



UPDATE ON A CASE OF ATYPICAL SCRAPIE IN A TSE FREE RESEARCH FLOCK

ISSUE

1. To update SEAC on investigations performed by the Veterinary Laboratories Agency (VLA) following the finding of a case of atypical scrapie in a research flock considered TSE-free. In addition, to update SEAC on the status of the United Kingdom Accreditation Service (UKAS) audit of procedures and biosecurity measures used at the research site.

BACKGROUND

2. At SEAC 95 members noted that Defra had issued an information bulletin¹ on 14th November 2006 concerning a case of atypical scrapie in a research flock considered free of TSEs. The founder animals for this flock had been imported from New Zealand, a country considered free of TSEs. Dr Danny Matthews (VLA) informed members of the details of the case and of investigations underway at VLA to inform on the cause and origin of the case. Members were informed that UKAS had been invited to perform an audit of procedures and biosecurity measures. No evidence of errors in the collection of the samples for testing, or a major breach in biosecurity, was found. Minor issues with sample handling and record keeping of post mortems were identified and there were a number of minor biosecurity issues that, over the 10 year period since the sheep were imported, may be relevant to the current outcome. The final UKAS report would be provided to SEAC in due course (see SEAC 95 draft minutes paragraphs 11 to 19, paper SEAC 96/1).
3. An update of investigations performed by the VLA is provided in Annex 1.

¹ <http://www.defra.gov.uk/news/2006/061114a.htm>

ADVICE SOUGHT FROM THE COMMITTEE

4. To note the progress of investigations into the possible cause and origin of the atypical scrapie case in the research flock.

ATYPICAL SCRAPIE IN THE VLA SCRAPIE-FREE FLOCK (PROJECT SE1931)

Update on investigations into the origin of the case.

Introduction

1. SEAC has previously been briefed following the diagnosis of a case of atypical scrapie in the VLA flock of New Zealand-derived sheep, maintained as a scrapie-free resource for research into prion diseases of sheep. This note summarises the initial events, and describes progress in investigating the possible origin of the case, with a view to enabling decisions to be taken in relation to the future of the flock.

Preliminary Action

2. Animal G320 (AFRQ/AFRQ), born May 2000, was confirmed as having been affected by “atypical scrapie” in November 2006. This result has been re-confirmed by Dr Sylvie Benestad of the Norwegian National Reference Laboratory. It conformed with the diagnostic phenotype of over 100 atypical cases already identified through surveillance programmes in the United Kingdom.

3. Animal G320 was born to a surrogate dam (C105) imported from New Zealand in 1998. Its natural mother (D301, ARQ/ARQ) and sire (D554, ARQ/ARQ) were also part of the same importation. DNA analysis of material from the natural parents and G320 has been carried out by Cellmark and confirms that relationship.

4. Unfortunately, because C105 was culled at another location during the course of the 2001 FMD epidemic, it was not been possible to collect and store brain tissue for examination. Also, D301 was transferred to another study where no brain tissue was retained after slaughter. Consequently the scrapie status of both natural and surrogate dams remains undetermined. The sire was re-examined and was negative.

5. None of the offspring of the natural and surrogate dams remain available to the VLA for re-examination.

UKAS audit

6. Detection of atypical scrapie in G320 prompted a request that procedures leading to that diagnosis be audited to ensure that the result was genuine. UKAS was invited to audit procedures, and did so at the end of November 2006.

7. The draft UKAS report was passed to Defra’s Science Directorate in January 2007, and the VLA and its subcontractor were given an

opportunity to comment on it with a view to ensuring that it did not contain errors or misinterpretations. Comments have been forwarded to UKAS.

8. As reported to SEAC on December 7th, 2006 the audit supported the VLA diagnosis for sheep G320. At the time of the audit the VLA made the auditors aware that it had preliminary data that might subsequently result in a diagnosis of atypical scrapie in another sheep (J19), which, if confirmed, would have influenced interpretation of risk factors in the flock. By the end of the audit week, internal investigations at the VLA clearly indicated that J19 was not infected with atypical scrapie, and, as a result, has not been taken into account in subsequent investigations.

Consequential investigations

9. Investigations into the circumstances surrounding this diagnosis have centred on:-

- Auditing the livestock, imported and homebred, with a view to determining the location of any samples that represented a potential opportunity to test for scrapie, and determine the prevalence of atypical scrapie in the flock of origin.
- To consider potential sources of infection in the flock, both temporal and geographical (NZ or UK origin), as well as reviewing local biosecurity procedures for evidence of potential routes of entry.

10. The starting point was to review the flock database, to identify all animals imported into the flock, and subsequently either still alive in the flock or departed for any reason. Priority was given to the tracing of imported animals that had entered the VLA diagnostic system, as well as any other animal that had been culled or entered VLA projects, again prior to post-mortem diagnosis, and potential storage of frozen tissues in the TSE Archive. It became necessary to transfer the database to the VLA, and to populate it with new identities/sample references that animals had acquired on entry into projects or the diagnostic system. This enabled the collation of data on samples available, both fixed and frozen, with the intention that testing be prioritised where possible.

11. In parallel with consideration of samples held by the VLA, all institutes that received live animals from the source flock were contacted, and provided with a master list of identities of all animals dispatched. They were asked to review their list and to notify the VLA of any potential tissues that could be made available for further examination, particularly from animals that were imported from New Zealand, and had not subsequently been exposed to scrapie or BSE.

12. Because of the time taken to fully populate the database, and to audit the collated data, the immediate availability of fixed tissue meant that the diagnostic status of culled sheep could be reviewed relatively quickly. This proceeded by either reviewing stained slides where IHC staining was carried out by current protocols (used for the detection of atypical scrapie), or by re-cutting and staining slides where past diagnostic protocols predated the introduction of current methods.

13. In the meantime, the database was populated with data from the TSE archive to confirm the identities of animals where frozen brain stem and/or cerebellum were likely to be available for testing with the BioRad TeSeE® ELISA.

Results

14. **Table 1** summarises the collated data for the source animals, imported from New Zealand, sorted by date of importation, date of birth, breed and sex. A total of 2003 sheep were involved.

15. Of these, 888 were listed as having left the nucleus flock for the VLA. **Table 2** summarises this information by year of departure. The expectation was that animals in shaded cells represented the optimal populations to target for testing, on the assumption that any evidence of infection would most probably have arisen from infection before importation. They left the flock within two years of arrival, but unfortunately were considered to be less than four years of age at the time. This is younger than normal for atypical cases found in the UK surveillance programme. At this time it had not been determined that these animals had actually been culled and were available to retest.

16. **Table 3** summarises the fate of all animals recorded as having entered the flock, either by importation or birth. Of the total of 5676 listed, 808 remained alive in the flock. The primary recipients of sheep from the flock were the VLA and Institute 3, although in the case of the VLA this would have included routine culls, and intercurrent deaths, while those sent to the Institute 3 were intended for projects.

17. Of the 2298 destined for the VLA, only 1082 were recorded as having a PG reference number on the Neuropathology Daybook (Table 4). This is issued on receipt into the diagnostic system. Some animals (372) were not issued with PG reference numbers despite being culled from the source flock. The majority would have been under 6 months of age and considered too young to warrant monitoring for the presence of scrapie. Another 489 were culled within specific projects, some exposed to scrapie by virtue of their locations, and others not, but where the project design did not require post-mortem examination. At the time of culling, their potential exposure to TSEs after leaving the scrapie-free

flock had been considered to compromise their use for monitoring the status of the source flock. These were therefore unavailable for testing as part of this exercise. A total of 355 animals were recorded as still alive in VLA projects.

18. Of the 1082 sheep for which fixed tissue samples are available for testing, 861 have been reviewed or retested as at 25th January (Table 5a). Only 54 samples remain to be reported on, while one, G320 was positive. These include 49 that were exposed to natural scrapie within the VLA scrapie-infected flock, and have tested negative previously using an older IHC protocol. A negative result on retesting using current protocols will still be of value. The balance of 166 samples are considered inappropriate for re-examination for a variety of reasons (Table 5b).

19. Care is needed in the interpretation of the results of IHC examinations. As indicated in tables 6 (Imports) and 7 (Homebred), the distribution of samples by age and genotype of the source sheep highlight the fact that the majority were under five years of age at death, and the AHQ and ARR alleles were relatively poorly represented, as indeed they are in the flock as a whole. Indeed 272 out of the total 1082 were less than one year old at death.

Epidemiology

20. In parallel with the UKAS audit, and testing of samples, the SoPs relating to the flock, and the age and genotype range of sheep tested are being analysed with a view to:-

- a) considering whether or not potential routes of entry can be identified, and
- b) estimating the power of the testing done to detect atypical scrapie if still in the flock at low prevalence.

21. In order to put potential risk factors into context, details of the local sheep population have been accessed from national databases. The farm is located in a county with one of the lowest sheep stocking densities in the country at under 30 sheep/km². In 2004, only 318 holdings were recorded as having sheep in the county, with a total of 20,947 sheep, and an average flock size of 66.

22. Only 33 of the holdings were recorded as being within a 10km radius of the sheep unit, and comprised a total of 2,506 sheep, an average flock size of 76, and a density of 8/km². This information is however based upon business address, and cannot identify the exact location of grazed sheep.

23. An examination of data arising from the fallen stock survey since 2002 reveals 67 suitable submissions from 45 holdings in the county, all of which tested negative for scrapie. Of the 33 in the 10km radius of the unit, six submitted a total of 7 suitable samples.

24. In the scrapie abattoir survey, only four tested sheep have been traced back to the county, two of which were from a single farm within 10km of the unit. Again results were negative.

Next steps

25. The next step, apart from completion of outstanding IHC examinations, is to carry out testing by BioRad TeSeE ELISA on those brain samples where brain stem and/or cerebellum is represented, targeting cerebellum wherever available (estimated to be approximately 750 sheep). It is inevitable that the majority of these samples will come from the 1082 subjected to IHC testing, but the ELISA is expected to be marginally more sensitive, especially when applied to cerebellum.

26. Additionally there will be a re-analysis of brain samples made available to us by receiving institutes. At the moment approximately 130 samples arising from imported stock have been identified for re-testing from this population.

D Matthews
Veterinary Laboratories Agency.

Table 1. Distribution of sheep imported from New Zealand by breed, sex and year of birth

Breed	Sex	Imported in 1998				Imported in 2001			Grand Total
		1994	1995	1996	1997	1998	1999	2000	
Cheviot	F				161	14	134	262	571
Cheviot	M				58			163	221
Dorset	F			157	189	4		1	351
Dorset	M		37	10	90			3	140
Suffolk	F	2	12	162	109			380	665
Suffolk	M		9	40				6	55
Total		2	58	369	607	18	134	815	2003

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Table 2. Distribution of sheep imported from New Zealand, destined for VLA by year of departure and age at departure

ImportYear	Age	1998	1999	2000	2001	2002	2003	2004	2005	2006	Total
1998	0	61									61
1998	1	27	60								87
1998	2	36	90	42							168
1998	3	3	7	18	53						81
1998	4				76	63					139
1998	5				34	44	22				100
1998	6					1	1				2
1998	7					2		5	6		13
1998	8								2		2
Sub-Total		127	157	60	163	110	23	5	8	0	653
2001	0				1						1
2001	1				54	10					64
2001	2				5	4	40				49
2001	3				2	1	11	17			31
2001	4						3	17	22		42
2001	5							3	11	24	38
2001	6								2	7	9
2001	7								1		1
Sub-Total		0	0	0	62	15	54	37	36	31	235
Total		127	157	60	225	125	77	42	44	31	888

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Table 3. Summary of Sheep by Destination

Destination	Total	Imported	HomeBred
Alive- at sheep unit	808	164	644
Institute 1	41	0	41
Institute 2	22	0	22
Institute 3	1756	750	1006
Institute 4	273	40	233
Slaughter**	74	0	74
Institute 5	280	37	243
Institute 6	124	124	0
VLA	2298	888	1410
TOTAL	5676	2003	3673

** - in one season, permission given by Defra for surplus lambs to be sent for human consumption, under 6 months of age

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Table 4. Summary of PG numbers for sheep destined for VLA

Destination	Alive	With PG Number	No PG Number	Grand Total
VLA Cull	0	265	372	637
VLA Project	355	817	489	1661
Total	355	1082	861	2298

A PG reference simply denotes entry into the diagnostic system for TSEs at VLA.

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Table 5.a Summary of Testing Fixed Tissue by IHC Results

New Result	Count Of PGnumber	Comment
N/A	166	tissue not to be re-tested or re-examined
Negative	861	
Pending	54	
Positive	1	
Total	1082	

The positive IHC result (PG0832/06 - for AR eartag G320) is for the index case for the investigation and so not a new positive that has cropped up during the investigation

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Table 5.b Summary of Fixed Tissue which will not be tested

Testing Status	Count Of PGnumber	Comment
Exp Positive	77	Tissue has been previously tested and result is scrapie positive - these sheep had exposure to scrapie experimentally
No tissue - A	1	Sheep used in a training PM and no tissues were kept for examination
No tissue - B	20	Tissues taken for Archive request 490 - no obex available for testing
No tissue - C	56	Dam in project at HM - no CNS taken at PM due to FMD restrictions, 2001
No tissue - D	12	i.c challenge study - +ve
Total	166	

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Table 6

Summary of sheep imported from New Zealand, Tested by IHC on Fixed Tissue by Genotype and Age

Destination	VLA Project
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Count of SheepID	Age(yr)										Grand Total
	0	1	2	3	4	5	6	7	8		
Genotype											
??/??/QQ		3									3
AA/HH/QQ			5	2			3				10
AA/RH/QQ		1	1	1							3
AA/RR/QQ			10	31	11	13	11	4	1		81
AA/RR/RQ	30	5	30	2		3	1				71
AA/RR/RR	8	15	19	6	1		1	1	2		53
AV/RH/QQ		2	11								13
AV/RR/QQ			6		2	2					10
AV/RR/RQ			7	1	1				1		10
VV/RR/QQ			2	1		1	1	3			8
Grand Total	38	36	112	24	17	17	10	6	2		262

Destination	VLA Cull
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Count of SheepID	Age(yr)										Grand Total
	0	1	2	3	4	5	6	7			
Genotype											
??/??/QQ		1									1
AA/HH/QQ	1		1		1						3
AA/RH/QQ	1										1
AA/RH/RQ	1										1
AA/RR/QQ	1	4	4	4	16	19		1			49
AA/RR/RH			1								1
AA/RR/RQ	7	6	13	3	6	8		1			44
AA/RR/RR	4	2	11	7	10	8	1	1			44
AV/RH/QQ			1								1
AV/RR/QQ	2	1	2			1		1			7
AV/RR/RQ	1			1							2
VV/RR/QQ			1		2	3		2			8
Grand Total	18	14	34	15	35	39	1	6			162

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Table 7 Summary of Home Bred sheep, Tested by IHC on Fixed Tissue by Genotype and Age

Destination	VLA Project
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Count of SheepID	Age(yr)									Grand Total
	0	1	2	3	4	5	6	7		
Genotype										
AA/HH/QQ	13	2				3				18
AA/RH/QQ	1									1
AA/RR/QQ	22		8	11	1	7	7	1		57
AA/RR/RQ	17	1	2							20
AA/RR/RR	41		8	8	4	3	10			74
AV/RR/QQ	72	1	1							74
AV/RR/RQ	28							1		29
VV/RR/QQ	16	1	6	3	4	7	3			40
Grand Total	210	5	25	22	9	20	21	1		313

Destination	VLA Cull
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Count of SheepID	Age(yr)									Grand Total
	0	1	2	3	4	5	6	7		
Genotype										
AA/RR/QQ	3	2	3	7	12	4	3			34
AA/RR/RQ							1			1
AA/RR/RR	2	5	9	6	7	6	2	1		38
AV/RR/QQ			2	1						3
AV/RR/RQ			2							2
VV/RR/QQ	1	3	2	4	5	4				19
Grand Total	6	10	18	18	24	14	6	1		97

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