MAFF Commissioned R&D

ROAME B

Contractor:

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Project Title:	*"Effect of oral inoculum dose on attack rate and incubation period of BSE in cattle"*
Project Code:	*SE1902*
CVL Order Book No.	*OB 195*
Project Leader:	*G A H Wells*
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CVL Programme Manager:	*G A H Wells*
Assessment Unit:	*SE19*
Assessment Unit Name:	*Mechanisms of pathogenesis in spongiform encephalopathies*
Sector name:	*Statutory and Exotic Diseases*
MINIM Code	*PP1: 04r*
MAFF customer:	*Animal Health (Disease Control) Division*
CSG Liaison point:	*Dr K J MacOwan*
Proposal date:	*April 1991*
Last revision date:	*May 1994*
Project start date:	*April 1992*
Project end date:	*December 1999*

B1. Summary of the Problem Addressed

The transmissible spongiform encephalopathies (TSE) are unusual, long incubation period diseases for which the infectious agent, and the dynamics of its replication relative to dose, have not yet been fully characterised.

In experimentally induced rodent models of TSE, the efficiency of infection varies depending on the route by which inoculum is administered, but the dose-response curve is consistent for a given route (Kimberlin and Walker, 1978).

Experimentally the oral route of inoculation is less efficient than parenteral routes for scrapie and TME (see Wilesmith and Wells 1991). Little is known however of the precise routes of transmission in naturally occurring TSE or of the important differences between single oral dosing and continuous dietary exposure as may be the case in BSE.

The infective dose of the ingested scrapie-like pathogen necessary to produce the perceived attack rate in the BSE epidemic is not known. Neither is there any understanding of incubation period relative to age of exposure or the possible effect of multiple exposures.

One aspect of the epidemic has focused particular attention on the dynamics of the exposure and how they relate to experimental rodent models.

An increase in incidence of BSE occurred in July 1989 which, given no change in the ascertainment rate at that time (comparable data from the Channel Islands support this assertion) is consistent with a real change in the exposure of the cattle population to the BSE pathogen in 1984 (Wilesmith 1992). But the increased incidence of BSE at this time was not reflected in within herd incidence, rather in a geographically proportional increase in the number of affected herds throughout Great Britain. It is suggested that this phenomenon resulted because there was a geographically uniform increase in the frequency with which batches of feed contained infective material but not an increase in titre within an infected batch.

At the levels of exposure which are producing BSE in the field, what is the relevance of this phenomenon to incubation period? Is there a dose-response relationship or an all or nothing effect? Can BSE be induced by oral inoculation with high titre infective material and is the risk of disease increased with increasing dose? The effect of age on the attack rate is also unknown.

B2. Objectives and completion dates

The proposed study has three main aims:

(1) To determine the attack rate and incubation period of BSE in cattle exposed orally to four different dose levels of brain homogenate from affected cattle.

(2) To determine also if there is a dose-response effect on the incubation period.

(3) To establish the effect of multiple exposures on attack rate and incubation period.

There are too many variables in the BSE epidemic to attempt to address all of the problems relevant to determining the dynamics of natural exposure in a single study. In this study age has been standardised within the estimated period of calfhood exposure in the epidemic (Wilesmith - personal communication).

Experimental transmission of BSE to cattle (CH25/003) has established that 100mg of brain i.c. plus 500mg brain i.v. is an infectious dose. Since in a cattle-to-cattle transmission there are no species-barrier effects, it is likely that 100mg i.c. would have been sufficient to produce disease. It is important that such data is established for experimental oral exposure of cattle to BSE.

By analogy with experimental rodent TSE and by extrapolation from the estimates of the exposure via feed in the natural epidemic of BSE it has been proposed that by the oral route of inoculation 10g of infected brain would probably cause clinical disease and that 100g would be certain to induce clinical disease (R Kimberlin - personal communications).

It involves the following objectives and approaches:

Ref	Title & Completion Date	Approaches
(1)	Oral exposure of calves to BSE brainstem homogenate. (31 January 1992)	Forty 4-month-old (approximately) calves are dosed orally with homogenate prepared from brainstem from BSE affected cattle as follows: i) 1g - 10 calves ii) 10g - 10 calves iii) 100g - 10 calves iv) 3 x 100g (on successive days) - 10 calves

Ref	Title & Completion Date	Approaches
(2)	Clinical monitoring and post mortem examination of experimental animals. If the cattle have not developed clinical disease by 7 years after exposure, they will be killed. (This end-point will be reviewed.) The calves were dosed in January 1992, giving a latest completion date of December 1999.	All the calves are monitored clinically using current methods developed at CVL to determine incubation periods and prevent welfare problems. They are kept until clinical disease develops, and slaughtered when a clinical diagnosis of BSE is established. The diagnosis is confirmed by histopathological examination of the brain, and by EM for fibril detection. If clinical disease does not develop by 7 years of age, cattle are either killed and examinations carried out as for clinically affected animals, or the project is reviewed in the light of current knowledge.

B3. Interdependence of objectives

The objectives of this experiment are independent.

B4. Possible delays

None envisaged. The calves have already been dosed. Intercurrent disease problems in experimental animals kept under conventional husbandry conditions must be considered an unavoidable hazard to the study.

B5. Connection with earlier work

Comparison can be made between the effects of oral dosing in cattle and those following exposure by i.c. and i.v. routes (CH25/003). This experiment is also closely linked with the pathogenesis study (SE1901). The 100g dose group provides a comparable group for that study in the unlikely event that the sequential kills preclude the development of clinical signs. The 100g dose group from this experiment would then provide an end-point study group, though the brain homogenate used was different from that used in the pathogenesis study (SE1901).

B6. Further research

If all doses are infectious in this study, it would be necessary to perform a further study to establish the LD50 for this route should this be required.

B7. Advancement of scientific/technical understanding

This study will furnish vital data on the effects of log increases in the level of exposure to BSE, on both the 'attack rate' and the length of incubation period.

The information provided by this study will influence future policy decisions regarding the feeding of ruminant-derived protein to ruminants.

B8. Publications

One full paper in a peer-reviewed journal at the end of the experiment.

B9. Monitoring progress and success

Research milestones will be:

Ref	Title	Due Date
01/01	Dosing of all calves .	31/1/92

No other milestones are predictable, although the anticipated duration of the study is 3-5 years.

Indicators of the success of the overall project will be:

a) Evidence of transmission (clinically affected cattle)

b) Evidence of a dose related incubation period

B10. Financial costs

Financial costs in each year of the project are estimated with reference to the current work plan and milestones, and are summarised as:

Financial Year	Staff time inputs (total man-days)	Staff time cost (£k)	Experimental animals costs (£k)	Total project costs (£k)
1994/95	113	47.0	160.2	207.2
1995/96	113	47.0	160.2	207.2
1996/97	113	47.0	160.2	207.2
1997/98	113	47.0	160.2	207.2
1998/99	113	47.0	160.2	207.2
1999/2000	7	3.6	0.0	3.6

Costs have been calculated assuming that the cattle will develop clinical disease in a given year. If this occurs costs will be reduced to 1999/2000 level in the subsequent year with no requirement for funding after this.

These cost estimates cover the agreed life of the project. The costs of laboratory consumables and capital equipment together with other miscellaneous costs are included in the figures for staff time.

Estimates for the life of the project were prepared by the CVL Business Unit on 12 January 1994 and are presented after calculation using staff time and other approved CVL cost rates current at that date.

References:

Kimberlin, R.H. and Walker, C.A. (1978). Journal of Comparative Pathology, 88, 39-47.

Wilesmith, J.W. (1992). Seminars in Virology, 2, 239-245.

Wilesmith, J.W. and Wells, G.A.H. (1991). Current Topics in Microbiology and Immunology, 172, 21-38.

ROAME SECTION B

PROJECT TITLE:	Effect of oral inoculum dose on attack rate and incubation period of BSE in cattle
PROJECT LEADER:	G A H Wells
PROJECT NUMBER:	SE 1902
SECTOR NAME:	Statutory and Exotic Diseases
PROGRAMME UNIT NAME:	Mechanisms of pathogenesis in spongiform encephalopathies
ORGANISATION:	Central Veterinary Laboratory
CONTACT ADDRESS:	Central Veterinary Laboratory New Haw Addlestone Surrey KT15 3NB
TELEPHONE:	0932 341111 ext 2498 (GTN 3956)
START OF PROJECT:	April 1992
COMPLETION OF PROJECT:	December 1999
MAFF ASSESSMENT UNIT:	AHVG Tolworth
DATE OF COMPLETION:	September 1993

B1. Summary of the Problem Addressed

The transmissible spongiform encephalopathies (TSE) are unusual, long incubation period diseases for which the infectious agent, and the dynamics of its replication relative to dose, have not yet been fully characterised.

In experimentally induced rodent models of TSE, the efficiency of infection varies depending on the route by which inoculum is administered, but the dose-response curve is consistent for a given route (Kimberlin and Walker, 1978).

Experimentally the oral route of inoculation is less efficient than parenteral routes for scrapie and TME (see Wilesmith and Wells 1991). Little is known however of the precise routes of transmission in naturally occurring TSE or of the important differences between single oral dosing and continuous dietary exposure as may be the case in BSE.

The infective dose of the ingested scrapie-like pathogen necessary to produce the perceived attack rate in the BSE epidemic is not known. Neither is there any understanding of incubation period relative to age of exposure or the possible effect of multiple exposures.

One aspect of the epidemic has focused particular attention on the dynamics of the exposure and how they relate to experimental rodent models.

An increase in incidence of BSE occurred in July 1989 which, given no change in the ascertainment rate at that time (comparable data from the Channel Islands support this assertion) is consistent with a real change in the exposure of the cattle population to the BSE pathogen in 1984 (Wilesmith 1992). But the increased incidence of BSE at this time was not reflected in within herd incidence, rather in a geographically proportional increase in the number of affected herds throughout Great Britain. It is suggested that this phenomenon resulted because there was a geographically uniform increase in the frequency with which batches of feed contained infective material but not an increase in titre within an infected batch.

At the levels of exposure which are producing BSE in the field what is the relevance of this phenomenon to incubation period? Is there a dose-response relationship or an all or nothing effect? Can BSE be induced by oral inoculation with high titre infective material and is the risk of disease increased with increasing dose? The effect of age on the attack rate is also unknown.

B2. Objectives, Approaches and Estimated Completion Dates

a) Objectives

01) To determine the attack rate and incubation period of BSE in cattle exposed orally to four different dose levels of brain homogenate from affected cattle.

02) To determine also if there is a dose-response effect on the incubation period.

03) To establish the effect of multiple exposures compared to a single exposure on attack rate and incubation period.

b) Approaches

There are too many variables in the BSE epidemic to attempt to address all of the problems relevant to determining the dynamics of natural exposure in a single study. In this study age has been standardised within the estimated period of calfhood exposure in the epidemic (Wilesmith - personal communication).

Experimental transmission of BSE to cattle (CH25/003) has established that 100mg of brain i.c. plus 500mg brain i.v. is an infectious dose. Since in a cattle to cattle transmission there are no species-barrier effects, it is likely that 100mg i.c. would have been sufficient to produce disease. It is important that such data is established for experimental oral exposure of cattle to BSE.

By analogy with experimental rodent TSE and by extrapolation from the estimates of the exposure via feed in the natural epidemic of BSE it has been proposed that by the oral route of inoculation 10g of infected brain would probably cause clinical disease and that 100g would be certain to induce clinical disease (R Kimberlin - personal communications). Forty 4-month-old (approximately) calves are dosed orally with homogenate prepared from brainstem from affected cattle as follows:-

i)	1g	10 calves
ii)	10g	10 calves
iii)	100g	10 calves
iv)	3 x 100g (on successive days)	10 calves

All the calves are monitored clinically. They are kept until clinical disease develops, and slaughtered when a clinical diagnosis of BSE is established. The diagnosis is confirmed by histopathological examination of the brain, and by EM for fibril detection. If clinical disease does not develop by 7 years of age, cattle are either killed and examinations carried out as for clinically affected animals, or the project is reviewed in the light of current knowledge.

c) Estimated completion date

If the cattle have not developed clinical disease by 7 years after exposure, they will be killed. (This end-point will be reviewed). The calves were dosed in January 1992, giving a latest completion date of December 1999.

B3. Interdependence of the Objectives

The objectives of this experiment are independent.

B4. Possible Delays

None envisaged. The calves have already been dosed. Intercurrent disease problems in experimental animals kept under conventional husbandry conditions must be considered an unavoidable hazard to the study.

B5. Connections with Earlier Work

Comparison can be made between the effects of oral dosing in cattle and those following exposure by i.c. and i.v. routes (CH25/003). This experiment is also closely linked with the pathogenesis study (SE 1901). The 100g dose group provides a comparable group for that study in the unlikely event that the sequential kills preclude the development of clinical signs. The 100g dose group from this experiment would then provide an end-point study group though the brain homogenate used was different from that used in the pathogenesis study (SE 1901).

B6. Further Research

If all doses are infectious in this study, it would be necessary to perform a further study to establish the LD50 for this route should this be required.

B7. Advancement of Scientific/Technical Understanding

This study will furnish vital data on the effects of log increases in the level of exposure to BSE, on both the 'attack rate' and the length of incubation period.

The information provided by this study will influence future policy decisions regarding the feeding of ruminant-derived protein to ruminants.

B8. Communication of the Results

One full paper in a peer-reviewed journal at the end of the experiment.

B9. Monitoring Progress and Success

01/01 Dosing of all calves (completed) January 1992.

No others are predictable, although the anticipated duration of the study is 3-5 years.

B10. Financial Costs

	Staff costs (+ 5% Seedcorn)	Animal costs
1993/94	£15,142	£223,000
1994/95	£53,486	£160,185
1995/96	£3,626	-

Costs have been worked out assuming that the cattle will develop clinical disease 1994/95. If this does not occur costs will have to be extended to 1999/2000.

References

Kimberlin, R H, and Walker, C A (1978)
Journal of Comparative Pathology, 88, 39-47.

Wilesmith, J.W. (1992)
Seminars in Virology, 2, 239-245.

Wilesmith, J W, and Wells, G A H (1991).
Current Topics in Microbiology and Immunology, 172, 21-38.