

**University of Cambridge
Animal Welfare and Ethical Review Board**

**Investigative report on the allegations and matters raised in the BUAV report:
Neurological Research on Sheep at the University of Cambridge – June 2014**

Background

On 8 June 2014, the British Union for the Abolition of Vivisection (BUAV) presented the University of Cambridge with a report entitled Neurological Research on Sheep at the University of Cambridge. The report details that during January and March 2013 a BUAV investigator worked undercover within the University as a research assistant. Her tasks primarily involved “carrying out behavioural tests, training sheep to do particular tasks and analysing data”. It makes several statements relating to alleged events and conditions of the animals during this time, the conclusion of which being that the animals used in this research “suffered greatly and at times unnecessarily”. The report also questions the translational benefit of the work and its validity.

The University instigated an immediate investigation into these allegations as soon as it became aware of them via contact with a journalist, and has worked closely with the Home Office regarding all allegations made in the report.

Background of the work

Huntington’s disease (HD) is a progressive neurodegenerative disease. HD symptoms begin insidiously, usually in middle age (although there are juvenile forms of the disease) and progress until an inevitable death 25-30 years after diagnosis. Inheritance is dominant, so if a person carries the gene, they will get the disease. Children of sufferers have a 50:50 chance of inheriting the disease. HD is the only neurodegenerative disease caused by a single gene mutation, which makes it a paradigm disease - if we can treat or cure HD, then it gives hope that we will be able to treat more complex disorders such as Alzheimer’s disease that are caused by multiple gene mutations. HD patients exhibit a complex of symptoms. HD patients’ motor function deteriorates, as does their cognitive function and most also have major psychiatric disorder. The disease imposes a heavy burden of care on families and on the health systems.

Batten’s disease is the collective name for a group of neuronal ceroid lipofuscinoses (lysosomal diseases). The disease studied at Cambridge is Late-infantile Batten’s disease. This is a very rare disorder that affects only 1/200 000 children in the UK, although together, the lysosomal diseases are much more common, affecting 1/5000 people in the UK¹. The form of Batten’s disease we study affects children. They are born normally, but then start missing their developmental milestones and within a few years are blind, epileptic and demented. There are two mutations of interest in the group in Cambridge (CLN5 and CLN6). For CLN5 mutations, the age of onset of dementia is around 9 years of age. For CLN6 mutations, the age of onset of dementia is younger, around 3-4 years of age. Batten’s disease is a recessive disease, so most people do not know they are carriers until they have a second child with the disease. (It is so rare that the first child in a family is rarely diagnosed correctly.) Batten’s disease children are severely disabled and require around-the-clock care. They usually die in their teens.

Justification for using sheep in research

There are three reasons sheep are potentially useful: they are long-lived, have large brains and have complex human-like brain anatomy.

Most research into HD is carried out in mice. However, mice have short lives (2 years), which means that only the early stages of HD can be modelled in mice. Thus while mouse models are extremely useful, they cannot recapitulate late-onset disease, and these aspects of human disease cannot be

¹ See a review on these diseases and their impact in a recent issue of Nature **510**,68-75 (2014)

studied currently. Mice also have small brains (1-2g) with smooth cerebral cortices. This poses a challenge for translating therapies to humans, who have large brains with convoluted cerebral cortices (which complicates therapeutic delivery). Therapeutic advances in HD mean that pre-clinical studies are ready to be tested in a larger brained animal. The obvious choice is non-human primates (monkeys), since they have large brains (100g), convoluted cortices and long lives (>10 years) and a number of HD models in monkeys are under development in the USA. The Principal Investigator has been testing the feasibility of using sheep instead of monkeys. Sheep have large brains (140g) and convoluted cortices. Furthermore, they are used extensively in the farming industry, and husbandry methods are well established. Finally, sheep are flock animals, which means they can be housed in natural environments, which will be challenging for monkey models of HD.

Batten's disease sheep carry a natural mutation that gives them their disease and although a transgenic HD sheep model exists, none of the HD sheep have ever been used in Cambridge.

As with humans, sheep carrying only one copy of a mutant Batten's disease gene are normal. There are at least three lines of sheep that are known to carry recessive mutations in the same genes that cause Batten's disease. Only one of these lines, sheep carrying CLN5 mutations, is used in Cambridge. Batten's disease sheep develop the same brain pathology as Batten's disease patients. The BUAV report describes animal models as being 'unreliable'. Yet this is a natural model of a human disease in which pathology, genetics and progression of the disease recapitulate the human disease. Sheep with natural genetic mutations that cause sheep versions of human diseases are invaluable. They do not need to be genetically manipulated, and it is likely that if the sheep form of the disease can be treated, this will be directly translatable to human patients. Batten's disease sheep already existed and there are at least 6 other sheep neurological diseases caused by mutations that cause human diseases. Using natural animal models of human diseases, particularly when they show the same symptoms and pathology as seen in people, should give better insight than transgenic models.

The BUAV report suggests that the models of Batten's disease are inappropriate because the most common form of the disease has a mutation in CLN3, whereas the mutations in CLN5 and CLN6 are rare. This is disingenuous. There are several hundred families with children carrying the CLN5 and CLN6 mutations. Even if one existed (which it doesn't), a sheep model of CLN3 will not be as useful for research into disease caused by mutations in CLN5 or CLN6 as the sheep models carrying mutations in the correct gene.

Development of the Work

Humans with neurodegenerative diseases are diagnosed by a number of neurological tests, both psychological (to test mental capacity) and physiological, such as EEG and MRI, to measure extent of brain damage. However, sheep cannot be used to best effect without similar tests that can translate results directly to humans. Such tests do not currently exist for sheep. The Principal Investigator started developing methods for direct testing of neurological function in sheep 5 years ago. This early phase of development is now complete, and they have data showing that they can use measures such as MRI and EEG, that are directly relevant to those used for humans, to measure physiological function in sheep. They developed these tests using normal sheep, and have recently been using the Batten's disease sheep as a 'positive control', since they knew that these sheep had brain and behavioural abnormalities that should be measurable.

A study examining the EEG from Batten's disease sheep has recently been completed and is been prepared for publication.

The group has characterised the sleep EEG (since this is the best characterised output of EEG), and finds that it is very similar to that seen in humans. They have found that the Batten's disease sheep develop abnormal brain waves very early in their disease, before they show any symptoms. This is a very important finding, since it means that Batten's disease patients probably show brain changes long before they start to show symptoms. This means that treating diseases once symptoms develop is likely to be less successful than treating the diseases early.

Future Development of the Research

Batten's disease is theoretically curable – it is a recessive disease, and only one copy of the gene is needed, so it is an ideal candidate for gene replacement therapy². Recessive monogenetic disorders such as lysosomal diseases are the perfect candidates for gene therapies. We have these opportunities; the UK should be leading the world in developing therapies. All of the sheep models identified have their origins in British flocks, yet not a single one has been identified in the UK apart from scrapie. We are potentially sitting on a goldmine of natural genetic models of human neurological diseases.

Animal Care and Monitoring

The University Animal Welfare Ethical Review Board requested a review of all direct allegations that animals experienced unnecessary suffering. The University has robust mechanisms in place for whistleblowing; however, no animal welfare concerns had been raised by any staff during the times noted in the reports, other than those referenced regarding the incident where a sheep's leg was broken. Note that several of the staff referenced have left the employ of the University and so are unable to be interviewed to add additional information to statements made.

The investigation confirmed that all incidents had been reported as appropriate to the University's Named Persons and to the Home Office. The facility at the centre of the allegations has provided copies of any and all documentation requested by the Home Office as part of its investigation to reaffirm that all appropriate reports were made, specifically with reference to the incidents quoted in the report.

A number of different allegations were made. Several of these related to work in the facility that was separate from the neurological studies that were the headline items in the BUAV report and the newspaper articles. In relation to the specific allegations made in the report:

- It is reported that six chickens suffocated overnight due to a faulty filter in the Chicken Isolator. This was a previously unreported malfunction in the equipment, which prevented the filters from working. A report on the incident was sent to the Home Office Inspector at the time and subsequent modifications to the Isolator were made with no reoccurrence. It was acknowledged that whilst regrettable, no preventative measures could have been taken. The Home Office found no infringement to the Establishment Licence had occurred.
- The report describes a single incident where a sheep suffered a broken leg whilst being handled for weighing. We were already aware of this incident and appropriate action was taken at the time. The member of staff referenced had been working with animals for 23 years without incident or accusations of mishandling. Senior management reviewed the incident in accordance with the University's own HR policies and agreed it was an accident. However, a written warning was issued to the member of staff and the matter reported to the Home Office for review. The member of staff was monitored to ensure good practice in handling was maintained. The member of staff has since retired from the University.
- As the report details, a stock animal (Orange 206) was found dead on 18 February 2013. This was recorded in line with standard operating procedures and was brought to the attention of the Named Veterinary Surgeon. No report was made to the Named Animal Care and/or Welfare Officer or Named Veterinary Surgeon regarding a lack of hay; however, sheep will often consume large quantities of quality hay overnight, leaving none in the morning.
- 6 stock ewes were subsequently been found dead or showed upper respiratory abnormalities over a period of 12 months. Post mortems suggested this was due to pleurobronchopneumonia. Following advice from the Large Animal Veterinary Unit, the

² Tomanin et al. (2012) Gene therapy approaches for lysosomal storage disorders, a good model for the treatment of Mendelian diseases *Acta Paediatrica* 101, 692–701

antibiotic treatment was updated, and so far has resulted in improved recovery. Animals that had not responded satisfactorily to veterinary treatment for foot rot or eye infections (contagious ophthalmia), or were otherwise 'poor doers', were euthanized to avoid unnecessary suffering. In some instances the bodies were forwarded for teaching purposes.

The other allegations in the report related to the HD and Batten's disease neurological studies.

- The report alleges that the sheep 'Janet' had lost half her body weight because she weighed only 27kg at time of death and that 'normal' body weight is 62kg. There was a sheep called Janet in the study. This sheep was euthanized as part of the study and her tissues sent for scientific analysis. Her highest body weight recorded during her life was 32 kg. There is a significant amount of variability in animal weights, which are dependent on factors such as sex, age, breed and quantity of fleece. The figure quoted for a normal sheep (62kg) would be the weight of a healthy adult male ram.
- The report described 'Janet' as 'sitting for days in her own faeces' and left for several days including over the weekend. Technicians are aware that the Batten's sheep tend to defecate where they lie during the night, so it is not unusual for faecal material to accumulate in close proximity to the animals. However, this is checked for and cleared as part of morning checks. All animals are routinely checked every day, including weekends.
- It is acknowledged in the project licence that the Batten's disease sheep will experience some side effects. However, all staff are trained to observe and record these in order to ensure the sheep do not exceed the moderate severity level. Where doubt exists, senior technical and veterinary staff are consulted.
- The report describes the use of ECG (electrocardiogram) implants in the sheep's heads. We would not implant an ECG device in the head; the report appears to be confusing this with an EEG (electroencephalogram). EEGs are being used as part of the study. For these, a series of electrodes are implanted on the skull and a small bundle of wires is exteriorised through the skin. An external amplifier is connected whenever a recording takes place. The amplifier is worn in a custom made jacket that allows the sheep to walk, lie and sit comfortably. The amplifier is not heavy; it is a human paediatric device that is typically used to measure EEG in a 24-hour test for epilepsy in children. (Children carry it in a backpack.)
- The implant technique is better described as requiring a skin incision rather than producing 'a large head wound' as described in the report. The site of the surgery is shaved to facilitate access and to allow good surgical and aseptic technique. Healing is quick, and, as with human surgery, post-operative analgesia is given for any pain relating to the wound. The animals are not, as it is alleged, starved for 24 hours before surgery. Food is removed the afternoon prior to surgery, to give the 'overnight fast' that is standard surgical practice for both humans and large animals before a general anaesthetic.
- The report alleges that a sheep called Jane was killed because she had a 'faulty implant'. There was a sheep called Jane that had an EEG implant; this was used successfully for 18 months Jane was eventually euthanized because another sheep chewed the wires connecting her electrodes and they could not be repaired without major surgery. It was felt the stress of Surgery to Jane would be disproportionate to the benefits, since a large amount of useful data had already been collected from this sheep and it was nearing the end of the study.
- The Batten's sheep are reported to have shown signs of distress at being 'sat down' for foot trimming. This is likely to be due to the fact that their tails have been docked above the vulva, a standard practice in New Zealand. The sling crate (incorrectly described in the report as a 'crush cage') was purchased to see if it would enable the animals to be immobilised for foot trimming with minimum distress.

- The report references sheep having seizures. The line of sheep used (CLN5) has been reported *not* to have convulsive seizures. New data from the unpublished EEG studies being conducted in Cambridge show that Batten's sheep probably have partial/absence seizures, where they momentarily become unaware of their surroundings. Triggers for these seizures include loud noises, and procedures are laid out to care for animals that suffer such seizures and prevention measures taken to avoid triggering them.
- We are careful to avoid causing stress to the Batten's disease sheep. As their disease develops, they become confused and can become agitated, particularly when approached by unfamiliar people or surroundings. Thus the animal care team is careful not to isolate any sheep from its flock-mates, allow interaction with strangers, or make sudden or unnecessary changes to their routines. It appears that the BUAV infiltrator not only disrupted their routines in the making of the undercover videos, but also isolated the animals. This will have made the sheep appear more agitated than they are when under routine care.
- The report questions the need for transporting the sheep to the UK. There is no flock in the UK with sheep identified to be carrying the Batten's disease mutations. The surgery and recording of EEG from sheep has only been done in Cambridge. Therefore, the sheep needed to be transported to the UK.
- It is alleged that a lamb had to be euthanized at a UK airport after becoming sick during transit from New Zealand. One of the lambs did appear disorientated on arrival in London, but was cleared by the Veterinary surgeon as being fit to continue his travels. No adverse effects were seen in any of the animals on arrival in Cambridge a few hours later.
- The report alleged that behavioural testing was carried out in which the sheep 'struggled' to carry out the tasks. The testing described refers to the use of a Gaitmat, an electronic mat used to test human gait. The sheep were only required to walk over the mat, and the Batten's sheep performed this task well. Data from these tests has now been analysed and show that there are minimal gait or locomotive differences between the Batten's and normal sheep, even at late stages of disease. This is not consistent with animals 'struggling' to complete the tasks.
- The BUAV report describes tests in a 'crush' trolley similar to a cage. This is the name of the trolley; the sheep are not crushed. Sheep are routinely transported around the site in such trolleys, and their visual responses are also tested in them, since the animals need to be kept in one place for this testing. They are also weighed in similar cage crates that incorporate scales. The sheep are habituated to these crates, and typically enter the cage without difficulty. It can be seen from the BUAV video that the sheep filmed by the infiltrator has clearly expected to be weighed, as she has voluntarily entered the weighing cage.

Ethical Review

The University follows a strict ethical review process before supporting any project licence application. The licence application was reviewed by an Ethical Review board consisting of lay people, researchers, and the Named Veterinary Surgeon and animal welfare officers, who considered and reviewed the harm:benefit of the project, by weighing up the potential benefits of the work against the likely adverse effects. The Review Board met with the lead researcher and questioned them on the work thoroughly before making its decision to support the project.