Royal Veterinary College
University of London

Minutes: AWERB summary minutes

Status: Chair approved

Meeting held: 15 September 2020 at 2pm by MS Teams

Present
Attendees: 10 plus 1 in attendance, 3 by invitation and 5 apologies

1 PPL PRESENTATION: NEW PROJECT LICENCE APPLICATION

The project licence holder was welcomed to the meeting. The scientist that had reviewed the application was also at the meeting to provide a scientist's perspective on the proposed work.

The project licence holder explained that the research focused on neurodegenerative diseases. The project aimed to increase understanding of diseases and the functions of the mutated proteins that caused these diseases as well as providing disease models for therapeutic discovery and development. The project would be focussing on rare inherited disease, which frequently cause premature death in childhood and the vast majority have no approved treatments. The project would also be studying diseases that have a more complex origin, in which the same genetic factors interact with environmental factors.

The project licence holder was involved in developing new disease models by generating new genetically-altered fish that were designed to be disease models. Experiments were then performed to potentially validate them as disease models. Where there was evidence that the treatment might have a wider use, then the novel therapeutics would be tested in one or more disease models.

The following queries were raised:

- **The licence mentioned that where fish would be re-used they would be maintained under the supervision of the NVS. Was this a new request or was this something that had been carried out by the previous NVS?** As this was a new request, the NVS and the project licence holder would discuss separately.

- **What was the current standard anaesthesia regime?** For recovery anaesthesia, a lower concentration of phenoxyethanol was used in which the fish was immersed, the gills were watched to see if there was a response, the experiment was then done and the fish placed back in fresh water, ensuring that the gills were covered. The fish were then monitored for the rest of the day to ensure that they recovered. If the fish did not recover within a few minutes they would be culled.

  For the euthanasia of fish which was required for tissue collection, chilling or perfusion fixation had been considered as the standard method. The project licence holder however was now aware of a schedule 1 culling method with anaesthesia and then exsanguination. The project licence holder was considering using the same method for some of the fish, but for younger fish where it was difficult to see the gills (for exsanguination), a different method was needed to

ensure that the brain wasn't destroyed as that was the tissue that needed to be preserved

- **The project licence mentioned that there would be no dangerous phenotypes, however as dealing with new models that were being characterised, what was the project licence holder's thoughts on how to monitor the welfare of these animals as they were being characterised?** The project licence holder advised that included in the licence was how often the animals should be monitored, how quickly the response should be to assess the phenotypes and how long they should be left at that stage. Once a phenotype had been spotted (which should come up slowly if due to a mutation as that was what happened in other models), they should be monitored for up to 4 days – it was a progressive neurodegeneration and likely to see some motor dysfunction or the retina looking smaller. The only phenotype that might be detrimental very quickly was if it went into bad seizures, but there were not many models that went into bad seizures right away. The usual pattern was to see changes in behaviour first.

- **To fully characterise the models was the idea to keep the fish longer to define the fish model and see what ages the fish were before they started to deteriorate or see the seizures? Was it about defining the model before using it?** The project licence holder advised that the plan was only to use initial phenotypes.

- **A query was raised were there occasions where other researchers' work might be duplicated?** The project licence holder advised that work would only be duplicated to verify the results of another group before extending that work further, especially if other results did not corroborate those findings or if they were using a smaller sample size.

- It was noted that the project licence made specific mention in relation to the timings of when the fish would be monitored or fed. It was felt that this could make the project licence too restrictive and could cause difficulties if later on changes to the routine were required. Although it was good to be informative (so a harm benefit analysis could be done), a balance was needed so as not to make the licence too restrictive, making it more difficult to carry out work. It was recommended that the wording be changed to be more flexible such as "checks would be done a minimum of twice a day" as that meant more checks could be carried out if required.

- Clear and defined humane end points were needed for each protocol to ensure that a fish was not unintentionally overlooked and welfare impacted.

- **A question was asked about how pain assessment of zebrafish for phenotypes was carried out.** The project licence holder advised that this was a developing area as there were not very many outwardly pain specific signs that could be seen, unless the pain was associated with something else such as loss of mobility. There were two ways that they had of assessing the health of a fish in the facility: body condition and also a green sheet that included 6 to 7 different features to look out for. As new models were developed, and more learnt about them, then it was possible additional points could be added into the scoring sheets for specific fish. Also for the humane end points these could be defined once the phenotype from that model had been seen. AWERB referred the project licence holder to a recent paper that had been published in Nature about pain assessment in fish (https://www.nature.com/articles/s41598-019-45464-w). There were also a number papers on nociception in fish, some using them as models, but there were a couple on fish welfare.

- It was suggested that developing the score sheets for new models should be flagged in the future potential refinements section.
- **Was genotyping of embryos done?** It was confirmed that this was done occasionally. AWERB mentioned that there was a Zebrafish Embryonic Genotyper (ZEG) that was an automated system that extracted DNA for PCR from zebrafish embryos without harming them. This would avoid having to grow all embryos to adulthood for genotyping.
- One of the protocols mentioned that fish could be injected up to 14 times but there was no mention of a minimal time period between the injections and whether it would be the same route of administration. Frequency and a minimum time period needed to be added so it could be reviewed as part of the harm benefit analysis.
- The same protocol mentioned an optokinetic response which would result in the fish being immobilised after they have recovered from the anaesthesia. Would this not be stressful for the animals? How long would it last? What adverse stress might there be? This should be included in the project licence.
- More information was requested about the human diseases**.** Were they mainly neurological diseases? It was confirmed that these were a group of conditions that affected the nervous system. Signs and symptoms varied widely between the forms but generally included a combination of dementia, vision loss, and epilepsy. The brain was generally hit first. It was an inherited disease. Could it be predicted who might be born with these mutations? The project licence holder advised that parents generally did not have symptoms but were carriers. They also would not know that they might be a carrier. If both parents were carriers then there was a 1:4 chance that their children would have the disease. At the same time each child also faced a one in two chance of inheriting just one copy of the defective gene (so becoming carriers).

The project licence holder was thanked for attending the meeting. The project licence would be amended as requested. This would then be circulated to AWERB for a final sign off.

After the project licence holder had left AWERB confirmed that they were overall happy with the licence and its principles.

2 PROJECT LICENCE AMENDMENT:

A project licence holder had submitted an amendment to the existing project licence in order to make changes to two of the protocols. The changes were required because the optional treatment of the mice carrying the inducible Cre gene with tamoxifen was not explicitly specified in protocols 1 and 4. An optional step for a tamoxifen injection was therefore being added to cover all the mice protocols in the licence where it might be required.

A query was raised whether tamoxifen was generally being used and the route of delivery? Was there a reason why the preferred route was via injections? The project licence holder explained that the mouse model had initially been generated by a collaborator in America who used this method and the same exact regimen needed to be followed as they were comparing data to keep it consistent. The project licence holder was encouraged that in the future she should move to using a more refined method. The data set should be built up now in order to be able to move to the more refined method. The project licence holder should also put in the refinement section that other methods of delivery for tamoxifen was being looked into.

The project licence holder was thanked for attending the meeting. The required changes to the project licence would be made before being forwarded to AWERB for a final sign off.

3 CONDITION 18 REPORTS

AWERB noted that two condition 18 reports had been submitted for one project licence. These were for 2 mice that had died/had to be euthanased within 2 days of each other. The situation had been

investigated and discussions held as it related to a lack of communication. Steps had been put in place to ensure that this did not happen again.

4 REHOMING

A presentation from one of the technicians was given on how the rehoming of the dogs was going.

The rehoming progamme had initially been put on hold during the lockdown but had recently restarted having been adapted to take into account social distancing requirements. A risk assessment had also been carried out. The playroom was being used for the families to meet the dogs.

There had been several challenges:

- There had been a lot of enquiries from people wanting to rehome dogs, which it was felt, was not always for the right reasons. The high demand could be due to prices of dogs having tripled since lockdown and rescue centres being closed, so leading people to come to us for a "free" dog.
- It was difficult to tell if the people enquiring about the dogs genuinely wanted to rehome them or just wanted something to do during lockdown.
- There was not much interest in the older dogs (above 2 years old).

This year 15 dogs had been rehomed (5 adults and 10 puppies). Two dogs had also been fostered.

AWERB discussed concerns and making sure that potential owners were aware of the risk that dogs could suffer from separation anxiety if they were left on their own. This it was felt could soon become a common problem as a lot of dogs had now got used to their owners being at home all the time and so might become anxious if they were left by themselves. This issue would be highlighted to prospective owners and they should be encouraged to get into a routine of leaving the dogs by themselves so they got used to it. This would be added to the rehoming programme when speaking to owners.

A fostering programme had also just started for those dogs that would not cope in going straight to a home, so that they were fostered by people they knew. The fostering programme gave the dogs opportunity to meet new people and get relaxed in a new environment.

The following comments were made:

- AWERB were very supportive of the programme
- It was important that prospective owners were made clear of the hard reality of looking after a dog and the level of commitment it took.
- Encouraging prospective owners to consider taking an older dog as opposed to a puppy was a common problem.

A query was raised that at a previous meeting, an issue had been discussed about how long the bitches in the breeding colony should be kept before being rehomed. AWERB had suggested that a standard policy be created that made it easier for decisions to be made about the dogs without there being arguments. Was this done? It was confirmed that there had been initial discussions but then the pandemic had happened. Although the technicians were involved more in the discussions they were still keen to have a standard policy that stated when a dog should be considered for rehoming, rather than decisions made on a case by case basis due to behavioural changes. As it was a breeding programme there should be cut off points so that subjective decisions were not made so discussions should start up again.

AWERB's advice was sought. As part of the socialisation programme, volunteers used to walk the dogs which gave them opportunity to meet with strangers. However this had been put on hold as it was necessary to keep biosecurity in place. As an alternative what were AWERB's views on allowing volunteers into the playroom to socialise and play with the dogs, as that would provide space to

maintain social distancing. It would give the dogs an opportunity to meet new people and expand their horizons and meet strangers. Were AWERB supportive of this? AWERB confirmed that they were.

The technician was thanked for her very interesting presentation and for all the effort that had been put into the rehoming programme.

5 3RS UPDATE

Due to time restrictions the update would be circulated by e-mail.

6 MINUTES OF PREVIOUS MEETING

The minutes of the meeting held on 25 August 2020 were agreed to be an accurate record.

7 ACTION LOG

7.1 Item 3.2: Project licence update (25 August 2020 meeting)

Data from the diet trial study were being processed.

7.2 Item 7: Mice study (25 August 2020):

Meetings had been held to review what went wrong with the first study. A plan had been agreed in order to move forward their next study in a more controlled environment.

7.3 Item 8: Retrospective assessment of project licence (25 August 2020)

No suggested changes had been received for the retrospective assessment. The retrospective assessment had therefore been submitted to the Home Office.

7.4 Item 3.5: Enrichment for the dogs (7 July 2020)

The technicians were pulling together their ideas into a presentation to be reviewed by the internal experts. For now though, nothing new was being placed into the paddocks, as the dogs were not in their usual routine. Once things were more back to normal then these enrichments could be put into place.

7.5 Item 4: Rehoming Programme (25 August 2020)

There would be a presentation at the next meeting focusing on rehoming the small animals and the challenges that were being faced.

8 NVS REPORT

8.1 Lame study dog

The dog was improving since his surgery and was using his leg more, but the improvement was slow.

8.2 Procedures

Several new procedures were being carried out which the NVS was observing.

9 NACWO REPORT

9.1 Camden

- **Humidity:** building parameters and humidity scores were being monitored
- **Sores had appeared on rodent tails**: possible causes were being investigated.

9.2 Hawkshead

- **Lame pig**: a pig that had been due to be used for a study had become lame shortly after delivery. Investigations had revealed that the pig had a bad injury so could not be used for the study and was therefore euthanased as it was not possible to treat the injury. There was uncertainty whether the injury had occurred as part of the delivery/transportation issue.
- **Teams:** the technicians were now back on full duties though with the rise in Covid-19 cases the situation was being monitored in case the emergency plan had to be implemented again.
- **Weekly reports**: Weekly reports were continuing to be provided to the Home Office Inspector.

10 PROJECT LICENCES AMENDED BY THE HOME OFFICE

AWERB noted that one project licence amendment had been approved by the Home Office since the previous meeting.

11 ANY OTHER BUSINESS

11.1 Thank you

AWERB members were thanked for having attended this additional meeting and giving up their time so that the additional project licences could be reviewed and discussed in detail.

11.2 Date of next meeting

This was scheduled for 14th October at 10am.

Secretary
24 September 2020