Unexpected atypical scrapie case: audit of sample handling and biosecurity

Statement from the Chief Scientific Adviser

In November 2006 the Veterinary Laboratories Agency (VLA) informed Defra they had detected atypical scrapie in a research flock considered to be free of Transmissible Spongiform Encephalopathies (TSEs). Defra issued an information bulletin¹ on the 14th November.

The origin of the atypical scrapie case was not clear. I initiated an independent audit of the research facilities concerned with the finding to investigate two aspects:

- Sample handling and identification procedures
- Biosecurity procedures at the site where the flock is held.

The audit was conducted by the UK Accreditation Service (UKAS)², which has now provided its report. I am confident that the audit has thoroughly investigated the issues concerned, insofar as it was able in the time available, and pleased that the findings will now be considered by the Spongiform Encephalopathy Advisory Committee (SEAC), together with additional evidence, in their scientific assessment of this case and its implications.

SIR HOWARD DALTON

9th May 2007

² http://www.ukas.com/
## ASSESSMENT REPORT

| Name & Address of Organisation | Defra  
| Area 1A  
| Nobel House  
| London  
| SW1P 3JR | Date(s) of Assessment | 27th November to 1st December 2006 |
| Contact(s) within Organisation | Dr W Reynolds  
| Dr S Hill | Assessment Criteria | As per Specification (Appendix I) |
| Organisations Assessed including Location(s) | VLA Weybridge  
| New Haw  
| Addlestone  
| Surrey  
| KT15 3NB | VLA Shrewsbury  
| Kendal Road  
| Harlescott  
| Shrewsbury  
| SY1 4HD | ADAS/VLA Sheep Unit | Presumed case of atypical scrapie in New Zealand derived sheep flock Audit of procedures and biosecurity in relation to the ADAS/VLA Sheep Unit and of sample handling and analysis at VLA. |
| Representative(s) from Organisations Assessed | VLA  
| Dr D Matthews  
| Dr H Simmons | ADAS  
| Mr M Heath  
| Dr G Povey | Name & Role of UKAS Assessment Team | Lead Assessor  
| Ms C Stewart | Veterinary Consultant  
| Mr B Hosie | Report Prepared by | Ms C Stewart | Report Authorised by | Dr J Beaumont |
| Report Issued Date | 10th January 2007 (amended report issued on 16th April 2007) | Report Issued by | Mr R Bettinson |
| Report Acknowledged Date | Email receipt requested | Report Acknowledged by | Email receipt requested |
1. Background

A case of atypical scrapie was confirmed in November 2006 in a home-bred Cheviot ARQ/ARQ ewe, PG 0832/06 (sheep ID G320), in the ADAS/VLA Sheep Unit, project reference SE 1931. The animal was born in 2000 as the result of embryo transfer using NZ-imported animals as both donor and recipient. It was noted as being apparently blind in June 2006 around the time of lambing and was maintained to raise its lamb. Prior to being euthanased at the Sheep Unit on 5th September 2006, the ewe was also found to be ataxic, therefore a differential post mortem was carried out at VLA Weybridge.

Initial screening for Transmissible Spongiform Encephalopathy (TSE) (histopathology and immunohistochemistry of the brainstem at the level of the obex) identified changes consistent with atypical scrapie. Further analysis of brainstem material by Bio-Rad ELISA and cerebellum by Western Blot confirmed the pathology results.

A second possible case was suspected in an ARR/ARR sheep, PG1097/04 (sheep ID J19) born to NZ parents. Brain tissue from this sheep tested negative for TSEs by immunohistochemistry (IHC) and Bio-RAD ELISA. Brain homogenate from this animal was inoculated as a negative control for an experiment designed to investigate the transmissibility of atypical scrapie in mice. Three out of seventeen mice in the negative control group have succumbed to a prion disease with characteristics of atypical scrapie on histological examination. The subsequent Western blotting profiles produced by the three mice affected by inoculum from the animal, demonstrate patterns that are similar, if not identical, to the atypical scrapie profiles shown for mice affected by inocula derived from sheep identified through scrapie surveillance in Great Britain.

UKAS was commissioned to assess the biosecurity and associated procedures relating to the Sheep Unit and of the sample handling, chain of custody and analysis at VLA Weybridge and Shrewsbury.

The full specification provided by Defra is appended to this report (Appendix I).

2. Executive Summary

It is the view of the assessment team that, having thoroughly challenged the sample labelling and record keeping at VLA Weybridge, it is highly unlikely that the samples examined and tested were from any sheep other than the ARQ/ARQ ewe (sheep ID G320) from the ADAS/VLA Sheep Unit (project reference SE 1931). Although some minor issues were raised regarding sample labelling and records, these are not considered to be significant with regard to the sample integrity.

It is understood that the VLA intends to perform further analysis in support of the above conclusion and will examine archived sample material from G320.

In addition, VLA is intending to perform further analysis of archived sample material from ARSU sheep, to ascertain the presence of any other cases of atypical scrapie. However a decision was taken in February 2006 not to examine the brains of culled resistant genotype sheep for scrapie, therefore it may be difficult to determine the extent of atypical scrapie in the flock if the brains have not been retained.

Scrapie and other transmissible spongiform encephalopathies (TSEs) are noted for their long and asymptomatic incubation period. Scrapie can be transmitted by contact from infected, apparently healthy sheep to uninfected sheep. Contact may be direct or indirect. Placenta and sheep faeces are considered important in transmission. Endoparasites, ectoparasites and hay mites have also been proposed as reservoirs of infection. Contaminated feedstuffs, vehicles, vermin and other animals including man may be important in the introduction of infection to susceptible sheep. The agents that cause these diseases are exceptionally robust and resist most routine methods of sterilisation procedures. For these reasons stringent biosecurity procedures are important.
With respect to the biosecurity procedures employed in relation to the Sheep Unit, no specific incident was identified as being the likely cause of the introduction of atypical scrapie to the project flock. However evidence of potential breaches in biosecurity was found and issues have been identified, which individually or collectively, may have contributed to the current situation. These are outlined in Section 4.3 of the report.

Contamination during the inoculation of brain homogenate into mice at VLA Weybridge cannot be excluded at this stage in the suspected second case of atypical scrapie in the ARR/ARR sheep (sheep ID J19).

If the flock is to be maintained, given the confirmation of atypical scrapie in the flock, consideration should be made with regard to future waste management plans in order to comply with legislation relating to flocks affected by scrapie (currently manure is spread on a commercial, arable farm).

Record keeping at VLA and ADAS was of a high standard, with files and records readily retrievable during the assessment.

UKAS would like to thank all personnel at VLA and ADAS for their full co-operation and professional approach throughout the assessment process.

Summaries of the findings from the assessment are detailed in the body of the report. Further specific detail is available in the records of the assessment.

3. Scope
The specification provided by Defra (Appendix 1) outlines the overall scope of the assessment.

The assessment comprised:

- **On-site examination at VLA Weybridge on 27th and 28th November and 1st December 2006 of:**
  - the paperwork relating to all aspects of the post mortem examination (sheep ID G320) and subsequent sample handling, storage, examination and analysis
  - relevant procedures/protocols
  - the post mortem preparation area
  - equipment sterilisation and storage areas
  - post mortem building 90
  - sample storage and laboratory areas within pathology and molecular biology
  - the containment level 3 laboratory in building 96
  - communal sample storage facilities
  - the mouse house

- **On-site examination at VLA Shrewsbury on 30th November 2006 of:**
  - the laboratory and sample storage facilities
  - test results for sheep IDs G320 and J19
  - QC results and the last round of Proficiency Test results

- **Office review ahead of the assessment of the Bio-Rad ELISA method used at VLA Shrewsbury**

- **On-site examination at ADAS ARSU on 29th and 30th November 2006 of:**
  - the procedures relating to the ADAS ARSU
  - records maintained within the ARSU
  - project meeting minutes from 1997 to 2006
  - a number of project files

Refer to Appendix II for a list of documents reviewed in preparing this report.
4. Observations & Assessment Findings

4.1 Veterinary Laboratories Agency (VLA)

4.1.1 Post Mortem Records & Procedures (G320)

On 5th September 2006 the ewe G320, subsequently confirmed as having atypical scrapie, was euthanased at the Sheep Unit and transported to VLA Weybridge for post mortem.

The ewe was allocated a unique reference number on receipt at VLA and the post mortem check sheet that was completed, indicated that the post mortem was scheduled to take place in building 270.

The post mortem logbook indicated that the post mortem had taken place in building 270 but the electronic VLA Pathology Planning Database indicated that the post mortem examination had taken place in building 90.

The logbook for building 90 showed that, with the exception of one person, the personnel involved in the post mortem of G320 signed-in on 5th September 2006.

In discussion with VLA personnel involved in the post mortem of G320 on 5th September and based on the entries in the logbook for building 90, it was concluded that the post mortem was carried out in building 90.

It is considered that this irregularity in the records relating to the post mortem does not give rise to concern regarding the integrity of the samples from sheep G320.

4.1.2 Sample Handling (VLA Weybridge)

The process for determining the samples to be taken at post mortem was explained during the assessment and the labelling of sample containers was demonstrated in relation to a future, scheduled post mortem. An internal VLA investigation/audit had previously noted that the unique ID was not on the sample pot containing the fixed half-brain of sheep G320 but the project number was written on the top of the pot, the metal ear tag was taped to the top of the pot, and the large, plastic ear tag was in the bottom of the pot. During the assessment the remainder of the fixed half-brain from G320 was available and examined by the Lead Assessor in the containment level 3 facility, in the original sample pot.

It is considered that the labelling of the sample was sufficient to provide traceability to the ewe ID G320.

Samples are placed into fixative or frozen, or both following post mortem and it is understood that both the fixed and frozen samples taken from G320 were transferred to the containment level 3 laboratories following the post mortem of G320.

The fresh tissues from G320 had been logged into the electronic sample archive/database and recorded as stored in freezers in the project store and a number of entries were checked and followed through the system. On checking the relevant project store freezers, the samples from G320 could not be found. The samples were subsequently traced by VLA and the database updated accordingly (memo from Head of TSE Archive to TSE Programme Manager dated 29th November 2006 and submitted to UKAS).

It is considered that this irregularity in the records of storage does not give rise to concerns about the integrity of the G320 samples.

The fixed brain from G320 was blocked on 12th October 2006 but there was no specific entry in any of the cabinet logbooks to indicate that this was the case.
It was confirmed by VLA during the assessment that the unique ID number (PG number) will be recorded in the equipment logbooks in future to provide full traceability.

The blocked samples from G320 were submitted to histology (submission reference 5547), where they were logged and cassetted on 23rd October and subsequently embedded and sectioned.

The paperwork associated with all samples received by Histology on 23rd October 2006 was reviewed to ascertain whether a possible substitution of samples could have occurred.

It is considered highly improbable that any sample substitution occurred.

Further testing of brain material from G320 was performed by VLA Weybridge and Shrewsbury and gave positive results for atypical scrapie by Western Blot and by Bio-Rad ELISA. The result of the open frame gene sequencing confirmed that the sample from G320 contained a phenylalanine at amino acid position 141, which is characteristic of atypical scrapie.

Unfixed tissue and stained sections examined and tested at VLA, were also sent to the National Veterinary Institute, Norway for examination and testing. Confirmation was received that the immunostaining was characteristic of Nor98 atypical scrapie and that the Western Blot results for the unfixed tissue were positive, characteristic of scrapie Nor98.

It is considered that there was sufficient traceability of the brain material to the ewe G320. The results from the range of tests performed provide alternative and independent confirmation of atypical scrapie. It is highly unlikely that the samples examined and tested were from any sheep other than the ARQ/ARQ ewe (sheep ID G320).

4.1.3 Sample Handling (VLA Shrewsbury)
Caudal medulla from G320 and cerebellum from J19 was despatched from VLA Weybridge, received and tested at VLA Shrewsbury. The paperwork associated with the Bio-Rad ELISA results for the samples from G320 and J19, including the QC results for the plates, were examined during the assessment. From the records available, it would appear that the testing, interpretation and reporting were performed in accordance with the documented procedure.

The result for G320 was confirmed as positive and the result for J19 was confirmed as negative.

Disease incidence in the flock is monitored against the bank of sera maintained at Shrewsbury for the original imports. The sample store was accessed on 30th November 2006 and it was confirmed that the samples are labelled and stored appropriately and are retrievable as required.

4.1.4 Project Reference SE 1850, sheep ID J19 (VLA Weybridge)
Sheep J19, apparently showing no signs of scrapie, was culled specifically to provide negative control material (brain homogenate inoculum) for projects designed to investigate the transmissibility of atypical scrapie in mice. The negative control reference for the inoculum is SE 1850/0024.

Six mice from the negative control group, inoculated with SE 1850/0024, had either been found dead or had been euthanased due to the clinical score assigned. Stained H&E and IHC slides and Western Blot results for three of the mice, have been confirmed characteristic of atypical scrapie. The H&E and IHC results for one mouse are understood to be negative and the results for the remaining mice are pending.

The brain tissue J19 was originally tested by Bio-Rad ELISA and IHC and the results were negative.

As a result of the examinations of the stained slides for the three positive mice, a sample of cerebellum from J19 was retrieved from the sample archive and sent to VLA Shrewsbury for Bio-Rad ELISA and tested negative (refer also to the Bio-Rad ELISA results in 4.1.3).
The procedure for the inoculation of mice was discussed during the assessment and it was confirmed that the mice were inoculated on day 210 in 2005. On the same day, another three inocula were injected into groups within the same study, inocula references SE 1850/0002, 0008 and 0009, all of which were positive.

The specific nature of inocula is not known by personnel in the mouse house at Weybridge and inocula were, therefore, injected in random order. As this is an ongoing blind study, this is intended to avoid the potential for bias. The negative control was the fourth inoculation performed on the day in question, preceded by three other positive atypical scrapie-infected inocula. This increases the potential for cross-contamination.

Examination of the records of the sequence of inoculations and the results available at the time of the assessment does not preclude the possibility of cross-contamination.

SE 1850 is a collaborative project, involving partner institutes in France and Germany. The French partner has confirmed that four mouse brains, inoculated with an aliquot of SE 1850/0024, have been checked and confirmed negative by Western Blot (this includes three mice sacrificed healthy at 400 days).

The German partner has confirmed that the brains of two mice euthanased at 366 and 426 days post inoculation with an aliquot of SE 1850/0024 have yet to be examined and tested.

At this stage, contamination during inoculation of mice at VLA Weybridge cannot be excluded as a possible cause of atypical scrapie in the mice that have been examined to date, but results that are still pending may provide further insight into this.

4.1.5 VLA Internal Investigation/Audit
Internal audits carried out by the VLA were available for review during the assessment.

The audits were comprehensive, as verified in the detailed audit reports prepared by the auditors and were effective in highlighting the deficiencies identified and opportunities for improvement.

In relation to G320, an extensive review of records, data, correspondence and laboratory facilities was conducted and the outcome of this assessment supports the findings from the VLA internal audit/investigation.

With regard to J19, the assessment of records, data and correspondence was more limited, however the outcome of this assessment supports the findings from the VLA internal audit/investigation.

4.2 ADAS/VLA Sheep Unit Site Assessment
Security at the ADAS/VLA Sheep Unit was found to be good on the dates of the assessment (29th and 30th November 2006), with access to the Unit well controlled.

The Unit is protected by a double, chain link fence around the perimeter of the unit. The outer-fence is around eight feet in height, with barbed wire at the top, increasing the overall height to around 10 feet. Additional security measures in the form of CCTV cameras, with hard drive recording, are installed to survey the Unit and the perimeter.

A number of procedures are in place, specifically in relation to the Unit (Appendix III), and the current and archive versions of these were referenced as required during the assessment.

Dedicated, appropriately maintained equipment is available for use within the Unit (such as that used for embryo transfer) and the facilities in general are maintained to a high standard. Records of disinfection and chloros use were available for the duration of the project and the level of record keeping in general was of a high standard.
In the review of the project files, it became apparent that the security fence was not always at its current height, which was a concern.

In addition to the site assessment, it was decided to review the minutes of the project meetings, which commenced in October 1997 to establish whether any potential breaches of biosecurity had been recorded. In addition, the Sheep Unit Health Reports were reviewed by Mr B Hosie, Veterinary Consultant to UKAS, to ascertain whether the history of disease incidence in the flock may indicate a breakdown in biosecurity.

With respect to the biosecurity procedures employed in relation to the Unit no specific incident was identified as being the likely cause of the introduction of atypical scrapie to the project flock. However there was evidence of potential breaches in biosecurity, which individually or collectively, may have contributed to the current outcome. These are included in Section 4.3 of the report.

The findings in this report are supported by the report prepared by Mr B Hosie, which is included in Appendix IV.

4.2.1 Site Suitability
It is understood that the site of the ADAS/VLA Sheep Unit had no known history of livestock since around 1960. Given the quality of the soil (high nitrogen and high organic matter content), the area was extensively arable hence the rationale for selecting the site for the New Zealand derived Defra flock.

The history of land use in each of the fields where the flock is maintained was requested and provided during the assessment. The cropping plans for Nat’s Meadow, Big Rough Lots and Little Rough Lots, dating back to 1960 were provided for review, confirming that no livestock had been maintained on the fields from that date.

The application of animal manure to the land was discussed and it is understood that no animal manure was applied to the fields from 1960.

With respect to the land use in Little Rough Lots, ADAS was involved in a trial with Anglian Water to compare sewage sludge and inorganic fertilizer as nutrient sources for Miscanthus sacchariflorus. Sewage sludge was applied each year from 1994 to 1997 at rates of application ranging from 25 to 200 cubic metres per hectare, with the last application in July 1997.

The flock is not maintained on Little Rough Lots, however the proximity of the field to the original sheep unit and the extended sheep unit, including any potential risks posed by run off, do not appear to have been considered in the overall risk assessment of the suitability of the site. The VLA staff member interviewed during the assessment was not aware that the sewage sludge had been applied to any of the fields on the ADAS site.

The water table is high and the implications of run-off are not known.

4.2.2 Importation
The original import, which took place in 1998 and a further import in 2001 appear to have been well planned and executed. Reports prepared for the respective imports were provided for comment and review.

No breaches of biosecurity were evident from the records available.
4.3 Review of Project Minutes, Project Files & Procedures

4.3.1 Incidents of Potential Concern

4.3.1.1 Security Fence

It was noted in the review of the project files that at the outset of the project there was a four-foot double security fence around the entire sheep unit and paddocks. The requirement for improving security, by means of increasing the height of the security fence and installing gates to prevent access to the track alongside the Unit (Southern perimeter) was raised in the project meeting of 22nd December 1997.

An eight-foot, chain link fence was constructed along the Southern perimeter of the sheep unit and paddocks, after the arrival of the first import of sheep; gates preventing access to the track were not installed at this stage. The chain link security fence to all other aspects of the sheep unit remained at a height of four-foot until the extension to the Unit was completed, understood to have taken place in December 2000.

CCTV cameras were positioned to survey the Unit and there was no documented evidence of intrusion by humans, deer or dogs in the project files. There were incursions by foxes (refer also to Appendix IV, Report from Mr B Hosie, Veterinary Consultant). The CCTV footage was not reviewed as part of the investigation.

A security fence at a height of four-foot would not be sufficient to prevent intruders (animal or human) from entering the Unit. This presented a potential risk to the security of the Unit from the outset.

4.3.1.2 Disease Security: Visitors & Contractors

Visitors to the Unit are categorized as low, medium and high risk. It is noted that only two categories are actually applied, high or medium/low risk. Entry to the Unit relies on a process of self-declaration, with visitors declaring whether or not they have been in contact with ruminants for the preceding 48 hours. There was no requirement to declare other contacts that could pose a potential risk (such as with laboratories handling TSE material). In practice the project team expected ‘showering in’ by high-risk visitors. A more robust mechanism of managing the potential risk to the project would be to implement a blanket policy of showering in for all visitors.

Access control was followed up in the review of completed, archived declarations made by shearers.

The records relating to a number of entries to the Unit were reviewed during the assessment. Records relating to the shearing that took place on 11th May 2001 were found to be lacking and there was no evidence that the team showered in to the Unit on this date. The self-declaration forms had the ‘medium/low risk’ category completed by the team of shearers.

There was no evidence in the project files that the shearers had not been in contact with ruminants for the preceding 48 hours, given that the self-declaration applies to all visitors.

It is generally accepted that shearers are a high-risk category.

Embryo transfer was being conducted within the Unit w/c 27th November 2006. It was noted during the assessment that a member of embryo transfer team, who was not wearing gloves, was assisting a sheep and his hands were observed to be in the mouth/airway of the animal. Given the close contact that embryo transfer personnel have with a range of animal species, especially sheep, this was considered to be of concern.

It has subsequently been confirmed that the individual had signed in to the unit as medium/low risk on Monday 27th November 2006 and had been at the Sheep Unit since that date. The incident is considered to be of very low risk under the circumstances.
Concerns had been raised at the project meeting in December 1998 regarding personnel involved in embryo transfer taking mobile phones and watches into the Unit and more specifically into the surgical area. It was confirmed in March 1999 that this issue had been addressed with the personnel involved.

4.3.1.3 Grass Nuts
Hauliers complete certificates, which are maintained on file within the Unit, declaring previous loads and the method of cleansing vehicles, prior to loading barley/grass nuts destined for the Unit. A number of completed forms were reviewed during the assessment. General conformity with Unit cleansing protocols was claimed in the majority, however there was no evidence that the vehicle delivering grass pellets (19th April 1999) had been sanitised between loads, with brushing entered as the only mechanism of cleaning.

The supplier of grass nuts to the project was approved for use on the project by ADAS in July 1997 and a signed agreement is in place with the supplier.

Six monthly audits of project related activities are performed by ADAS. An audit of suppliers of barley grain, feed straw and grass nuts in 1999 revealed that sheep are grazed on fields, which are used to supply grass nuts to clients, other than ADAS/VLA. It was confirmed with the supplier that the fields used to supply the Unit were not grazed by sheep. That said, the same tractors are used to maintain the fields in which sheep are grazed and those where sheep are not grazed. The tractors are not cleaned with chloros before entering the fields used to supply the project. In addition, the lorries used to transport the grass were not cleaned with chloros between accessing fields where sheep are grazed and fields in which sheep are not grazed. Corrective actions were agreed between the supplier and ADAS for implementation in time for the late summer 2000 supply to the Unit.

An audit to ascertain conformity with the agreed, revised procedures does not appear to have been undertaken for the late Summer 2000 supply to the Unit.

An audit of the supplier was to be undertaken at the time of the next supply of grass nuts to the project but this had not been possible due the FMD outbreak.

It was agreed that the supplier would be audited as a priority in 2003. An audit was performed in June 2003, some three years after the integrity of the feed was questioned, and the type and concentration of chloros used for disinfection purposes was queried. The follow-up action was to ensure that the supplier was using the chloros specified by the Unit. It has subsequently been confirmed that the type and concentration of chloros used was verified as acceptable, following the audit.

It was subsequently agreed that suppliers of straw, barley grain, grass nuts and soyabean meal would be assessed bi-annually.

The supplier of grass nuts to the project had been audited in May 2005 and full conformity with the ARSU supply procedures was confirmed.

No immediate action was taken when the issue was raised in 1999 and, on the basis that the supplier was in breach of contract, no effort was made to change suppliers or discard potentially contaminated feed.

It is considered that the situation with the grass nuts supplier should have been regarded as a breach of biosecurity and a potential risk to the project.
4.3.1.4 Disease Security: Animals
A number of potential breaches of biosecurity were identified by ADAS including problems with crows, foxes and vermin. Crows were reported as problematic in September 1998 and remained an outstanding issue in December 1998. However by March 1999, bird traps had been set up to address the problem with the crows. It was also reported in March 1999 that a fox had been found and shot in the sheep paddocks and that although the mice population had decreased, there was a subsequent increase in the rat population.

Rat droppings were seen in the feed during the site visit to the Unit on 30th November 2006.

Three disease incidents point to potential breaches of biosecurity in relation to the Unit and these are highlighted in the report prepared by Mr Brian Hosie (Appendix IV).

4.3.2 Incidents considered as potential breaches of biosecurity but rejected having received further confirmation

4.3.2.1 Veterinary Procedure
In May 2000, it was agreed that the Unit would source skin care products from New Zealand, given the perceived (high) risk that the use of products containing animal constituents posed to the project especially during lambing as gloves are not worn.

It is of concern that this policy was only implemented two years after the start of the project rather than at the outset.

It has subsequently been confirmed that the hand creams sourced from New Zealand for shepherding staff were found to be identical to products sold in UK, which is fortunate.

4.3.2.2 Boluses
It was noted in November 2001 that half the flock had been bloused and that the boluses are packed on a commercial farm in Somerset. At the time of writing, the project has no written confirmation on the integrity of the boluses. A VLA project member has audited the farm and no risk to disease security at the ADAS/VLA Sheep Unit was perceived.

4.3.2.3 Molasses
At the time of writing it would appear that ADAS has no verbal or written confirmation with regard to the country of origin, the processing and transportation procedures for the supply of molasses, however the source of molasses and the transportation procedures have subsequently been confirmed.

5. References
Refer to Appendix II.

6. Appendices
Appendix I: Specification provided by Defra
Appendix II: References
Appendix III: ADAS Procedures
Appendix IV: Report prepared by Mr B Hosie
Appendix V: Names and/or Contact Details for Suppliers to the Project
Appendix I

Specification of Requirements
Auditing of procedures and biosecurity in sheep reference flock – presumed case of atypical scrapie.

Preamble
Defra maintains a flock as a source of scrapie free sheep for research purposes. ADAS, the UK’s largest provider of environmental and rural solutions and policy advice, are sub-contracted by the Veterinary Laboratories Agency (VLA) to manage the flock. The VLA provides a range of applied research and consultancy, diagnosis and surveillance on livestock diseases to Government and commercial customers. The VLA has recently reported cases of atypical scrapie, from the Arthur Rickwood flock, to Defra.

Background
Investigation into the atypical scrapie cases in SE 1931

A case of atypical scrapie has recently been confirmed in a home-bred Cheviot ARQ/ARQ ewe in the flock (SE 1931). The animal was born in 2000 as the result of embryo transfer using NZ-imported animals as both donor and recipient. It was noticed as being apparently blind in June of this year around the time of lambing, but it was maintained to raise its lamb. It was euthanased at the ADAS/VLA Sheep Unit on 5th September 2006 and transported directly to VLA Weybridge for an investigative post mortem. Initial routine cull screening for Transmissible Spongiform Encephalopathy (TSE) (histopathology and immunohistochemistry of the brainstem at the level of the obex) identified changes suggestive of atypical scrapie.

Results from current screening tests (histopathological and immunohistochemical analysis of the brainstem and whole brain) from this animal was consistent with a diagnosis of atypical scrapie. This has subsequently been confirmed by Bio-Rad ELISA of brainstem material and Western Blot analysis of the cerebellum. The VLA are in the process of further investigating this ewe and the issues that surround her.

A second case now appears probable in an ARR/ARR sheep born to NZ parents. Brain tissue from this sheep tested negative for TSEs by immunohistochemistry and BioRAD ELISA. Brain homogenate from this animal was inoculated as a negative control for an experiment designed to investigate the transmissibility of atypical scrapie in mice. Three out of 17 transgenic mice succumbed to a prion disease with characteristics of atypical scrapie on histological examination. This indicates the presence of a possible low level TSE infection in the sheep inoculum. Characterisation of brain material by Western Blot is in progress.

The VLA has identified the following as possible explanations for these initial findings:
A) samples examined were from wrong sheep.
B) brain samples had been switched with those from other animals during the sampling/testing process
C) breakdown in biosecurity at the Sheep Unit
D) infection was brought in from New Zealand with the original import animals
E) atypical scrapie is a spontaneous disease

Project scope
To conduct an audit of biosecurity and associated procedures in the ADAS/VLA Sheep Unit and of the subsequent analysis of tissues and associated procedures in relation to suspected cases of atypical scrapie. The audit should consider gaps in biosecurity and/or standard operating procedures in the context of probable routes of transmission for prion diseases in small ruminants for all the requirements.

Detailed requirements
1. To examine all procedures associated with the choice of the biosecure site and identify any areas of uncertainty over its suitability.

2. To examine all procedures associated with the importation of the founder animals and identify any areas where biosecurity could have been breached.

3. To examine all standard operating procedures in use with the NZ flock since 1998 on the site of the Sheep Unit and identify any areas of potential, or actual, breach of their use.

4. To identify any potential gaps in biosecurity associated with the standard operating procedures.

5. To document and examine the procedures for ongoing TSE surveillance and monitoring of TSE freedom in the flock.

6. To record the handling of the tissues from this case at post mortem and when sampled for diagnostic testing and comment on the likelihood of any sample substitution.

7. To examine the documentation for sampling of blood, and any other tissues, from live animals and provision to other laboratories and comment on the likelihood of any sample substitution.

\(^1\) To include a review of the history of disease incidence in the flock that may indicate a breakdown in biosecurity
APPENDIX II

References

Documents reviewed during the Assessment of the ADAS/VLA Sheep Unit and the post mortem and laboratory facilities at VLA include:

- ADAS Procedures (refer to Appendix III)
- VLA post mortem procedures, test methods and support procedures (as required)
- Minutes from Project Meetings held on:

  1st October 1997  4th July 2001
  3rd November 1997 13th November 2001
  1st December 1997  4th March 2002
  22nd December 1997  1st July 2002
  2nd February 1998  7th November 2002
  9th March 1998  17th March 2003
  27th April 1998  8th August 2003
  6th July 1998  21st November 2003
  7th September 1998  24th February 2004
  7th December 1998  15th June 2004
  15th March 1999  18th October 2004
  21st June 1999  28th February 2005
  27th September 1999  6th June 2005
  24th January 2000  17th October 2005
  8th May 2000  8th February 2006
  25th September 2000  14th June 2006
  25th January 2001
### APPENDIX III

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Appendix IV

Review of Disease Incident Reports for the ADAS/VLA Sheep Unit
1998 – 2006

B D Hosie, Veterinary Consultant to UKAS

Summary
Three disease incidents point to potential breaches of the biosecurity of the ADAS/VLA Sheep Unit. These are:

- Occurrence of tapeworm cysts in the brains of two sheep. A fox, a definitive host for the tapeworm, was shot on the Sheep Unit.
- Leptospirosis is believed to be associated with a recognised vermin (rat) problem.
- Salmonella abortion occurred in 2006. There is good evidence that wild birds contaminated water and/or feed.

Background
Investigation of disease incidents is of interest because they may indicate the quality of management, veterinary care and biosecurity. Scrapie and other transmissible spongiform encephalopathies (TSEs) are noted for their long and asymptomatic incubation period. Scrapie can be transmitted by contact from infected, apparently healthy sheep to uninfected sheep. Contact may be direct or indirect. Placenta and sheep faeces are considered important in transmission. Endoparasites, ectoparasites and hay mites have also been proposed as reservoirs of infection. Contaminated feedstuffs, vehicles, vermin and other animals including man may be important in the introduction of infection to susceptible sheep. The agents that cause these diseases are exceptionally robust and resist most routine methods of sterilisation procedures. For these reasons I understand that the Sheep Unit had physical barriers and detailed procedures in place to prevent the introduction of TSE disease. For example I understand that care was taken to use veterinary pharmaceuticals either sourced from New Zealand (a country considered free of disease caused by TSEs) or guaranteed free of animal protein or products of fermentation. Therefore the occurrence of any disease that might point to a potential breach of the biosecurity of the Sheep Unit is of concern as it could provide early warning of the subsequent occurrence of scrapie or other TSE.

Methodology
I reviewed the disease incident reports provided by Carol Stewart, UKAS, for evidence of a possible breach in the biosecurity of the Sheep Unit. I also spoke to Chris Lewis, MRCVS on Monday 11 December 2006. Chris is a Veterinary Consultant to the Unit and prepared the reports. Chris visits about eight times a year and provides written reports, two or three times a year. The reports I have received and examined are listed in Table 1 attached. They provide a useful summary of the health status of the Unit over a period of eight years.

I read the report from N Woodger, MRCVS of 22nd June 2006 to ADAS, concerning his visit of 12th May 2006 to investigate an outbreak of Salmonella Havanna in the flock.
Findings
The disease incidents reported can be summarised under the following headings

1. Infectious diseases introduced from New Zealand
2. Nutritional problems
3. Neonatal losses
4. Incidental diseases
5. Incidents considered as potential breaches of biosecurity but rejected
6. Disease incidents of potential concern

I will consider each in turn and consider the “Security Serology” undertaken periodically on the unit.

1. Infectious Disease introduced from New Zealand
   I understand that the sheep were sourced from different farms in New Zealand and were transported to England in two batches. They were received in 1998 and 2001. The health status of the New Zealand sheep was generally high but there are still significant levels of endemic disease. These diseases are

   - Johnes (OJD) or Mycobacterium avium paratuberculosis infection
   - Caseous Lymphadenitis (CLA)
   - Pneumonia due to bacteria (*Mannheimia* spp) and mycoplasma (*Mycoplasma ovipneumoniae*).
   - Coccidiosis

2. Nutritional Diseases
   The copper status and Vitamin E status of the flock was a concern to the veterinary consultant. These are not biosecurity issues but they are a consequence of the management system practised (housing the sheep for most of the year).

3. Neonatal Losses
   The level of losses experienced in the Sheep Unit appears to be consistent with that of a well-run, modern sheep unit. Neonatal mortality up to 15 – 20% is to be expected. None of the incidents reported cause me concern. I note that acid fast organisms were reported in a ewe which aborted on 16 February 1999 and in the last foetus to be aborted in the report from April to July 2002. A final report for the later case does not appear to be given in the summary reports but I was informed that the VLA Regional Laboratory did not specifically report final negative results. While there is no evidence of EAE in the flock, the occurrence of these isolated acid fast organisms is of interest.

   Chris Lewis informed me that he considered these findings to be non-specific and not evidence of a breach in biosecurity. In my experience non-specific, acid fast organisms are occasionally found in sheep abortion material. The fact that few losses were associated with these findings suggests it is not an infectious condition.

4. Incidental Diseases
   Even a well-run, sheep unit will have isolated incidents, such as losses due to the torsion of the intestine (termed “red gut”), trauma and production diseases such as metritis and mastitis. Most of these incidents of diseases do not cause me concern.
5. **Incidents considered as potential beaches of biosecurity but rejected**

I considered the following reports in more detail and discussed them with Chris Lewis.

5.1 **Nasal Bot Fly**

The presence of the nasal bot (Oestrus ovis) in two ewes in the May to September report 2002 is of interest. I concur with Chris Lewis’s conclusion that the bots are most likely to have been introduced with the sheep from New Zealand. It is possible that the flies came into the unit from neighbouring sheep flocks but since the sheep were treated with an avermectin parasiticide (Dectomax; Pfizer) there has not been a recurrence.

5.2 **Orf**

Orf is a pox-like condition caused by a virus. The disease is common in sheep flocks in both New Zealand and the UK. I was surprised that the first report of an outbreak of Orf in the unit was in the report April to July 2003, some five years after the introduction of the sheep. The virus is highly resistant to cleansing and disinfection and can cause a cutaneous infection in man. Chris Lewis stated that he believes that the outbreak in July 2003 was not the first occurrence of the disease on the Sheep Unit. He believes it came in with the 2001 importation and occurred at an insignificant level in the unit. The shepherds would not have reported mild cases to him. This is a reasonable hypothesis.

5.3 **Ovine keratoconjunctivitis**

This disease is typically caused by Mycoplasma conjunctivae. It is a specific pathogen of sheep, goats and other small ruminants (Hosie, 2000). During outbreaks, a high proportion of susceptible sheep are affected. The disease is endemic in both UK and New Zealand. The only report of the condition is a case which required protracted treatment, period September 2005 to February 2006. I suspect this case was not the infectious form of ovine keratoconjunctivitis but was a result of an injury or irritation from a grass seed. No samples were taken for culture. It does not appear to indicate a breach of biosecurity.

6. **Incidents of Potential Concern**

There are three diseases reported which on their own may not appear significant but they merit further consideration given the occurrence of atypical scrapie in the Sheep Unit.

6.1 **Tapeworm Cyst (Reported 10/3/99)**

The sheep is the intermediate host for the dog and fox tapeworm, Multiceps (Taenia) multiceps. The final host (dog or fox) acquires infection by ingesting the intermediate stage (Coenurus cerebralis) when scavenging the brain of infected sheep. The tapeworm develops in the small intestine of the dog or fox and eggs are deposited in the host’s faeces. Eating pastures or foodstuffs contaminated by dogs or foxes may infect sheep. The embryos, after hatching in the sheep’s intestine, pass via the bloodstream to various parts of the body. Only those that reach the central nervous system develop into cysts (Soulsby, 1968).
The time taken from hatching, migration to the brain and evidence of nervous disease takes between 2 and six months (Scott, 2000). The presence of the tapeworm cyst in the brain of a sheep sold to SAC is of concern as Chris Lewis reported in his Health Report of 10th March 1999 that these tapeworms are not believed to be present in New Zealand. He stated that it will be important to prevent foxes entering the paddocks. Further he reported that there was a second case submitted to the CVL and a fox was shot in a paddock on the Sheep Unit. The report of the brain examination at CVL is not given in that report or in any subsequent reports. The panel that reported to Dr W Reynolds accepted that these cases could have represented contamination that occurred prior to construction of the Sheep Unit. Equally contamination may have resulted from any of the subsequent incursions by foxes.

6.2 *Leptospira bratislava*

Low antibody titres to various leptospires including *L. bratislava* are noted regularly over the years covered by these reports. Of concern is the detection of *L. bratislava*, normally a leptospire associated with disease in pigs. Also there are comments that the leptospires titres probably reflect the incursion of rodents particularly rats which contaminated feeding stuffs. I was informed in March 2007 that the titre of 1/200 in 2000 alerted management to introduce harsher rodent control. They rightly recognised this as a potential breach in biosecurity from the establishment of the flock in 1998 until at least that time. Vermin may come from infected premises and introduce infectious diseases.

6.3 *Salmonella Havana*

In the February to June 2006 report, two abortions were reported due to a group G *Salmonella* infection; the second case was typed as *Salmonella Havana*. I noted that the routine mortalities and abortions were screened for salmonella in the proceeding eight years and *Salmonella* was not cultured. Therefore I conclude that this incident is evidence of a probable breach of security.

Mr N Woodger concluded in his report that *Salmonella Havanna* was reasonably prevalent in the sheep sheds. Wild birds and vermin appeared to be a problem and he found bird droppings, and accumulations of mouse and occasional rat droppings. *Salmonella Havanna* was isolated from two samples of bird droppings.

There is good evidence that wild birds contaminated water and/or feedstuffs on the unit. Mr N Woodger noted that the door of the small barn storing the feed straights was open and it was in this barn that the accumulation of rodent droppings was found.

Chris Lewis informed me that feed is blown into sealed feed hoppers and is not accessible to wild birds. However there was potential for feed to be contaminated after delivery to the sheep pens. Further, in the spring of 2006 starlings congregated about water troughs and *Salmonella Havana* was cultured from bird droppings in that area. Also wild birds including seagulls and corvids are seen on the paddocks.
7. **Routine security serology**

There are regular reports on the “Security Serology” undertaken at intervals on the unit. I presume that this serology was undertaken to provide early warning of a serious breach of biosecurity. The veterinary consultant monitored for evidence of diseases that are transmitted by direct and indirect contact, vermin and placental material. Scrapie and other TSEs are highly infectious and are caused by agents that are exceptionally resistant to disinfection. Therefore the failure of routine serology to provide evidence of a breach in biosecurity cannot be taken as evidence that scrapie or another TSE was not introduced to the Sheep Unit. Routine serology is at best a pointer to the biosecurity situation on the unit.

Tests were carried out for four disease agents, these are

1. Maedi Visna (MV)
2. Enzootic abortion of ewes (EAE)
3. Leptospira Pooled serovars
4. Border Disease

MV and EAE do not occur in New Zealand. For a breach of biosecurity to involve MV, such a breach would entail the Unit’s sheep having very close contact with infected animals (e.g. nose to nose or sharing dosing guns or needles). A rule of the Sheep and Goat Health Scheme (SGHS) for MV Accreditation is that MV Accredited sheep should not come within two metres of non-accredited animals. The double fencing round the Sheep Unit should provide protection against the introduction of MV virus. Also a breach indicated by the detection of antibody to MV would be exceptionally unfortunate, as MV is still relatively rare in the UK. The SGHS finds that less than two percent of flocks are infected. Never the less the results of MV serology provides no evidence of such an unfortunate and serious breach of biosecurity.

EAE is an important cause of abortion in sheep in the UK. It is now well controlled by the use of highly effective vaccines. However the disease is readily spread at lambing time when infected placentas can transfer infection within and between units. Wildlife such as crows and foxes are sometimes accused of spreading disease tissues from one flock or another. Again the results of EAE serology provides no evidence of such a fundamental breach of biosecurity.

Both Leptospira and Border Disease virus are present in New Zealand and the UK. Chris Lewis related the ongoing, low-grade, MAT titres to Leptospira to rodents contaminating feedstuffs. Border Disease is a congenital virus disease of sheep caused by a pestivirus (Nettleton, 2000). The disease is characterised by barren ewes, abortion, stillbirths and the birth of small weakly lambs, some of which show tremor. Direct contact is the principal route of transmission but pestiviruses from pigs and other ruminants (principally cattle) can also cause Border Disease in sheep. When compared with the occurrence of the pestivirus Bovine Viral Diarrhoea (BVD in cattle, Border Disease is comparatively rarely diagnosed in sheep in the UK. The results of Border Disease serology are believed to be consistent with antibody to the virus having been introduced with sheep from New Zealand. Also the results of Border Disease serology provides no evidence of such a fundamental breach of biosecurity.
7. **Post Mortem Reports**
I reviewed a selection of post mortem reports including those listed below and consider that standard, veterinary diagnostic laboratory practice was followed.

S0015: Animal ID C317 (7\textsuperscript{th} January 1999)  
SAC report of two tapeworm cysts in the brain of the ewe sold to SAC (Chris Lewis report of 10 March 1999)

S0426/02/99: Animal ID D682 Dead Lamb (17\textsuperscript{th} February 1999)  
Sheep abortion results equivocal

14/S0596/09/98: Sheep ID C115 (16\textsuperscript{th} September 1998)  
Ewe with head tilt euthanased in September 1998 – no neurohistopathology result provided to me in December 2007. I was informed in March 2007 that the case was submitted via VLA Bury St Edmunds on 18 September 1998, and a brain sample was examined for the presence of Scrapie Associated Fibrils (SAF). It was test negative. Brain was subsequently examined by histopathology and reported as negative. The obex has been re-stained on 2 January 2007, with mAb 2G11, and again reported negative for scrapie (classical and atypical). Therefore I conclude that the cause of the head tilt was not determined.

14/S0389/10/06: Senders reference: G207059 (16\textsuperscript{th} October 2006)  
Adult Sheep CNS Listeriosis

14/S0251-05-06: Sheep ID H45 (8\textsuperscript{th} May 2006)  
Sheep with Peritonitis associated with uterine tear

8. **References**


Scott, P.R.(2000). Other nervous diseases. In Diseases of Sheep, 3\textsuperscript{rd} Ed Martin & Aitken pages 228-239

Table 1

Disease Incident Reports from ADAS/VLA Sheep Unit

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