Short Communications

Infectivity in the ileum of cattle challenged orally with bovine spongiform encephalopathy

G. A. H. Wells, M. Dawson, S. A. C. Hawkins, R. B. Green, I. Dexter, M. E. Francis, M. M. Simmons, A. R. Austin, M. W. Horigan

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UNIFORMITY of the pathology of bovine spongiform encephalopathy (BSE), defined by the pattern of distribution and severity of the vacuolar changes in the brains of affected cattle provided the first indication that the epidemic in Britain involved a single strain of scrapie-like pathogen (Wells and others 1992). Subsequently this view was supported by the uniformity of incubation periods in cattle parenterally infected with BSE (M. Dawson and G. A. H. Wells, unpublished) and the consistency of data on seven primary transmissions of BSE to mice (Bruce and others 1994). The uniform pathological response observed in cattle provided the necessary predictibility required for an experimental study of the pathogenesis of BSE in the natural host (Wells and others 1992, 1994). Such a study was initiated with the major aim of determining the spatial and temporal development of infectivity and pathological changes following the oral exposure of calves to a single dose of BSE affected cattle brain homogenate. This experiment is in progress and although no definitive results are available, preliminary evidence is reported here of infectivity in the distal small intestine of calves killed early in the study.

A status report of transmission studies of BSE in cattle, pigs and domestic fowl (Dawson and others 1994) has previously outlined the materials and methods of the experiment. Briefly, 40 Friesian/Holstein calves, born in 1991, were assembled from farms with no history of BSE. At four months old 30 were each dosed orally with 100 g of a homogenate of pooled brain stems from 75 cases of BSE, confirmed in 1991. Ten calves received no treatment and served as controls. All cattle were clinically monitored throughout the study. Starting at six months old and thereafter at four-month intervals, three challenged calves and one control calf were killed and tissues collected for infectivity assays, and for histopathological examinations. After each of the sequential cattle kills inocula were prepared from a range of tissues, representing principally the lymphoreticular system, the peripheral and central nervous systems, alimentary tract, striated muscles and major viscera (for a full list see Table 1, Dawson and others 1994). Single tissue pools were made from the three challenged calves and inocula prepared as 10 per cent suspensions in saline with antibiotics. Inocula were prepared also from single tissues of the control animal. Test and control tissue inocula were injected by intracerebral and intraperitoneal routes into RIII inbred mice for standard qualitative assay of infectivity.

No clinical evidence of BSE has yet developed in the cattle; seven groups have been killed and those remaining have survived to 30 months after inoculation. Early evidence of the oral experimental transmission of BSE to the cattle has been obtained from the mouse assay of the distal ileum of challenged calves from the second (six months after inoculation) and third (10 months after inoculation) sequential kill groups, but not from the first (two months after inoculation) group to be killed. The assays of the distal ileum from all challenged cattle are, as yet, incomplete. Of the assays of the distal ileum tissue from the controls only that of the calf killed at two months after inoculation is complete.

The possibility that the infectivity detected in the intestine of the challenged cattle represents residual inoculum, sequestrated in this tissue after its passage along the alimentary tract, is remote for two reasons. First, of the mice inoculated with distal ileum from the challenge calves killed two months after inoculation there has been no histopathological evidence of transmission in those necropsied and 10 of 20 inoculated survive at 655 days after inoculation. Secondly, it is extremely unlikely that inoculum could persist in the tissue for 10 months after inoculation. Further data are required, including the results of quantitative assays of infectivity in this and possibly also related tissues of each of the sequentially killed groups before this can be clarified.

Nevertheless, these preliminary findings are consistent with the infection of cattle with a scrapie-like agent by the oral route, albeit with an exposure dose several orders of magnitude greater than that estimated to cause natural cases of BSE (Kimberlin and Wilesmith 1994). The findings are also consistent with our knowledge of the pathogenesis of natural scrapie in sheep and experimental scrapie in rodents when infection is by non-neural peripheral routes. In these it is well established that there is early replication and life-time persistence of infection in certain lymphoreticular system tissues, including Peyer's patches (Hadlow and others 1982, Kimberlin and Walker 1988, 1989) which are concentrated in the distal ileum. However, previous assays of distal ileum of confirmed natural cases of BSE have failed to detect infectivity (Fraser and Foster 1994). Although these results appear at variance both to the previous understanding of the pathogenesis of scrapie and to the observation presented here it is possible that the difference is a consequence of the relatively large effective exposure of calves in the present study. Thus natural effective exposures of cattle to BSE may well have resulted in infection via intestine and replication of agent in lymphoreticular tissues but with infectivity levels below the limits of detection by bioassay in mice. These limits are estimated at approximately $10^{2.0}$ intracerebral LD50/g of tissue (Kimberlin 1994). Alternatively, it may ultimately prove necessary to postulate that the dynamics of agent replication and spread might be different in BSE than is observed in the rodent models of scrapie or in natural scrapie.

This study is necessarily protracted but the observations reported here suggest that it may now provide considerable information on the pathogenesis of BSE, particularly regarding the development of infection prior to entry to the central nervous system.

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G. A. H. Wells, M. Dawson, S. A. C. Hawkins, R. B. Green, I. Dexter, M. E. Francis, M. M. Simmons, A. R. Austin, M. W. Horigan Central Veterinary Laboratory, New Haw, Addlestone, Surrey KT15 3NB

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References

BRUCE, M. E., CHREE, A., McCONNELL, I., FOSTER, J., PEARSON, G. & FRASER, H. (1994) *Philosophical Transactions of the Royal Society of London* 343, 405

DAWSON, M., WELLS, G. A. H., PARKER, B. N. J., FRANCIS, M. E., SCOTT, A. C., HAWKINS, S. A. C., MARTIN, T. C., SIMMONS, M. M. & AUSTIN, A. R. (1994) BSE Update. Proceedings of a Consultation on BSE with the Scientific Veterinary Committee of the Commission of the European Communities, 14-15 September 1993, Brussels (in press)

FRASER, A. & FOSTER, J. D. (1994) BSE Update. Proceedings of a Consultation on BSE with the Scientific Veterinary Committee of the Commission of the European Communities, 14-15 September 1993, Brussels, (in press)

HADLOW, W. J., KENNEDY, R. C. & RACE, R. E. (1982) *Journal of Infectious Diseases* 146, 657

KIMBERLIN, R. H. (1994) BSE Update. Proceedings of a Consultation on BSE with the Scientific Veterinary Committee of the Commission of the European Communities, 14-15 September 1993, Brussels (in press)

KIMBERLIN, R. H. & WALKER, C. A. (1988) Novel Infectious Agents and the Central Nervous System, Ciba Foundation Symposium 135. Chichester, John Wiley & Sons. p 37

KIMBERLIN, R. H. & WALKER, C. A. (1989) *Virus Research* 12, 213

KIMBERLIN, R. H. & WILESMITH, J. W. (1994) *Annals of the New York Academy of Sciences* 724, 210

WELLS, G. A. H., HAWKINS, S. A. C., CUNNINGHAM, A. A., BLAMIRE, I. W. H., WILESMITH, J. W., SAYERS, A. R. & HARRIS, P. (1994) BSE Update. Proceedings of a Consultation on BSE with the Scientific Veterinary Committee of the Commission of the European Communities, 14-15 September 1993, Brussels (in press)

WELLS, G. A. H., HAWKINS, S. A. C., HADLOW, W. J. & SPENCER, Y. I. (1992) Prion Diseases of Humans and Animals. Eds S. B. Prusiner, J. Collinge, J. Powell and B. Anderton Chichester, Ellis-Horwood. p 256

Superovulation of beef cows and heifers with a single injection of FSH diluted in polyvinylpyrrolidone

T. Suzuki, M. Yamamoto, M. Oe, M. Takagi

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THE superovulatory responses of cows and heifers were compared after single intramuscular injections of refined follicle stimulating hormone (FSH) (Denka Pharmaceutical) diluted with either polyvinylpyrrolidone (PVP) (Denka Pharmaceutical) or saline. An additional objective of the study was to determine the relationship between the concentration of progesterone in the plasma of the superovulated cattle and the numbers of palpable corpora lutea and recoverable embryos.

Forty-two Japanese black parous cows (average age 4·6 years and average weight 530 kg) and 12 sexually mature heifers (average age 14 months and average weight 360 kg) were used.

In experiment 1, two groups of eight cows received single intramuscular injections of 30 mg FSH, diluted with either 10 ml of 30 per cent PVP of molecular weight 40,000 (group 1) or saline (group 2).

In experiment 2, FSH diluted with saline was administered twice daily for five days in declining doses (30 mg; 5:5, 4:4, 3:3, 2:2 and 1:1 mg) to 11 cows (group 1) and 15 cows (group 2), and 12 heifers (group 3) received only a single injection of 30 mg FSH diluted with 10 ml 30 per cent PVP.

All the animals were treated on days 9 to 13 after oestrus and received 30 mg prostaglandin $F_{2\alpha}$ (Lutalyse; Upjohn) on the third day of their five days of treatments. The animals were inseminated with frozen thawed semen at 12 and 24 hours after the onset of oestrus, and the ova and embryos were collected seven days after insemination by a modification of the techniques described by Suzuki and others (1984), Hasler and others (1983) and Donaldson (1983). Each ovary was examined with an ultrasound sector scanner (type 500; Aroka) for the number of corpora lutea and unovulated follicles larger than 10 mm in diameter.

Blood samples were collected from a jugular vein via an indwelling catheter at 09.00 just before the treatment with FSH, and three and seven days after insemination to determine plasma progesterone concentrations. The blood samples were transferred into centrifuge tubes and cooled on ice immediately after collection and stored overnight at 4°C. Progesterone concentrations were assayed according to the procedures described and validated by Troxel and others (1980) and Wiseman and others (1983). Data were analysed using Student's *t* test and the means are given as (mean ± se).

In experiment 1, the mean ± se number of ova plus embryos recovered from the cows treated with FSH diluted with PVP was 9·8 ± 2·4 (Table 1). These results indicated that a single injection of FSH diluted with PVP produced multiple ovulations, and that the method may be useful for the induction of multiple ovulation in cows. It was not possible to calculate the half-life of the FSH, but it was estimated to be approximately three days. In contrast, several of the cows treated with FSH diluted in saline showed multiple follicular growths which failed to ovulate. Thus, a single injection of FSH diluted with saline did not induce ovulation and/or the nuclear maturation of the oocytes. The half-life of FSH in cows after a single injection has been estimated from the decay curve as only 301 ± 23 minutes (Demoustier and others 1991) and as a result multiple injections are necessary to induce superovulation in cows treated with FSH diluted with saline.

In experiment 2 (Table 2), the combined average number of total ova and transferable embryos was significantly higher ($P \leq 0{\cdot}01$) in the cows of groups 1 and 2 than the heifers of group 3. In the heifers, a considerable number of unovulated follicles were present in the ovaries on day 7 but, despite this large ovarian response, the number of transferable embryos recovered was comparatively low, possibly indicating a degree of ovarian overstimulation. This concept is supported by the observations of McGowan and others (1985) who found the high levels of follicular stimulation usually produced poor quality embryos and larger numbers of unfertilised ova. It is possible that a dose of up to 20 mg of FSH may be more satisfactory in heifers.

The plasma progesterone concentrations were highly correlated with the number of corpora lutea palpated, and with the number of ova and embryos recovered, as has been reported by Wubishet and others (1991). Fig 1 shows the mean plasma progesterone

TABLE 1: Influence of PVP as a dilution medium on the superovulatory activity of FSH administered as a single injection to cows

Dilution medium	n	Number of corpora lutea	Number of follicles	Recovery (%)	Recovered ova plus embryos	Transferable embryos
Saline	8	0·7 ± 1·3[a]	4·2 ± 2·8[a]	0[a]	0[a]	0[a]
PVP	8	12·9 ± 1·5[b]	1·8 ± 1·6[a]	76·6 ± 3·7[b]	9·8 ± 2·4[b]	7·0 ± 2·2[b]

ab ($P<0{\cdot}01$)
PVP Polyvinylpyrrolidone
FSH Follicle stimulating hormone

TABLE 2: Mean ± se numbers of palpable corpora lutea and the characteristics of the embryos collected from beef cows and heifers treated with FSH diluted with PVP or saline

Dilution medium	Animals	Number of corpora lutea	Number of follicles	Recovery (%)	Recovered ova plus embryos	Transferable embryos
Saline	11 cows	13·8 ± 2·5[a]	1·8 ± 2·0[c]	87·0 ± 0·8	12·0 ± 8·4[a]	6·0 ± 4·7[c]
PVP	15 cows	12·0 ± 3·9[a]	2·1 ± 1·6[c]	86·7 ± 4·1	10·4 ± 7·6[a]	5·2 ± 2·2[c]
PVP	12 heifers	3·8 ± 3·7[b]	5·2 ± 4·9[d]	73·7 ± 7·5	2·8 ± 3·1[b]	1·1 ± 1·9[d]

ab ($P<0{\cdot}01$), cd ($P<0{\cdot}05$)
PVP Polyvinylpyrrolidone
FSH Follicle stimulating hormone

T. Suzuki, M. Yamamoto, M. Oe, M. Takagi, United Graduate School of Veterinary Sciences, Yamaguchi University, 753 Yamaguchi, Japan

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