Natural transmission of BSE between sheep within an experimental flock

SIR, – The recognition of bovine spongiform encephalopathy (BSE) in a French goat (Eloit and others 2005) has heightened the debate in Europe as to whether BSE has been maintained in small ruminants following historical exposure via feed. Key to the debate and associated risk assessments, especially in the UK, is whether BSE can transmit naturally between infected sheep. Here, we report preliminary evidence that natural transmission can take place between sheep in an experimental flock.

Thirty six-month-old ewe lambs of a PrP ARQ/ARQ genotype from transmissible spongiform encephalopathy (TSE)-free sources were dosed orally with 5 g of BSE cattle brain inoculum. This genotype has previously been shown to be fully susceptible to this inoculum. Approximately six months after infection, the BSE-dosed sheep were mixed with 20 matched undosed animals of the same age and genotype, and kept as a single group under strict biosecurity. Normal intensive commercial practices were followed as far as possible, while avoiding iatrogenic spread of infection.

The ewes were bred from 18 months of age by natural mating using breed/genotype-matched sires from the same TSE-free flocks that had been introduced to the unit. Placental cotyledons were collected at birth. All sheep had unrestricted access to the lambing area to maximise the potential for transmission of disease. Clinical observation, weight recording and tonsil and third eyelid biopsies were used to monitor disease progression. At clinical end point, the sheep were euthanased and examined postmortem, and tissues were collected for a range of immunohistochemical (IHC) and biochemical tests.

Twenty-four of the original 30 dosed sheep reached clinical end point between 655 and 1056 days postinfection (dpi), with a mean (sd) incubation of 797 (105) dpi. Two of the lambs born in 2003 also died of BSE. The first clinical disease in the flock occurred in a dosed ewe, the dam of lamb 2, just 73 days after the birth of its lamb, with clinical end point at 655 days after dosing. The dam of lamb 1 reached clinical end point 198 days after its birth. The first positive tonsil biopsies occurred in these dosed ewes at 369 dpi and in lambs 1 and 2 at 546 days of age. IHC examination of tissues from lambs 1 and 2 using monoclonal antibodies R145 and P4 showed the typical reduction in intracellular labelling with P4 in the obex previously associated with BSE (Fig 1). Lymphoid tissues also showed the typical BSE-associated pattern of reduced labelling of tingible body macrophages with the P4 antibody (Fig 2).

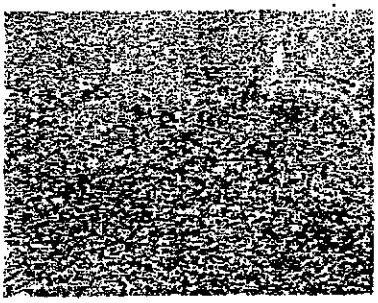

FIG 1: Vagus nucleus in the obex of lamb 1 stained with P4, showing abundant extracellular labelling with very little or no labelling of neurons or microglia. x 230

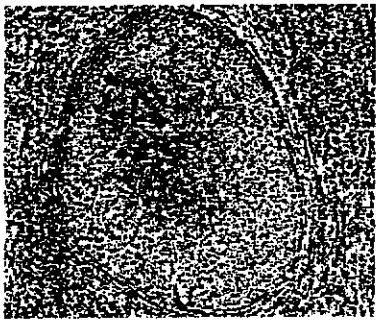

FIG 2: Secondary follicle in the tonsil of lamb 1 stained with P4, showing immunolabelling of follicular dendritic cells within the light zone but no labelling of tingible body macrophages within the dark or light zones. x 230

To date (June 2005), 22 lambs from the 2003 lamb crop born to both dosed and undosed ewes remain alive at 781 to 786 days of age and there are no clinical cases in the original undosed ewes three years after their introduction.

Although scrapie is known to transmit between sheep under natural and experimental conditions, there have been no previous reports of BSE being transmitted naturally between sheep. Foster and others (2004) failed to demonstrate transmission, but this may be explained by lower infection pressures in their experimental design and the use of less susceptible genotypes. At this stage in our study, it is impossible to determine whether infection was acquired from the dam in utero or during the perinatal period, but the incubation period of the affected lambs suggests infection occurred at or just before birth. Previous studies in experimentally infected sheep of the same genotype resulted in incubation periods of 628 to 1132 dpi in animals dosed orally with 5 g of the same inoculum at six months of age (Bellworthy and others 2005) and 525 to 723 dpi in lambs dosed orally with 1 g of the inoculum at two weeks of age (S. J. Bellworthy, unpublished data). The incubation period in the lambs in the present study would preclude the extremely unlikely potential of iatrogenic infection associated with tonsil biopsy. The absence of disease in unrelated, but susceptible, lambs introduced both before and after the first lambing period suggests that transmission may have been restricted to mother and lamb, rather than also horizontally, but it would be premature to conclude at this stage of the study that horizontal transmission had not occurred. Age and closeness of contact may play critical roles in determining likelihood of transmission, although in studies with scrapie we have demonstrated that adult sheep do become infected following introduction to an infected flock, albeit with longer incubation periods than lambs. Horizontal or vertical transmission is clearly a major factor in the spread of scrapie, and transmission may even occur in the absence of direct sheep-to-sheep contact. It remains to be seen whether this is confirmed also with BSE in sheep.

This is the first confirmation that BSE can transmit either in utero or perinatally in sheep. It indicates that if BSE had entered the sheep population at the start of the BSE epidemic, it could have propagated within the flock if the level of infection was sufficient in the presence of susceptible sheep. However, an extensive survey of the UK flock has shown no evidence of the classic BSE phenotype (Stack and others 2005).

S. J. Bellworthy, G. Dexter, M. Stack, M. Chaplin, S. A. C. Hawkins, M. M. Simmons, *Veterinary Laboratories Agency (VLA) – Weybridge, Addlestone, Surrey KT15 3NB*

M. Jeffrey, S. Martin, L. Gonzalez, *VLA – Lasswade, Pentlands Science Park, Bush Loan, Penicuik, Midlothian EH26 0PZ*

P. Hill, *ADAS DEFRA Drayton, Alcester Road, Stratford-upon-Avon, Warwickshire CV37 9RQ*

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別紙 3-5

医薬品
医薬部外品　研究報告　調査報告書
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識別番号・報告回数		回	報告日 年　月　日	第一報入手日 2005 年 6 月 8 日	新医薬品等の区分 該当なし	厚生労働省処理欄
一般的名称			研究報告の公表状況	Detection of ultra-low levels of pathologic prion protein in scrapie infected hamster brain homogenates using real-time immuno-PCR Janet M. Barletta, Daniel C. Edelman, W.E. Highsmith, Niel T. Constantine Journal of Virological Methods, 2005; 127: 154- 164	公表国 米国	
販売名（企業名）						

研究報告の概要

伝染性海綿状脳症に関与する病原性プリオン蛋白 (PrP^{Sc}) は，プリオン病の診断確定のための抗体検査またはバイオアッセイにより検出される。現在では，PrP^{Sc} の検出目的でのウシ脳幹のスクリーニングには，ウェスタンブロットまたは ELISA が公的に使用されている。イムノ PCR (IPCR) は，ELISA 法の抗体を用いた蛋白検出法の特異性と PCR の指数関数的な増幅能とを組み合わせた技術である。本方法を，超微量のプリオン蛋白を検出するためのリアルタイム PCR 変法に応用した。IPCR を用いたところ，ハムスターの組み換え PrPC が，10^{-15}g/ml～10^{-18}mg/ml という極めて微量の PrP^{Sc} が検出可能であることが示された。また，プロテイナーゼ K (PK) で処理したスクレイピー感染ハムスター脳ホモジェネート (10^{8} 倍に希釈，約 10－100 感染単位) が半定量用量反応的に検出された。この感度は，ウェスタンブロットまたは ELISA の検出限界の 100 万倍以上であり，臨床症状発現前の段階で PrP^{Sc} を検出できる方法となり得るものである。一方，検体中の PrP^{C} が PK により完全に処理されなければ，残存する PrP^{C} が混在してしまい結果が不正確になる可能性がある。
今後はヒトおよび動物の血中の PrP^{Sc} を検出できる IPCR の開発に取り組む予定である。現時点では規制当局の要求を満たす実用段階ではないが，本 IPCR 法をさらに標準化すれば臨床症状発現前に PrP^{Sc} を検出することが可能になり，血液供給や動物および動物由来品のより安全な供給に寄与できるであろう。

使用上の注意記載状況・その他参考事項等

報告企業の意見	今後の対応
弊社ではプリオン検出技術の改良に関する文献を随時検索している。IPCR 法の確実性が確認されかつ大規模な製造工程に適用可能となれば，血漿分画製剤の製造工程においてプール血漿レベルでの PrP^{Sc} の検出能が顕著に向上すると思われる。	現時点で弊社が新たな安全対策上の措置を講じる必要はないと考える。引き続き超高感度プリオン検出技術の関連情報の収集に努める。