M03057 – final technical report

Background

Since the discovery of atypical scrapie (1) and its subsequent identification, mostly through active surveillance, in a number of countries including those with no previous history of transmissible spongiform encephalopathies (TSEs) including New Zealand (*2*), and Australia (*3*) there has been scientific debate as to whether this form of TSE is in fact spontaneous or acquired (*1,4,*) rather than contagious.

The epidemiological studies that have been undertaken suggest that atypical scrapie does not appear to transmit between animals in the field situation (*1,5*). Although the routes by which natural transmission occurs have never been fully established for TSEs, it is widely accepted that ingestion of infective material, i.e. the oral route, is a key component in some TSEs, for example kuru (6), variant Creutzfeldt-Jakob Disease (vCJD) (*7)*, bovine spongiform encephalopathy (BSE) (*8* and transmissible mink encephalopathy (TME) (*9*).

Within the sheep population, susceptibility to particular strains of TSE has been shown to be heavily affected by polymorphisms of the prion protein gene (*PRNP)* of the sheep (10*,11,12*). Successful transmission of atypical scrapie to sheep following intracerebral inoculation has been previously reported for sheep of one genotype ($A_{136}H_{154}Q_{171}$/ $A_{136}H_{154}Q_{171}$) (*13,14*) and challenges in other homologous and heterologous genotype combinations are ongoing (Defra project SE1847). However, it is also known that successful intracerebral transmission of a particular TSE agent in a particular species does not necessarily indicate susceptibility by the oral route (*15*).

The tissue distribution of infectivity and/or disease-specific prion protein (PrP^{Sc}) in classical scrapie and to a lesser extent BSE has led to extensive public health control measures based on the known pathogenesis and distribution of PrP^{Sc} in edible tissues, and their removal from carcases over a certain age. Although early studies in atypical scrapie were unable to reveal PrP^{Sc} or infectivity outwith the brain recent data indicate that peripheral tissues from natural cases can harbour infectivity either in the presence or absence of detectable PrP^{Sc} (*16*). However, it is not known yet if this infectivity is established before or after the agent has propagated in central nervous system.

The first aim of the current study was to look at the distribution of infectivity in peripheral tissues in animals at and beyond the ‘cut-off’ point for the current European Commission (EC) meat hygiene regulations (i.e. 12 months of age). The second aim was to investigate the potential for oral transmission of atypical scrapie.

Materials and Methods

The scale of this proposal was necessarily restricted by the availability of both naturally-occurring donor material and appropriate recipient sheep, and the current thinking that challenge of sheep with tissue from single affected cases (not pooled material) of the same genotype (homologous genotype combinations) is preferable. In this context, there was sufficient material from three atypical scrapie cases (two ARR/ARR and one AHQ/AHQ) identified through the national surveillance schemes, which would enable the challenge of 6 homologous recipients each (i.e. 12 sheep in total) from the Defra NZ-derived classical scrapie-free flock (SE 1931; (*4*)).

Inoculum

The characterisation of inoculum prior to dosing would be best practise, however the restricted amounts of donor tissue available limited the degree to which this could be achieved. Also, due to the use of neonatal lambs, there had to be contingency for the loss of some lambs in the perinatal period, so any inoculum not initially assigned for dosing was held in reserve in case additional challenges were required, with the intention of characterising this residual inoculum more fully following successful completion of the dosing. This was of particular importance given the very small group sizes in this project. The original proposal incorporated inoculum titration in mice, however insufficient material remained from any of the donors following the lamb challenges. With the agreement of the FSA Project officer, the costs for inoculum titration were removed from the project and a revised pricing schedule submitted in September 2007.

Details of the donor animals are given below.

ARRa

This was a passive surveillance case (PG 829/04) from 2004, which had previously been used to supply Defra project SE 1441 (HuTg mice at Roslin/Univ Ed). It has also been used for the oral dosing of VRQ/VRQ and AHQ/AHQ sheep, and the intracerebral challenge of AHQ/AHQ sheep (i.e. not homologous transmissions) within Defra project SE1847. All sheep are currently healthy 6 years post-challenge. A total of 25ml of neat brain homogenate was available, which was used to make 10 aliquots: exactly sufficient for 5 lambs. No initial BioRad ELISA screening result was available for this case, since it was submitted through the passive surveillance stream before ELISA was a routine part of the diagnostic screening process. An aliquot of inoculum used for SE 1847 (i.c. challenge) was submitted for BioRad ELISA, but the small volume of tissue recovered from the inoculum meant that it had to be made up in normal brain (20% dilution) to run the test and this was negative (OD 0.020). Since this stored SE1847 inoculum was not directly referable to the inoculum given to the lambs within this study, mouse titration was not performed. However, a single panel of Tg338 mice was challenged with this inoculum to confirm that the donor animal contained infectivity with a profile which is representative of atypical scrapie

(see Figure 1). Full PrP distribution mapping (by IHC) was not undertaken on the donor sheep because most of the brain was frozen. However, immunolabelling in the obex, cerebellum and thalamic areas were consistent with atypical scrapie (17).

ARRb

This donor brain (PG 1403/04, identified through active surveillance (fallen stock)) has also been used to dose sheep orally and intracerebrally (homologous recipients) within project SE 1847. It was bioassayed within project SE 1850 (see figure 1). No inoculum remained for titration. BioRad ELISA of this SE1850 inoculum gave positive OD readings of 1.554 and 0.761. The initial diagnostic screening sample OD was 1.074. Full PrP distribution mapping revealed immunolabelling consistent with atypical scrapie throughout the neuraxis, with emphasis on the cerebellum, the substantia nigra area of the midbrain, the thalamus and basal ganglia, with less pronounced involvement of the cerebral cortex.

AHQ

The amount of brain available from the AHQ donor animal (PG 1088/06, identified through active surveillance (fallen stock)) was sufficient to make 12 x 2.5ml aliquots, which were all assigned to lamb challenges. One lamb died on the day after the first dose, so the remaining dose for this animal was used as the first dose for a replacement lamb challenge. Small remnants of brainstem and cortex from this donor were still available in the tissue store, and these were used to make one further aliquot to use as a second dose for the final lamb. Sufficient residual material from this second homogenate remained for basic biochemical analysis (ELISA OD 2.272) and inoculation into a panel of Tg338 mice The initial diagnostic screening sample from this case gave an ELISA OD of 0.948. Full PrP distribution mapping (by IHC) revealed immunolabelling consistent with atypical scrapie throughout the neuraxis, but with a preponderance of white matter staining and substantially less neuropil staining than has been seen in other AHQ atypical cases. Intracerebral inoculation of this donor into sheep (reference laboratory tissue production) has resulted in the expected level of neuropil labelling associated with atypical pathology, together with a pronounced white matter involvement.

Challenge of lambs

All dosing was carried out in accordance with the Animal (Scientific Procedures) Act 1986, under Licence from Home Office.
Lambs were dosed with 2.5gms in the first 24 hours of life and then 2.5gms at 14 days of age in accordance with previously published methodology (18) which accommodates data on age-related susceptibility of sheep to prion infection (19). To enable this, pregnant ewes were moved from the NZ-derived sheep unit to VLA 2 months prior to lambing. Out of hours monitoring of the ewes at lambing time was undertaken to ensure accurate recording of

the time of lambing and better survival of lambs in the event of any perinatal problems. See table 1 for a summary of animals and challenge details.

Blood samples were taken monthly from 3-6 months of age, and deposited in the TSE Archive (see tables 2-5 for details of all samples currently held in the AHVLA Biological Archive. The numbers indicate the number of aliquots held). There is currently no test available for the screening of blood, so these unique time-course samples have been stored for future use should such an assay become available.

Of the 12 dosed animals, six animals (three of each genotype) were killed at the current time cut-off for removal of specified risk materials from sheep (12 months) and the remaining six were kept alive for a further 12 months.

Post mortem examination

At post-mortem examination a range of neural and non-neural tissues (see tables 6-9 for a comprehensive list) were taken using aseptic techniques and either placed into 10% formal saline (neural tissues), 10% buffered formalin (non-neural tissues) or frozen and held at -80ºC for subsequent examination by immunohistochemistry (IHC) and/or ELISA depending on the size and nature of the sample.

The tissues collected were selected to provide a range of samples relevant to the food chain, and/or the pathogenesis of known forms of TSE. Brain and spinal cord were examined in detail to determine if disease had been successfully transmitted. Various gut-associated tissues (eg tonsil, distal ileum, RAMALT (recto-anal mucosa-associated lymphoid tissue) mesenteric lymph node and celiaco-mesenteric nerve plexus) were collected to see if there was evidence of peripheral accumulation of PrP^{Sc} following challenge by the oral route, and therefore to give a crude indication of the possibility of infectivity being shed in faeces. Salivary gland was also sampled to investigate the possibility that infectivity could be shed trough saliva. Other tissues (such as pre-scapular lymph node, thymus, kidney, liver, extra-ocular muscles) were selected to represent edible tissues not directly linked to the gastro-intestinal tract. Extra-ocular muscles are frequently used to represent skeletal muscle in TSE, since PrP^{Sc} accumulation occurs in muscle spindles specifically, and these small eye muscles have a very high concentration of spindles. Spleen was collected to investigate the possibility of haematogenous dissemination of the agent.

Selected positive tissues were also subject to Western blotting; i.e. the current UK active surveillance screening and confirmatory tests, to ensure that the Western blot ‘signature’ of atypical scrapie was unchanged by being transmitted orally. Other tissues were retained for review pending the outcome of the initial testing. Negative control tissues were included in assays, and positive controls were derived from project SE1847 (animals positive for atypical scrapie following intracerebral challenge).

Positive cases were assayed in mice to assess the stability of agent following passage. A range of tissues were also collected for potential bioassay in Tg338, as this mouse model is more sensitive than biochemical testing ((20) VLA SE1850 data). Irrespective of the results of biochemical testing brain, spleen and distal ileum were bioassayed for evidence of infectivity using these tg338 mice.

The remaining tissues were stored for future testing and/or bioassay, if appropriate (see table 10. The numbers indicate the number of aliquots retained). Some bioassays are now being undertaken on this material within Defra project SE 1847 (see tables 6-9 for details of which tissues).

Testing methods[1]

All of the methods used to test tissues from these animals are well-established diagnostic methods which are used routinely at AHVLA.

IHC:

Fixed samples were processed into paraffin wax, sectioned and stained with haematoxylin and eosin as described in detail elsewhere (*21*). Immunohistochemical detection of PrP^{Sc} was performed as previously described (*13*) using mouse monoclonal antibody 2G11 (Institut Pourquier, Montpellier, France), raised against ovine PrP peptide sequence 146-R154 R171-182. Immunohistochemistry profiles were created using standard subjective method as previously described (*17*) in which the type of PrP^{Sc} immunolabelling is assessed in a standard range of precise neuroanatomical areas. Tissues from the lymphoreticular system of each challenged animal were examined by IHC, using the same method described above.

ELISA:

All tissues were analysed by the Bio-Rad TeSeE kit for TSE detection in sheep and goats according to the manufacturer's instructions. The cut-off value was calculated as the average absorbance (AU) reading of the negative control values plus a value of 0.14 AU. In order to directly measure PrP^{Sc} content in the samples that were used to inoculate the recipient animals, the inocula were prepared for the Bio-Rad protocol prior to analysis to account for variation in tissue and buffer content. Samples were centrifuged at high speed to concentrate insoluble material. The weight of the pellet was calculated, and then resuspended in homogenisation buffer to

[1] The laboratory facilities are UKAS accredited to BS EN ISO 17025:2000 (Lab Nos. 0941, 1769 and 2112) for an extensive range of tests supported by proficiency testing accredited to ISO/IEC Guide 43-1 1997 (Lab No. 0004). VLA is certificated to BS EN ISO 9001:2000 (Certificate Nos. LRQ 4000436, 4001071, 0962413 and 4001392). Additionally VLA holds Good Laboratory Practice approval and complies with Good Clinical Veterinary Practice quality standards.

prepare a 20 % w/v homogenate and continued as described from the ribolysation stage. Pellets that contained insufficient weight to prepare 250 µls of 20% w/v homogenate were supplemented using brain homogenate or relevant tissue prepared from negative sheep.

Western immunoblot:

Fresh brain samples were subjected to the TeSeE Universal WB (Bio-Rad Cat No: 355 1169 Marnes-la-Coquette, France) as previously described (*13*). Molecular mass markers were included at either end of the gel. A single lane each of a known UK classical scrapie, known UK bovine BSE, and a known UK atypical scrapie case were included for profile comparisons.

Mouse Bioassay:

Samples from the donor and recipient animals were treated in the same way. Tissue homogenate (10%) was prepared w/v in normal saline, screened for microbiological sterility using standard methods, treated using ampicillin and gentamycin if contamination was identified, and re-checked before use.

Panels of 10 transgenic mice over-expressing ovine *PRNP* (Tg338 (*22*)) were inoculated intracerebrally with 20µl and, when sufficient inoculum was available, intraperitoneally with 100µl of homogenate. Mice were monitored weekly and were killed when they had shown clinical signs on two out of three consecutive monitoring days or natural lifespan. Brains were then fixed, processed and lesion profiles produced as described in detail elsewhere (*23*).

Bioassay of a minimum of three samples from each challenged sheep was planned. The distal ileum, to represent the hypothetical 'point of entry' through the gut-associated lymphoid tissue following oral challenge; the spleen, for evidence of haematogenous dissemination of infectivity, and the cerebellum to establish if neuroinvasion had occurred.

Results

12 month cull:

Clinical observations

Of the six animals killed after 12 months, five were clinically normal at the time of culling. PG 152/08 appeared nervous during handling, although its behaviour at previous blood sampling sessions had been unremarkable, and it displayed a bilaterally absent menace response (inconclusive with regards to scrapie).

Post mortem testing
None of the sheep presented with detectable PrP^{Sc} in any of the tissues examined by IHC and/or ELISA (see Tables 6-9).

Bioassay
Bioassays in mice (see table 11) demonstrated infectivity in the cerebellum of PG 151/08, but only in a single mouse with a long incubation time, suggestive of a very low titre. Bioassays of the cerebella from all other 12 month culls were negative. All spleen bioassays were negative. The distal ileum samples from the ARR/ARR animals were all contaminated with a residual bacterial toxin which could not be cleared from the inoculum, so these could not be assessed by bioassay. Bioassay of the distal ileum from the three AHQ/AHQ culls gave positive results for cases 154/08 and 155/08. The mouse lesion profiles for these distal ileum isolates is the same as that of the donor (See example in figure 2B), demonstrating that the biological properties of the agent are the same in the lymphoid tissue as in the central nervous system.

24 month cull:

Clinical observations
Of the six sheep culled at 24 months post inoculation, three appeared clinically normal. PG108/09 displayed alopecia suggestive of pruritus although no pruritic behaviour was observed (inconclusive with regards to scrapie). PG110/09 appeared nervous when approached and handled, and displayed a wide-based hind limb posture (inconclusive with regards to scrapie). PG109/09 appeared nervous with a fine head tremor during handling, had a wide-based hind limb posture and was ataxic with uncoordinated jumps, swaying and loss of balance (*Movie file 1* in reference 24). At blood sampling two months earlier, it was observed circling clockwise when left alone, which was not seen at the final examination. This animal was considered to be a scrapie clinical suspect. None of the sheep displayed a positive scratch test, and none of these six sheep had lost weight prior to cull.

Post-mortem testing
PrP^{Sc} was detected in two cases only (PG 108/09 and PG 109/09), both of which were AHQ homozygotes. This immunolabelling was confined to the central nervous system. In PG 108/09 (see Figure 3), which was clinically normal, PrP^{Sc} was observed in the caudal thalamus, restricted to the lamina medullaris externa, in the caudate nucleus, the amygdala and external capsule, and there was minimal labelling in the basal and septal nuclei. Positive immunolabelling was present in the hippocampus, together with extensive incidental ‘thready’ staining (25). There was white matter labelling in the olfactory tract and rostral commissure. No PrP^{Sc} was detected in the medulla or cerebellum, which are the areas currently used for statutory surveillance purposes.

In PG109/09 (Figure 4) PrP^{Sc} was distributed widely throughout the brain, and was detectable by IHC (Figure 2), ELISA and Western blot. There was widespread white matter labelling and mild to moderate granular labelling consistent with that described for natural cases of atypical scrapie (17). There was no evidence of PrP^{Sc} accumulation in any of the examined tissues from the other cases.

Western blot profiles of PrP^{Sc} from these cases were compatible with atypical scrapie, and consistent with the donor profiles (Figure 5).

Bioassay

All three screened tissues from the three ARR/ARR animals were negative by bioassay. PG110/09 was also negative in all three tissues. In PG108/09 there was confirmation of the IHC results, with infectivity being demonstrated in both the basal ganglia and the hippocampus, and infectivity was also present in the cerebellum (with longer incubation periods, and therefore lower titre) despite the absence of detectable PrP with the *in vitro* tests. These samples all had lesion profiles consistent with the donor animal. Only one animal (PG109/09) had demonstrable infectivity in the distal ileum.

Discussion

Although all TSEs are transmissible after intracerebral challenge to a susceptible host only some are infectious under natural conditions. So it was important from a pathogenesis and disease control perspective to establish whether or not oral transmission can be successful. This study has resulted in the first successful oral transmission of atypical scrapie, confirms that the disease phenotype is retained following transmission by this route in AHQ/AHQ sheep, and indicates that infectivity can be demonstrated in the gut in the absence of detectable PrP^{Sc} at least as early as 12 months after exposure.

One sheep (PG109/09) culled at 24 mpi displayed abnormalities in behaviour and movement suggestive of atypical scrapie. Signs like ataxia with head tremor and circling have been described in experimental and natural) disease, which was attributed to lesions in the cerebellum and forebrain, respectively, corresponding with PrP^{Sc} accumulation in these areas. In this regard, the animal resembled natural field case atypicals, with the notable exception of the incubation period, which at 24 months is approximately a third of the normally observed age at onset of natural cases. This could be attributed substantially to the age at challenge and the large volumes given (see below).

By contrast, PG 108/09, with confirmed atypical scrapie based on post mortem tests was considered clinically normal. The less severe and more restricted PrP^{Sc} accumulation in the brain of this sheep compared to PG

109/09 may explain the absence of clinical abnormalities. It also means that this animal would have escaped detection with the current surveillance regime, since it would not have presented through passive surveillance, and testing of the obex and cerebellum regions with the current screening tests would have been negative, despite the presence of infectivity in the cerebellum.

The challenge model in this study exposed animals as neonates, when the oesophageal groove is operational and the lambs are physiologically monogastric. Exposure of three month old ruminating animals to similar amounts of positive brain by the oral route have so far been unsuccessful, with all animals still alive at approximately 2000 days post challenge (Defra project SE1847), but most natural cases have been recorded in animals older than this, so these animals may yet progress to disease. It is likely that transmission is more efficient in newborn animals; the incubation periods of sheep orally infected with classical scrapie were significantly shorter in sheep challenged at 14 days compared with 6 months of age (*26*). If, however, oral transmission is only effective in such young animals, then field exposure would most likely have to be through milk, which is known to be a very effective route of transmission in classical scrapie (*27*). There is currently no data available on the potential infectivity of milk from animals with atypical scrapie.

Successful oral transmission also raises questions regarding the pathogenesis of this form of disease. There must be passage of the infectious agent from the gastro-intestinal tract to the brain through one of several possible routes, most likely those that have been suggested and discussed in detail for other TSEs, for example retrograde neuronal transportation either directly or via lymphoid structures or hematogenously, as reviewed elsewhere (*28*). Infectivity in the absence of readily demonstrable PrP^{Sc} has been reported previously (*29, 30, 31*), and the mouse bioassay may detect evidence of disease in other tissues, but these data may not be available for at least another two years. It may be that more protease-sensitive forms of PrP^{Sc} can be broken down more efficiently within cells and so do not accumulate in peripheral tissues, as has been proposed previously (*13*), thus enabling atypical PrP^{Sc} to transit the digestive tract and disseminate through other systems in small amounts before accumulating detectably in central nervous system.

Although there is no epidemiological evidence to support efficient spread of disease in the field these data imply that disease is potentially transmissible under field situations, and that spread through animal feed may be possible if the current feed restrictions were to be relaxed.

The bioassays performed in this study are not titrations, so the infectious load of the positive gut tissues cannot be quantified, although it is unequivocal that infectivity is present. There is currently no experimental data available on the zoonotic potential of atypical scrapie, either through experimental challenge of humanised mice, although such studies are ongoing in several laboratories, or

any meaningful epidemiological correlation with human forms of TSE. However, the detection of infectivity in the distal ileum of animals as young as twelve months, in which all the tissues tested were negative by the currently available screening and confirmatory diagnostic tests, indicates that the diagnostic sensitivity of current surveillance methods is suboptimal for the detection of atypical scrapie and potentially infectious material may be able to pass into the human food chain undetected.

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Project outputs

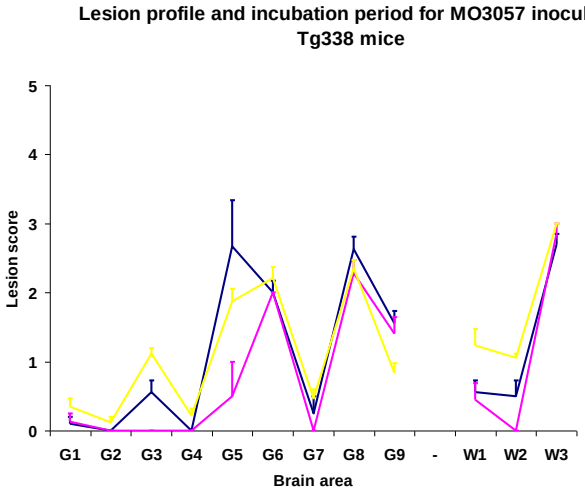
This project has two main outputs:

One peer-reviewed publication
Simmons MM, Moore SJ, Konold T, Thurston L, Terry LA, Thorne L, Lockey R, Vickery C, Hawkins SAC, Chaplin MJ, Spiropoulos J (2011) Experimental oral transmission of atypical scrapie to sheep. Emerging Infectious Diseases 17, 848-854. *(Appended)*

Archived blood and post-mortem tissues
These tissues are listed in the attached tables. Their retention will be reviewed on a regular basis as part of the routine management of this unique archive, and if there has been no demand for the tissues they will ultimately be discarded, although current policy will allow the retention of a representative panel of single aliquots.

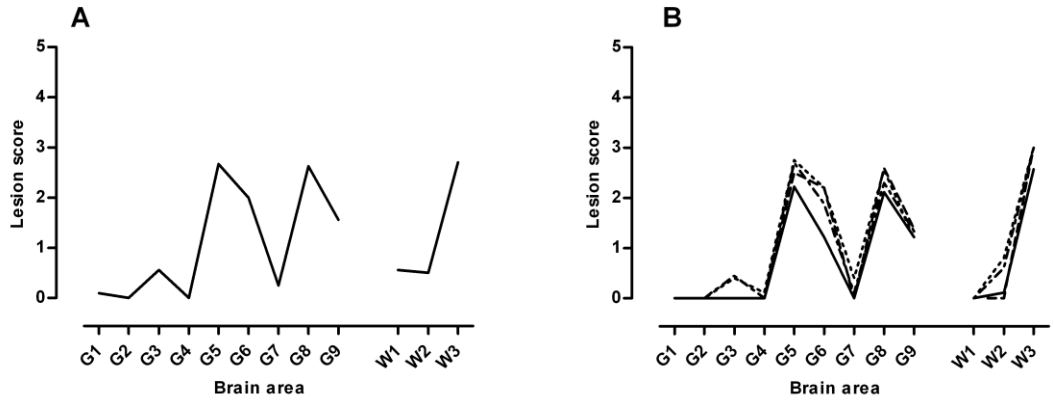
Figures

Figure 1.
Vacuolation lesion profiles for the three donor inocula, profiled in Tg338 mice. This shows that all three inocula were able to transmit disease in this model, and the biological signature was the same from all three sources.



Key:
Blue = ARRa (mean ip 191d);
Cyan = AHQ (mean ip 177d);
Yellow = ARRb (mean ip 200d)

Figure 2.
Comparison of the mouse lesion profiles from donor inoculum and recipient sheep tissues

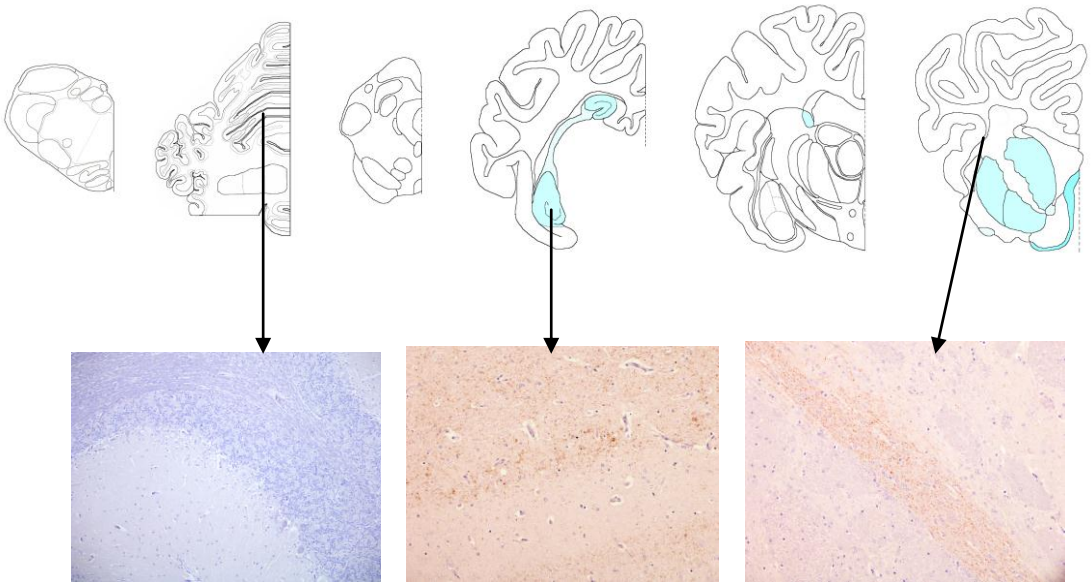


A) The donor AHQ/AHQ sheep (same profile as 'yellow' in figure 1)

B) Recipient sheep brain and distal ileum.

Cerebellum from PG109/09
Basal ganglion from PG108/09
Hippocampus from PG108/09
Distal ileum from PG155/08

Figure 3.
Distribution of PrP immunolabelling in case PG 108/09.



(negative)

Figure 4

Distribution of PrP immunolabelling in case PG109/09

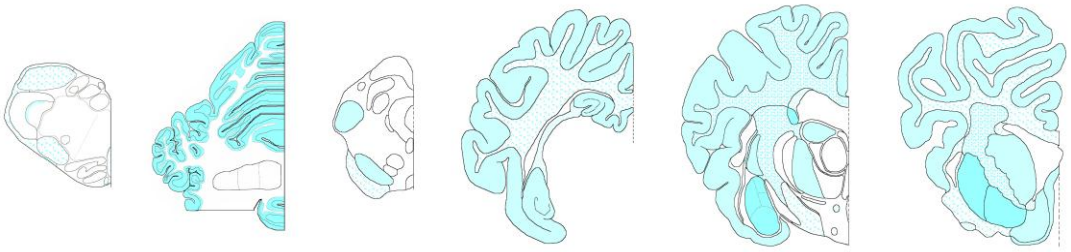
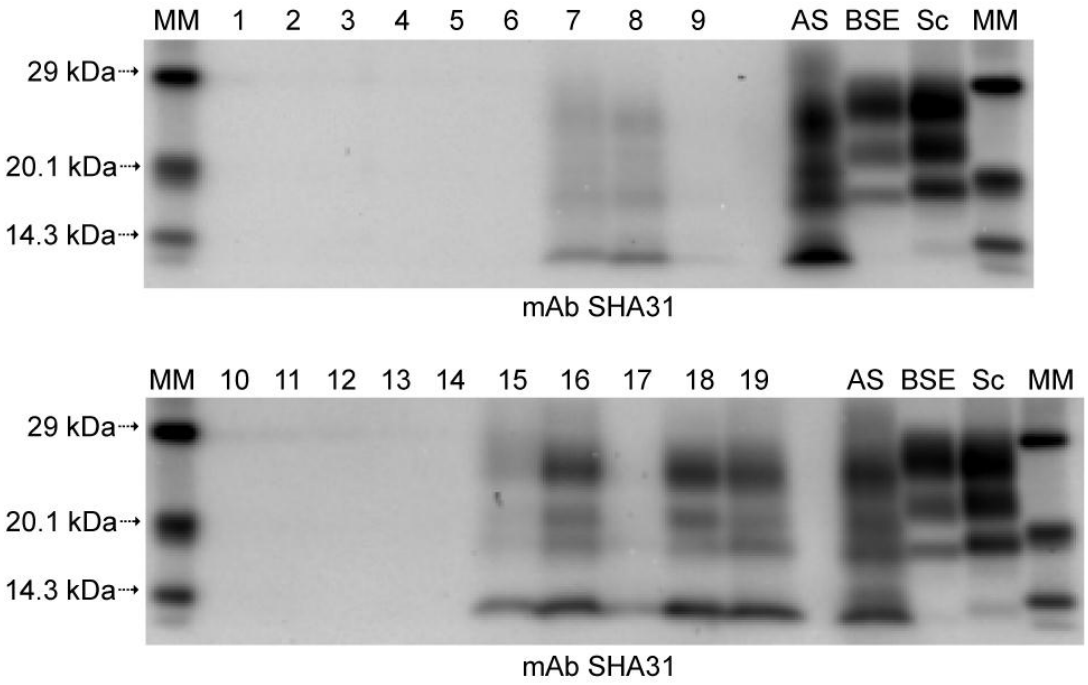


Figure 5.

Western blot showing the characteristic 4 band pattern of atypical scrapie in the samples of both donor and recipient samples



Key

Lanes 1-6:	obex regions from 12 month culls.
Lane 7:	Donor ARRa (rostral brainstem)
Lane 8:	Donor ARRb (frontal cortex)
Lane 9:	Donor ARRb (caudal medulla)
Lanes 10 -13:	obex region of negative animals at 24 month cull
Lane 14	PG 108/09 (obex)
Lane 15	PG 108/09 (hippocampus)
Lane 16	PG 108/09 (basal nuclei)
Lane 17	PG 109/09 (obex)
Lane 18	PG 109/09 (cerebellum)
Lane 19	Donor AHQ (frontal cortex)
MM	Molecular Mass Marker
AS	Atypical Scrapie +ve
BSE	Classical BSE +ve
Sc	Classical Scrapie +ve

Table 1 – Details of animals, inocula, challenges and post-mortem dates

MO3057 Animal Details

Animal ID:	Sex:	Breed	Genotype:	DOB:	Date of 1st Dose:	Date of 2nd Dose:	PM Date:	Inoculum Code:	Inoculum source
PG0150/08	M	Dorset	ARR/ARR	06/02/2007	07/02/2007	21/02/2007	18/02/2008	MO3057/BP0001	PG829/04 (ARRa)
PG0091/09	F	Dorset	ARR/ARR	06/02/2007	07/02/2007	21/02/2007	16/02/2009	MO3057/BP0001	PG829/04 (ARRa)
PG0092/09	M	Dorset	ARR/ARR	15/02/2007	15/02/2007	01/03/2007	23/02/2009	MO3057/BP0001	PG829/04 (ARRa)
PG0151/08	F	Dorset	ARR/ARR	15/02/2007	15/02/2007	01/03/2007	21/02/2008	MO3057/BP0001	PG829/04 (ARRa)
PG0152/08	M	Dorset	ARR/ARR	18/02/2007	18/02/2007	04/03/2007	25/02/2008	MO3057/BP0001	PG829/04 (ARRa)
PG0093/09	F	Dorset	ARR/ARR	17/02/2007	18/02/2007	04/03/2007	24/02/2009	MO3057/BP0003	PG1403/04 (ARRb)
PG0153/08	F	Cheviot	AHQ/AHQ	20/03/2007	20/03/2007	03/04/2007	27/03/2008	MO3057/BP0002	PG 1088/06 (AHQ)
PG0108/09	M	Cheviot	AHQ/AHQ	20/03/2007	20/03/2007	03/04/2007	30/03/2009	MO3057/BP0002	PG 1088/06 (AHQ)
PG0154/08	M	Cheviot	AHQ/AHQ	21/03/2007	22/03/2007	05/04/2007	28/03/2008	MO3057/BP0002	PG 1088/06 (AHQ)
Died 24/03/07	F	Cheviot	AHQ/AHQ	22/03/2007	22/03/2007	N/A	N/A	MO3057/BP0002	PG 1088/06 (AHQ)
PG0109/09	F	Cheviot	AHQ/AHQ	23/03/2007	23/03/2007	06/04/2007	301/03/2009	MO3057/BP0002	PG 1088/06 (AHQ)
PG0155/08	M	Cheviot	AHQ/AHQ	23/03/2007	23/03/2007	06/04/2007	31/03/2008	MO3057/BP0002	PG 1088/06 (AHQ)
PG0110/09	M	Cheviot	AHQ/AHQ	23/03/2007	24/03/2007	07/04/2007	01/04/2009	MO3057/BP0002 and 4	PG 1088/06 (AHQ)

Table 2 – Blood samples retained in the AHVLA Biological archive from the ARR/ARR animals culled at 12 months. All blood samples were fractionated and stored as aliquots of different blood fractions. The numbers refer to the number of aliquots of each sample type

PG Number	Sampling Date	Blood Clot	Buffy Coat (Citrate)	Buffy Coat (EDTA)	Buffy Coat (Fl Ox)	Buffy Coat (Hep)	Plasma (Citrate)	Plasma (EDTA)	Plasma (Fl Ox)	Plasma (Hep)	Serum	Umbilical Cord (foetal)	Whole Blood (Citrate)	Whole Blood (EDTA)	Whole Blood (Fl Ox)	Whole Blood (Hep)
PG0150/08	07/02/2007	1									1			1		
	22/10/2007		1	1	1	1	1	3	1	1	4		1	2	1	2
	26/11/2007		1	1	2	1	1	2	2	2	4		1	2	2	2
	20/12/2007		1	1	1	1	1	2	1	2	4		1	2	1	2
	22/01/2008			1		1		2		2	4		1	2	2	2
PG0151/08	22/10/2007		1	1	1	1	1	2	1	1	3		1	2	1	2
	26/11/2007		1	1	2	1	1	2	2	2	4		1	2	2	2
	20/12/2007		1	1	1	1	1	2	1	2	4		1	2	1	2
	22/01/2008		1	1	1	1	1	2	2	2	4		1	2	2	2
PG0152/08	22/10/2007		1	1	1	1	1	3	1	1	4		1	2	1	2
	26/11/2007			1		1		2		2	4			2		2
	20/12/2007		1	1	1	1	1	2	1	2	4		1	2	1	2
	22/01/2008		1	1	1	1	1	2	2	2	4		1	2	2	2

Table 3 - Blood samples retained in the AHVLA Biological archive from the AHQ/AHQ animals culled at 12 months. All blood samples were fractionated and stored as aliquots of different blood fractions. The numbers refer to the number of aliquots of each sample type

PG Number	Sampling Date	Blood Clot	Buffy Coat (Citrate)	Buffy Coat (EDTA)	Buffy Coat (Fl Ox)	Buffy Coat (Hep)	Plasma (Citrate)	Plasma (EDTA)	Plasma (Fl Ox)	Plasma (Hep)	Serum	Umbilical Cord (foetal)	Whole Blood (Citrate)	Whole Blood (EDTA)	Whole Blood (Fl Ox)	Whole Blood (Hep)
PG0153/08	20/03/2007	2									2			4		
	22/10/2007		1	1	1	1	1	3	1	1	3		1	2	1	2
	26/11/2007		1	1	2	1	1	2	2	2	4		1	2	2	2
	20/12/2007		1	1	1	1	1	2	1	2	4		1	2	1	2
	22/01/2008		1	1	1	1	1	2	2	2	4		1	2	2	2
	25/02/2008		1	1	1	1	1	2	1	2	4		1	2	1	2
	25/03/2008		1	1	2	1	1	2	2	2	4		1	2	2	2
PG0154/08	21/03/2007											1				
	23/03/2007	4									3			6		
	22/10/2007		1	1	1	1	1	3	1	1	4		1	2	1	2
	26/11/2007		1	1	1	1	1	2	2	2	4		1	2	2	2
	20/12/2007		1	1	1	1	1	2	1	2	4		1	2	1	2
	22/01/2008		1	1	1	1	1	2	2	2	3		1	2	2	2
	25/02/2008		1	1	1	1	1	2	1	2	4		1	2	1	2

Table 3 *[contd]*

	25/03/2008		1	1	2	1	1	2	2	2	4		1	2	2	2
PG0155/08	23/03/2007	3									3					
	22/10/2007		1		1	1	1	3	1	1	4		1	2	1	2
	26/11/2007		1	1	1	1	1	2	2	2	4		1	2	2	2
	20/12/2007		1	1	1	1	1	2	1	2	4		1	2	1	2
	22/01/2008		1	1	1	1	1	2	2	2	4		1	2	2	2
	25/02/2008			1		1		2		2	4			2		2
	25/03/2008		1	1	2	1	1	2	2	2	4		1	2	2	2

Table 4 - Blood samples retained in the AHVLA Biological archive from the ARR/ARR animals culled at 24 months. All blood samples were fractionated and stored as aliquots of different blood fractions. The numbers refer to the number of aliquots of each sample type

PG Number	Sampling Date	Blood Clot	Buffy Coat (Citrate)	Buffy Coat (EDTA)	Buffy Coat (Fl Ox)	Buffy Coat (Hep)	Plasma (Citrate)	Plasma (EDTA)	Plasma (Fl Ox)	Plasma (Hep)	Serum	Umbilical Cord (foetal)	Whole Blood (Citrate)	Whole Blood (EDTA)	Whole Blood (Fl Ox)	Whole Blood (Hep)
PG0091/09	07/02/2007	2									1			2		

Table 4 *[contd]*

	22/10/2007		1	1	1	1	1	3	1	1	2		1	2	1	2
	26/11/2007		1	1	2	1	1	2	2	2	4		1	2	2	2
	20/12/2007		1	1	1	1	1	2	1	2	4		1	2	1	2
	22/01/2008			1	1	1		2	1	2	4		1	2	2	2
	25/02/2008		1	1	1	1	1	2	1	2	4		1	2	1	2
	25/03/2008		1	1	2	1	1	2	2	2	4		1	2	2	2
	23/04/2008		1	1	2	1	1	2	2	2	4		1	2	2	2
	22/05/2008			1		1		2		2	4			2		2
	24/06/2008		1	1	2	1	1	2	2	2	4		1	2	2	2
	23/07/2008		1	1	2	1	1	2	2	2	4		1	2	2	2
	26/08/2008		1	1	2	1	1	2	2	2	4		1	2	2	2
	24/09/2008		1	1	2	1	1	2	2	2	4		1	2	2	2
	23/10/2008		1	1	2	1	1	2	2	2	4		1	2	2	2
	26/11/2008		1	1	2	1	1	2	2	2	4		1	2	2	2
	18/12/2008		1	1	2	1	1	2	2	2	4		1	2	2	2
	22/01/2009		1	1	2	1	1	2	2	2	4		1	2	2	2
PG0092/09	15/02/2007															
	22/10/2007		1	1	1	1	1	3	1	1	3		1	2	1	2
	26/11/2007		1	1	2	1	1	2	2	2	4		1	2	2	2
	20/12/2007		1	1	1	1	1	2	1	2	4		1	2	1	2

Table 4 *[contd]*

	22/01/2008		1	1	1	1	1	2	2	1	4		1	2	2	2
	25/02/2008		1	1	1	1	1	2	1	2	4		1	2	1	2
	25/03/2008		1	1	2	1	1	2	2	2	4		1	2	2	2
	23/04/2008		1	1	2	1	1	2	2	2	4		1	2	2	2
	22/05/2008		1	1	2	1	1	2	2	2	4		1	2	2	2
	24/06/2008		1	1	1	1	1	2	2	2	4		1	2	2	2
	23/07/2008		1	1	2	1	1	2	2	2	4		1	2	2	2
	26/08/2008		1	1	2	1	1	2	2	2	4		1	2	2	2
	24/09/2008		1	1	1	1	1	2	1	2	4		2	2	1	2
	23/10/2008		1	1	2	1	1	2	2	2	4		1	2	2	2
	26/11/2008		1	1	2	1	1	2	2	2	4		1	2	2	2
	18/12/2008		1	1	2	1	1	2	2	2	4		1	2	2	2
	22/01/2009		1	1	2	1	1	2	2	2	4		1	2	2	2
PG0093/09	22/10/2007		1	1	1	1	1	3	1	1	3		1	2	1	2
	26/11/2007		1	1	2	1	1	2	2	2	4		1	2	2	2
	20/12/2007		1	1	1		1	2	1				1	2	1	
	22/01/2008		1	1	1	1	1	2	2	2	4		1	2	2	2
	25/02/2008		1	1	1	1	1	2	1	2	4		1	2	1	2
	25/03/2008		1	1	2	1	1	2	2	2	4		1	2	2	2
	23/04/2008		1	1	2	1	1	2	2	2	4		1	2	2	2

Table 4 *[contd]*

22/05/2008		1	1	2	1	1	2	2	2	4		1	2	2	2
24/06/2008		1	1	2	1	1	2	2	2	4		1	2	2	2
23/07/2008		1	1	2	1	1	2	2	2	4		1	2	2	2
26/08/2008		1	1	2	1	1	2	2	2	4		1	2	2	2
24/09/2008		1	1	2	1	1	2	2	2	4		1	2	2	2
23/10/2008		1	1	2	1	1	2	2	2	4		1	2	2	2
26/11/2008		1	1	2	1	1	2	2	2	4		1	2	2	2
18/12/2008		1	1	2	1	1	2	2	2	4		1	2	2	2
22/01/2009		1	1	2	1	1	2	2	2	4		1	2	2	2

Table 5 - Blood samples retained in the AHVLA Biological archive from the AHQ/AHQ animals culled at 24 months. All blood samples were fractionated and stored as aliquots of different blood fractions. The numbers refer to the number of aliquots of each sample type

PG Number	Sampling Date	Blood Clot	Buffy Coat (Citrate)	Buffy Coat (EDTA)	Buffy Coat (Fl Ox)	Buffy Coat (Hep)	Plasma (Citrate)	Plasma (EDTA)	Plasma (Fl Ox)	Plasma (Hep)	Serum	Umbilical Cord (foetal)	Whole Blood (Citrate)	Whole Blood (EDTA)	Whole Blood (Fl Ox)	Whole Blood (Hep)
PG0108/09	21/03/2007	2									2			4		
	22/10/2007		1	1	1	1	1	3	1	1	4		1	2	1	2
	26/11/2007		1	1	1	1	1	2	2	2	4		1	2	2	2
	20/12/2007		1	1	1	1	1	2	1	2			1	2	1	2
	22/01/2008		1	1	1	1	1	2	2	2	4		1	2	2	2
	25/02/2008		1	1	1	1	1	2	1	2	4		1	2	1	2
	25/03/2008		1	1	2	1	1	2	2	2	4		1	2	2	2
	23/04/2008		1	1	1	1	1	2	2	2	4		1	2	2	2
	22/05/2008		1	1	2	1	1	2	2	2	4		1	2	2	2
	24/06/2008		1	1	2	1	1	2	2	2	4		1	2	2	2
	23/07/2008		1	1	2	1	1	2	2	2	4		1	2	2	2
	26/08/2008		1	1	2	1	1	2	2	2	4		1	2	2	2
	24/09/2008		1	1	2	1	1	2	2	2	4		1	2	2	2

Table 5 *[contd]*

	23/10/2008		1	1	2	1	1	2	2	2	4		1	2	2	2
	26/11/2008		1	1	2	1	1	2	2	2	4		1	2	2	2
	18/12/2008		1	1	2	1	1	2	2	2	4		1	2	2	2
	22/01/2009		1	1	2	1	1	2	2	2	4		1	2	2	2
	23/02/2009		1	1	2	1	1	2	2	2	4		1	2	2	2
PG0109/09	23/03/2007	4									3					
	22/10/2007		1	1	1	1	1	3	1	1	4		1	2	1	2
	26/11/2007			1		1	1	2		2	4			2		2
	20/12/2007		1	1	1		1	2	1		4		1	2	1	
	22/01/2008		1	1	1	1	1	2	2	2	4		1	2	2	2
	25/02/2008		1	1	1	1	1	2	1	2	4		1	2	1	2
	25/03/2008		1	1	2	1	1	2	2	2	4		1	2	2	2
	23/04/2008			1		1		2		2	4			2		2
	22/05/2008		1	1	2	1	1	2	2	2	4		1	2	2	2
	24/06/2008		1	1	2	1	1	2	2	2	4		1	2	2	2
	23/07/2008		1	1	2	1	1	2	2	2	4		1	2	2	2
	26/08/2008		1	1	2	1	1	2	2	2	4		1	2	2	2
	24/09/2008			1	1	1		2	1	2	4			2	2	2
	23/10/2008		1	1	2	1	1	2	2	2	4		1	2	2	2
	26/11/2008		1	1	2	1	1	2	2	2	4		1	2	2	2

Table 5 *[contd]*

	18/12/2008		1	1	2	1	1	2	2	2	4		1	2	2	2
	23/02/2009		1	1	2	1	1	2	2	2	4		1	2	2	2
PG0110/09	23/03/2007											1				
	22/10/2007		1	1	1	1	1	3	1	1	4		1	2	1	2
	26/11/2007		1	1	1	1	1	2	2	2	4		1	2	2	2
	20/12/2007		1	1	1	1	1	2	1	2	4		1	2	1	2
	22/01/2008		1	1	1	1	1	2	2	2	4		1	2	2	2
	25/02/2008		1	1		1	1	2		2	4		1	2		2
	25/03/2008		1	1	2	1	1	2	2	2	4		1	2	2	2
	23/04/2008		1	1	2	1	1	2	2	2	4		1	2	2	2
	22/05/2008		1	1	1	1	1	2	2	2	4		1	2	2	2
	24/06/2008		1	1	2	1	1	2	2	2	4		1	2	2	2
	23/07/2008		1	1	2	1	1	2	2	2	4		1	2	2	2
	26/08/2008		1	1	2	1	1	2	2	2	4		1	2	2	2
	24/09/2008		1	1	2	1	1	2	2	2	4		1	2	2	2
	23/10/2008		1	1	2	1	1	2	2	2	4		1	2	2	2
	26/11/2008		1	1	2	1	1	2	2	2	4		1	2	2	2
	18/12/2008		1	1	1	1	1	2	2	2	4		1	2	2	2
	22/01/2009		1	1	2	1	1	2	2	2	4		1	2	2	2
	23/02/2009		1	1	2	1	1	2	2	2	4		1	2	2	2

Table 6 – Tissues collected and tests performed ARR/ARR 12 month kill. (ITT = insufficient tissue to test; SE1847 = material has been put into bioassay in Defra project SE1847)

ARR/ARR animals. 12 month kill

	PG 0150/08			PG 0151/08			PG 0152/08		
	IHC	**ELISA**	**Bio-assay**	**IHC**	**ELISA**	**Bio-assay**	**IHC**	**ELISA**	**Bio-assay**
obex	neg			neg			neg		
cerebellum	neg	neg	neg	neg	neg	**pos**	neg	neg	neg
rostral medulla	neg			neg			neg		
caudal midbrain	neg			neg			neg		
rostral midbrain	neg			neg			neg		
thalamus	neg	neg		neg	neg		neg	neg	
occipital cortex	neg			neg			neg		
parietal cortex	neg			neg			neg		
frontal cortex	neg	neg		neg	neg		neg	neg	
C5	neg			neg			neg		
T1	neg			neg			neg		
T6	neg			neg			neg		
T12	neg	neg		neg	neg		neg	ITT	
L5-6	neg			neg			neg		
palatine tonsil	neg	ITT		neg	ITT	SE1847	neg	ITT	
spleen	neg	neg	neg	neg	neg	neg	neg	neg	neg
MLN	neg	neg		neg	neg	SE1847	neg	neg	
distal ileum	neg	neg	NA	neg	neg	NA	neg	neg	NA
RAMALT	neg			neg			neg		
C-M plexus	neg			neg			neg		
extraocular muscles	neg	ITT		neg	ITT		neg	ITT	
MRPLN		neg			neg	SE1847		neg	
submandibular LN		ITT			ITT			ITT	
LRPLN									
Submand salivary gland		neg			neg			neg	
pharyngeal tonsil		ITT			ITT			ITT	
cervical thymus		neg			neg			neg	
prescap LN		neg			neg			neg	
jejunum									
kidney		neg			neg			neg	
liver		neg			neg			neg	
respiratory epithelium									
triceps									
cranial cervical gang									
nodose gang									
facial n									
trigeminal gang									
sciatic n									
buffy coat		neg			neg			neg	

Table 7– Tissues collected and tests performed AHQ/AHQ 12 month kill (ITT = insufficient tissue to test; SE1847 = material has been put into bioassay in Defra project SE1847)

AHQ/AHQ animals. 12 month kill

	PG 0153/08			PG 0154/08			PG 0155/08		
	IHC	ELISA	Bio-assay	IHC	ELISA	Bio-assay	IHC	ELISA	Bio-assay
obex	neg			neg			neg		
cerebellum	neg	neg	neg	neg	neg	neg	neg	neg	neg
rostral medulla	neg			neg			neg		
caudal midbrain	neg			neg			neg		
rostral midbrain	neg			neg			neg		
thalamus	neg	neg		neg	neg		neg	neg	
occipital cortex	neg			neg			neg		
parietal cortex	neg			neg			neg		
frontal cortex	neg	neg		neg	neg		neg	neg	
C5	neg			neg			neg		
T1	neg			neg			neg		
T6	neg			neg			neg		
T12	neg	neg		neg	neg		NS	NS	
L5-6	neg			neg			neg		
palatine tonsil	neg	ITT		neg	ITT		neg	ITT	SE1847
spleen	neg	neg	neg	neg	neg	neg	neg	neg	neg
MLN	neg	neg		neg	neg		neg	neg	SE1847
distal ileum	neg	neg	neg	neg	neg	**pos**	neg	neg	**pos**
RAMALT	neg			neg			neg		
C-M plexus	neg			neg			NS		
extraocular muscles	neg	ITT		neg	neg		neg	neg	
MRPLN		neg			neg			neg	SE1847
submandibular LN		ITT			ITT			ITT	
LRPLN									
Submand salivary gland		neg			neg			neg	
pharyngeal tonsil		ITT			ITT			ITT	
cervical thymus		neg			neg			neg	
prescap LN		neg			neg			neg	
jejunum									
kidney		neg			neg			neg	
liver		neg			neg			neg	
respiratory epithelium									
triceps									
cranial cervical gang									
nodose gang									
facial n									
trigeminal gang									
sciatic n									
buffy coat		neg			neg			neg	

Table 8 – Tissues collected and tests performed ARR/ARR 24 month kill (ITT = insufficient tissue to test)

ARR/ARR animals. 24 month kill

	PG 91/09			PG 92/09			PG 93/09		
	IHC	**ELISA**	**Bio-assay**	**IHC**	**ELISA**	**Bio-assay**	**IHC**	**ELISA**	**Bio-assay**
obex	neg			neg			neg		
cerebellum	neg	neg	neg	neg	neg	neg	neg	neg	neg
rostral medulla	neg			neg			neg		
caudal midbrain	neg			neg			neg		
rostral midbrain	neg			neg			neg		
thalamus	neg	neg		neg	neg		neg	neg	
occipital cortex	neg			neg			neg		
parietal cortex	neg			neg			neg		
frontal cortex	neg	neg		neg	neg		neg	neg	
C5	neg			neg			neg		
T1	neg			neg			neg		
T6	neg			neg			neg		
T12	neg	neg		neg	neg		NS	NS	
L5-6	neg			neg			neg		
palatine tonsil	neg	ITT		neg	ITT		neg	ITT	
spleen	neg	neg	neg	neg	neg	neg	neg	neg	neg
MLN	neg	neg		neg	neg		neg	neg	
distal ileum	neg	neg	neg	neg	neg	neg	neg	neg	neg
RAMALT	neg			neg			neg		
C-M plexus	neg			neg			NS		
extraocular muscles	neg	neg		neg	ITT		neg	neg	
MRPLN		neg			neg			neg	
submandibular LN		ITT			ITT			neg	
LRPLN									
Submand salivary gland		neg			neg			neg	
pharyngeal tonsil		neg			neg			ITT	
cervical thymus		neg			neg			neg	
prescap LN		neg			neg			neg	
jejunum									
kidney		neg			neg			neg	
liver		neg			neg			neg	
respiratory epithelium									
triceps									
cranial cervical gang									
nodose gang									
facial n									
trigeminal gang									
sciatic n									
buffy coat		neg			neg			neg	

Table 9 – Tissues collected and tests performed ARR/ARR 24 month kill (ITT = insufficient tissue to test; SE1847 = material has been put into bioassay in Defra project SE1847)

AHQ/AHQ animals. 24 month kill

	PG 108/09			PG 109/09			PG 110/09		
	IHC	**ELISA**	**Bio-assay**	**IHC**	**ELISA**	**Bio-assay**	**IHC**	**ELISA**	**Bio-assay**
obex	neg			neg			neg		
cerebellum	neg	neg	**pos**	**pos**	**pos**	**pos**	neg	neg	neg
rostral medulla	neg			neg			neg		
caudal midbrain	neg			neg			neg		
rostral midbrain	neg			neg			neg		
thalamus	**pos**	High neg		**pos**	**pos**		neg	neg	
occipital cortex	neg		**pos**	neg			neg		
parietal cortex	neg			neg			neg		
frontal cortex	**pos**	**pos**	**pos**	**pos**	**pos**		neg	neg	
C5	neg			neg			neg		
T1	neg			neg			neg		
T6	neg			neg			neg		
T12	neg	neg		neg	neg		neg	neg	
L5-6	neg			neg			neg		
palatine tonsil	neg	ITT		neg	ITT		neg	ITT	
spleen	neg	neg	neg	neg	neg	neg	neg	neg	neg
MLN	neg	neg		neg	neg		neg	neg	
distal ileum	neg	neg	neg	neg	neg	**pos**	neg	neg	neg
RAMALT	neg			neg			neg		
C-M plexus	neg			neg			neg		
extraocular muscles	neg	neg	SE1847	neg	ITT		neg	neg	
MRPLN		neg	SE1847		neg			neg	
submandibular LN		ITT			ITT			neg	
LRPLN						SE1847			
Submand salivary gland		neg			neg	SE1847		neg	
pharyngeal tonsil		neg	SE1847		neg			ITT	
cervical thymus		neg			neg			neg	
prescap LN		neg			neg	SE1847		neg	
jejunum									
kidney		neg			neg			neg	
liver		neg			neg			neg	
respiratory epithelium									
triceps						SE1847			
cranial cervical gang									
nodose gang									
facial n									
trigeminal gang									
sciatic n									
buffy coat		neg			neg			neg	

Table 10 – post mortem tissue aliquots retained in the AHVLA Biological Archive

PG Number	150/08	151/08	152/08	153/08	154/08	155/08	91/09	92/09	93/09	108/09	109/09	110/09
Basal Nuclei	1	1	1	1	1	1	1	1	1	1	1	1
Buffy Coat (EDTA)						2	2	6	2	4	4	4
Buffy Coat (Hep)	2	1	2		2	2		2	3	2	4	4
Caudal Medulla	2	2	2	2	2	2	2	2	2	2	2	2
Cervical Thymus	3	3	3	3	3	3	3	3	3	3	3	3
Coeliac Mesenteric Plexus	1	2	2	1	2	1		1	1	1	2	2
CSF									1			
CSF Cells									1			
CSF Supernatant									3			
Distal ileum (excluding peyer's patches)	5	5	4	6	5	6	3	4	1	5	4	2
Distal Ileum (Peyer's Patches)	4		2	3	5	5	6	6	3	6	4	5
DRG C3,4,5,6	1	1	1	1	1	1	1	1	1	1	1	1
DRG T5,6,7,8	1	1	1	1	1	1	1	1	1	1	1	1
Extraocular muscles	1	1	1	1			1	1		1		1
Facial Nerve	1	3	3	3	3	3	2	3	3	2	2	2
Frontal Cortex	1	1	1	1	1	1	1	1	1	1	1	1
Jejunum (excl Peyer's Patches)	6	6	6	6	6	6	5	6	6	6	5	6
Jejunum (Peyer's Patches)	6	6	6	6	6	6	2	4	3	5	4	6
Kidney	6	6	6	6	6	6	6	6	6	6	6	6
Lateral Retropharyngeal LN	1	2	2	2	2	2	2	2			1	1
Liver	6	6	6	6	6	6	6	6	6	6	6	6
Medial Retropharyngeal LN	3	2	2	3	3	2	1	1	1	2		1
Mesenteric LN	5	7	8	8	8	8	8	8	7	7	5	7
Midbrain & Brain stem	1	1	1	1	1	1	1	1	1	1	1	1
Obex						1						
Occipital cortex	1	1	1	1	1	1	1	1	1	1	1	1
Palatine Tonsil	1	2	2	2	2	2	1	1	1	1	1	1
arietal cortex	1	1	1	1	1	1	1	1	1	1	1	1
Pharyngeal Tonsil	2	2	2	1	2	2	2	1	1	1	1	1
Plasma (EDTA)	5	3	5	5	5	5	5	5	5	5	5	5
Plasma (Hep)	10	5	10	10	10	10	10	10	10	10	10	10
Prescapular LN	1	1	1	1	1	1	3	4	3	4	3	3
Rectal Tonsil	2	2	2	2	2	2	2	2	2	2	2	2
Red Blood cells (EDTA)	4	2	4	4	4	2	4	4	4	4	4	4
Red Blood Cells (HEP)	8	4	8	6	8	7	8	8	8	8	8	8
Respiratory Epithelium	4	4	4	4	4	3	4	4	3	3	1	4
Sciatic Nerve	1	3	3	3	3	3	3	3	3	2	1	3
Serum	5	5	3	3	5	5	5	4	5	3	5	5
Spinal Cord C1-2	1	1	1	1	1	1	1	1	1	1	1	1
Spinal Cord C2-3	1	1	1	1	1	1	1	1	1	1	1	1
Spinal Cord L2-3	1	1	1	1	1	1	1	1	1	1	1	1
Spinal Cord T10-11		1	1				1					1
Spinal Cord T9-10	1	1	1	1	1	1	1	1	1	1	1	1
Spleen	7	7	7	7	7	7	8	8	8	8	7	8
Submandibular LN	2	2	2	1	2	2	1	1		1	1	
Submandibular salivary gland	4	4	4	4	4	4	4	3	4	4	4	3

Table 10 *[contd]*

Thalamus	1	1		1	1	1	1	1	1	1	1	1
Tongue	1	1	1	1	1	1	1	1	1	1	1	1
Triceps	6	6	6	6	6	6	6	6	6	6	6	6
Trigeminal Ganglia	1	1	1	1	1	1	1	1	1			1

Table 11 – Mouse bioassay results. Each box represents a single mouse, each number its incubation period or survival time

MO3057 12 month cull bioassay final report 23/01/2012												
Inoculum code	Sample ID	Tissue	Mouse strain	Histology result/days PI											Mean incubation period or survival time in dpi were applicable
MO3057/0003	PG0150/08	Cerebellum	Tg338	0	487	629	640	640	777	785	861	868	875		729
MO3057/0004	PG0150/08	Spleen	Tg338	336	466	494	609	641	672	735	742	815	830		634
MO3057/0005	PG0151/08	Cerebellum	Tg338	0	322	383	721	721	760	802	802	802	823		
MO3057/0006	PG0151/08	Spleen	Tg338	360	371	550	595	675	686	795	795	865	872		656
MO3057/0007	PG0152/08	Cerebellum	Tg338	426	481	595	609	700	728	749	749	795	851		668
MO3057/0008	PG0152/08	Spleen	Tg338	108	414	428	479	583	658	653	770	805	889		579
MO3057/0009	PG0153/08	Cerebellum	Tg338	683	722	722	722	782	802	808	861	861	861		793
MO3057/0010	PG0153/08	Spleen	Tg338	492	503	552	681	755	755	769	790	811	860		720
MO3057/0011	PG0154/08	Cerebellum	Tg338	486	653	705	714	721	805	805	839	839	877		773
MO3057/0012	PG0154/08	Spleen	Tg338	385	508	622	622	655	673	710	714	731	853		647
MO3057/0013	PG0155/08	Cerebellum	Tg338	433	524	524	538	538	622	649	781	781	845		624
MO3057/0014	PG0155/08	Spleen	Tg338	405	427	566	566	649	671	794	796	797	797		647
MO3057/0018	PG0153/08	Distal ileum	Tg338	529	644	644	672	690	774	796	802	868	868		729
MO3057/0019	PG0154/08	Distal ileum	Tg338	237	285	300	305	316	344	549	816	868	868		310
MO3057/0020	PG0155/08	Distal ileum	Tg338	220	229	232	243	243	243	244	278	291	358		258
MO3057 24 month cull bioassay final report 23/01/2012												
Inoculum code	Sample ID	Tissue	Mouse strain	Histology result/days PI											Mean incubation period or survival time in dpi were applicable
MO3057/0024	PG0091/09	Cerebellum	Tg338	415	453	610	617	617	671	705	743	775	789		640
MO3057/0025	PG0091/09	Spleen	Tg338	657	657	702	724	752	760	780	801	832	832		750
MO3057/0026	PG0091/09	Distal ileum	Tg338	405	429	608	664	678	788	788	788	788	788		672
MO3057/0027	PG0092/09	Cerebellum	Tg338	507	566	580	691	745	832	832	832	832	832		725
MO3057/0028	PG0092/09	Spleen	Tg338	252	420	500	545	645	705	724	815	832	832		627
MO3057/0029	PG0092/09	Distal ileum	Tg338	507	507	619	622	671	678	780	788	788	832		679
MO3057/0030	PG0093/09	Cerebellum	Tg338	98	463	552	569	616	642	678	760	780	832		599
MO3057/0031	PG0093/09	Spleen	Tg338	419	610	680	680	689	752	767	767	773	794		693
MO3057/0032	PG0093/09	Distal ileum	Tg338	257	312	557	637	774	805	805	805	805	805		656
MO3057/0033	PG0108/09	Cerebellum	Tg338	249	263	284	296	312	318	322	333	384	573		307
MO3057/0034	PG0108/09	Spleen	Tg338	466	629	724	759	773	794	811	811	811	811		739
MO3057/0035	PG0108/09	Distal ileum	Tg338	270	538	542	720	733	755	807	807	807	807		679
MO3057/0036	PG0109/09	Cerebellum	Tg338	93	154	163	164	170	178	178	184	185	199		175
MO3057/0037	PG0109/09	Spleen	Tg338	464	598	701	709	776	807	807	807	807	807		728
MO3057/0038	PG0109/09	Distal ileum	Tg338	225	257	257	294	299	299	357	360	406	805		306
MO3057/0039	PG0110/09	Cerebellum	Tg338	293	458	680	706	714	714	714	783	797	800		666
MO3057/0040	PG0110/09	Spleen	Tg338	86	242	283	392	567	669	678	776	800	800		529
MO3057/0041	PG0110/09	Distal ileum	Tg338	567	567	678	687	687	774	800	800	800	800		716
MO3057/0042	PG0108/09	Basel ganglia	Tg338	179	183	189	197	197	197	197	197	197	197		193
MO3057/0043	PG0108/09	Hippocampus	Tg338	183	183	183	197	197	203	203	208	211	211		198

222	Positive result - terminal disease
222	Positive result - non terminal disease
222	Inconclusive result
222	Negative result
0	No tissue available