic Laboratory, Laramie and at CSU Fort Collins but were unsuccessful.

Also at the Wyoming State Diagnostic Laboratory, Laramie, transmission to goats was attempted. In 1984 three goats were inoculated intracerebrally with a 10% CWD brain suspension. One goat, untreated, was placed in contact with the CWD inoculated goats and three controls, housed separately, received saline intracerebrally. To date these animals remain healthy.

Epidemiology of CWD

Descriptive epidemiological data has been collected from the two wildlife facilities and a publication is in preparation.

The occurrence of CWD must be viewed against the context of the locations in which it occurred. It was an incidental and unwelcome complication of the respective wildlife research programmes. Despite its subsequent recognition as a new disease of cervids, therefore justifying direct investigation, no specific research funding was forthcoming. The USDA viewed it as a wildlife problem and consequently not their province! Thus

there have been no specific epidemiological studies, other than information gained from noting the occurrence of cases. Because of the relatively short term nature of the programmed research at the facilities it has not been possible to keep Mule Deer under the appropriate experimental circumstances or for sufficient periods to establish horizontal or maternal transmission. Beth Williams is of the view that the occurrence of CWD at Sybille is entirely related to propagative spread by contagion. Investigations have failed to identify any common source of infection and the incident has presented a protracted time course with sporadic cases throughout. There is no evidence that wild born deer were responsible for introduction of the disease to the facility.

I asked Hughie Dawson about the nutritional aspects of the deer kept at Sybille. Mule Deer calves are reared on condensed milk and homogenised or pasteurised domestic cow's milk from birth to 1 month or to 6 months. some would be given "Lamb milk replacer" which has a higher butter fat content than either of the former products, but is derived also from domestic cow's milk. It was thought that at the Colorado facility calves would receive only "evaporated milk". Calves are weaned on to a pelleted feed containing corn, wheat bran and linseed meal with no crude mineral supplement. Salt licks ("sulphur blocks") which have a specific mineral composition are supplied.

CWD has occurred or is suspected to have occurred in establishments supplied with Mule Deer from the Colorado facility. In some cases evidence for this is tenuous. For example, it is understood that Denver Zoo state that "they have not had cases of CWD" and yet they have had cases of Mule Deer succumbing to a chronic wasting disorder which was not diagnosed. A case of CWD occurred in a Mule Deer in Toronto Zoo in 1976. The animal in

question came from Denver Zoo but was originally from the Colorado wildlife facility.

Pathology of CWD

A paper (Williams et al) is in preparation on the distribution of brain lesions in CWD. Vacuolar changes occur predominantly in the dorsal nucleus of the vagus nerve (this nucleus is invariably affected), the hypothalamus and the olfactory cortex with occasional vacuolation of the olfactory tract white matter.

Cerebellar lesions are sometimes present but there are very few changes in the spinal cord which probably accounts for the rarity of ataxia clinically. As in sheep scrapie the hypothalamic lesions correlate with the common clinical occurrence of polydipsia. Beth Williams is aware of occasional neuronal vacuoles occurring in the red nucleus of clinically normal deer! Spraker has added that he has experienced vacuoles in neurons of Gasserian ganglia and at the level of the obex in normal deer.

It has never been reported but Pat Merz carried out SAF detection on CWD brain material. Work may be undertaken with NIH on the immunohistological demonstration of PrP in sections but to date there has been no PrP work.

Does CWD occur in free-living cervids?

There is some, mostly circumstantial, evidence that CWD occurs in free-living cervids but to what extent, if at all, this represents an established reservoir of infection in the wild is not known.

At Sybille two Mule Deer orphans (wild caught) and a White-tail Deer (*Odocoileus virginianus*) hybrid developed clinical signs when only 2½ years of age.

An Elk (*Cervus canadensis*) wild caught as an adult, presumed 2 years old, developed signs when 3-4 years old.

Another group of elk, wild caught 400 miles from the facility, with an age range 2-8 years, old subsequently developed the disease in the facility (?period of captivity). The location of capture relative to the facility did not apparently rule out that they may have at some time had fence-line nose contact with animals in the facility!

Cases have also occurred in Mule Deer that were obtained from the wild within one hour of birth but these were never kept completely isolated through to maturity.

Also at Sybille there has been one case of CWD diagnosed in a free ranging Elk. It was killed in Sybille Canyon 3 miles from the facility. It could have had fence-line contact with captive Mule Deer in the facility.

Similar incidents had occurred in Colorado. In 1985 a free-ranging affected Elk was caught in the Rocky Mountain National Park within a 2 mile radius of the Colorado Division of Wildlife Research Pen. In 1986 and again in 1987 a single affected Mule Deer on each occasion was caught within a 5 mile radius of the Pen. These latter cases occurred within 2 years of the cervid cull at the Pen (?1985). Brain tissue from the free-ranging Elk brain was inoculated into mice but for some reason these were kept for only 6 months and then the experiment was abandoned.

A specific exercise has been carried out by Beth Williams with the Wyoming State Diagnostic Laboratory and Fish Department to sample the brains of healthy wild Mule Deer for histological examination. On two separate occasions the first in 1985 and again in 1987 a total of 150 Mule Deer

brains were collected from areas of, and adjacent to, Sybille Canyon. These deer would have been shot under a game permit by local hunters. As they were brought down from the hills to the Game station for the mandatory registration of the kill the heads were removed and ages estimated. Most were 2-5 year old with a few 6 year old. For obvious reasons hunters were reluctant to give up stag heads. Thus, but for 15-20 brains from stags, examinations were on brains from females. No evidence of CWD lesions was found in any of these brains. However, it was considered that sporadic cases of CWD, should they occur in the wild population, would soon become separated from the herd and fall prey to coyotes (*Canis latrans*).

The possibility of any reservoir of infection in wild cervids originating from scrapie in domestic sheep flocks seems remote. Scrapie has been recorded in only three flocks in Wyoming since 1947 and Beth Williams could recall only one previous occurrence in 1966. This had involved a Suffolk flock close to the border with Nebraska. However, there has been one new confirmed and a suspected affected flock this year in Wyoming. In the latter a ewe bought-in from an Illinois flock is incriminated.

Spraker suggested an interesting explanation for the occurrence of CWD. The deer pens at the Foot Hills Campus were built some 30-40 years ago by a Dr Bob Davis. At or about that time, allegedly, some scrapie work was conducted at this site. When deer were introduced to the pens they occupied ground that had previously been occupied by sheep. Whether they were scrapie infected sheep or not is unclear. There were domestic sheep and goats present in the facility also in the 1960's but there is no evidence that these animals developed scrapie. During the 60's hybridization studies between the Bighorn and domestic sheep were carried

out, again, without evidence of scrapie. Domestic goats were also kept at Sybille in the 1960's.

Spraker considers that the nasal route is responsible for transmission of CWD through nose to nose contact, which may well occur also between captive and free-living individuals.

In domestic cattle of which about 15-20 adults were necropsied per year at the Diagnostic Laboratory, CSU., Spraker had not encountered any lesions suggesting BSE. Polioencephalomalacia (PEM) and Encephalic Listeriosis were the most common morphologic neuropathological diagnoses. No bovine rabies was seen.

VISIT TO USA - DR A E WRATHALL - INFO ON BSE AND SCRAPIE

1. Dr Clark lately of the Scrapie Research Unit, Mission Texas has successfully transmitted ovine and caprine scrapie to cattle. The experimental results have not been published but there are plans to do this. This work was initiated in 1978. A summary of it is:-

Expt A 6 Her x Jer calves born in 1978 were inoculated as follows with a 2nd Suffolk scrapie passage:-

i/c 1ml; i/m, 5ml; s/c 5ml; oral 30ml.

1/6 went down after 48 months with a scrapie/BSE-like disease.

Expt B 6 Her or Jer or HxJ calves were inoculated with angora Goat virus 2/6 went down similarly after 36 months.

Expt C Mice inoculated from brains of calves/cattle in expts A & B were resistant, only 1/20 going down with scrapie and this was the reason given for not publishing.

Diagnosis in A, B, C was by histopath. No reports on SAF were given.

2. Dr Warren Foote indicated success so far in eliminating scrapie in offspring from experimentally- (and naturally) infected sheep by ET. He had found difficulty in obtaining embryos from naturally infected sheep (cf SPA).
3. Prof. A Robertson gave a brief account of BSE. The US approach was to

accord it a very low profile indeed. Dr A Thiermann showed the picture in the "Independent" with cattle being incinerated and thought this was a fanatical incident to be avoided in the US at all costs. BSE was not reported in USA.

4. Scrapie incidents (ie affected flocks) have shown a dramatic increase since 1978. In 1953 when the National Control Scheme was started there were 10-14 incidents, in 1978 - 1 and in 1988 so far 60.
5. Scrapie agent was reported to have been isolated from a solitary fetus.
6. A western blotting diagnostic technique (? on PrP) shows some promise.
7. Results of a questionnaire sent to 33 states on the subject of the national sheep scrapie programme survey indicated
 - 17/33 wished to drop it
 - 6/33 wished to develop it
 - 8/33 had few sheep and were neutral

Information obtained from Dr Wrathall's notes of a meeting of the U.S. Animal Health Association at Little Rock, Arkansas Nov. 1988.