Royal Veterinary College
University of London

Summary Minutes for the website: AWERB: PPL review meeting

Status: FINAL

Meeting held: 09 July 2024 at 10.30am

Present: 15 plus 1 in attendance, 9 by invitation, 15 apologies

1 WELCOME

Three observers were welcomed to the meeting.

2 PPL AMENDMENT FOR REVIEW

The project licence holder (PPLH) and two colleagues were welcomed to the meeting. It was explained that the PPLH was seeking approval to add a new protocol to the licence.

Idiopathic epilepsy is the most common neurological condition affecting pet dogs in the UK, with one third of dogs showing continued seizure activity despite anticonvulsant medications. There are also often quality of life inhibiting adverse effects from these drugs, that result in many of the dogs ultimately being euthanised. Novel treatment approaches were therefore urgently needed both for veterinary medicine and human medicine, as epilepsy has a similar incident rate in both.

A novel strategy for the treatment of epilepsy that involves antiseizure gene therapy has been developed, with the hypothesis that this novel gene therapy should be well tolerated and reduce seizure frequency and severity in dogs. A proof of concept study to provide pilot data on the safety of intrathecal administration of this gene therapy was therefore required. The client-owned dogs that would be recruited would be those whose seizures were significantly compromising their quality of life and viable alternative treatments have been exhausted.

The following queries were raised by AWERB:

- **If a dog was euthanised after receiving the gene therapy, what steps would be taken to ensure that the researchers were able to obtain the cadaver to check how the gene had worked?**
  Ideally the dog would be euthanised at the Queen Mother Hospital for Animals (QMHA).

  The researchers were advised that owner information and consent forms would be needed, making it clear that if a dog had to be euthanised that this should be done at the QMHA and ensuring that the owners were 100% on board about what would be required.

- **It had been mentioned that the gene therapy has been tested in both human cell models and rodent models. What was the scalability of these types of therapies between rodents and larger animal species? How would the high and low dose be determined?**
  The construct was currently rodent or human specific. External collaborators were relatively confident that these would be ample for the dog as the promotor and the transgene have very similar sequences to dogs. Modifying the constructs specifically to canine sequences in case that

improves expression, durability or efficacy had been discussed though and this would be added to the project plan. In terms of scaling and dosing, the work done so far by collaborators was purely intraparenchymal injections in mice and a dose response to intrathecal administration. They should have data on the precise construct of different intrathecal doses in the mouse brain, which would be used to define a dose for the dogs. However, they would be very careful to ensure that they worked within what has been reported in the current literature and to err on the side of caution whilst they documented the tolerability of this treatment.

- **As gene therapy is very expensive, is the primary aim of this study to create a therapy for veterinary use or to translate this into human clinical practice?**
  It was both. It was currently a long way off becoming clinically feasible until the costs of producing these viruses comes down, however there was confidence that this should happen in the next 5 to 10 years. It was hoped that this work would complement both veterinary and human development.

- AWERB recommended that for this type of study it was important to have objective inclusion criteria that can be quantified, such as how many drugs dogs needed to be on; how many seizures to be classed as resistant. It should not be left open to individuals' interpretation and ambiguity. The criteria needed to make it clear that selected dogs were severely epileptic and resistant to alternative treatments. Once it was shown the therapy was safe then the criteria could be loosened.

- **The licence mentioned that the dogs would be monitored and followed up for a minimum of 6 months. If however no beneficial effect from the treatment was seen, are owners likely to keep the dogs alive for a further 6 months?**
  The licence would be amended to say followed up for a minimum of 6 months or until euthanasia if the treatment does not improve quality of life.

- **What consideration would be given to drug interactions between medications that the dogs were currently on and what was being done procedure wise and whether there could be any interference between them?**
  The dogs would remain on exactly the same anti-seizure medication regimen pre and post gene therapy, unless the seizures worsened, in which case the medications would be modified following advice from the attending vet in order to optimise control.

- **Could the procedures cause any complications for animals that are prone to epileptic seizures?**
  Epileptic dogs were routinely anaesthetised, so this was already a routine part of the care of managing an epileptic patient. Worsening of seizures because of the anaesthesia was very rare. What was important was that the dogs needed to receive their anti epileptic medication on the morning of the procedure whilst taking into account the need to starve the animals pre general anaesthesia. The dogs would also have regular serum monitoring as was standard in epileptic dogs to make sure there were no adverse effects. The literature on myelogram would be reviewed though.

AWERB advised that before a dog was recruited onto the trial it had to be made clear to the owner that this was a very new experimental therapy which might produce adverse effects in their dog resulting in euthanasia. With this knowledge were the owners still willing to give their permission for the dog to be recruited onto the study.

The researchers were thanked for attending the meeting.

3 STUDY REQUEST

A study request that was linked to a grant application had been received. The applicants were welcomed to the meeting, along with a scientist who had been invited to provide a scientist's perspective on the project.

The applicants were studying the effects of green versus white light exposure during incubation on embryo development and physiology. Previous work has shown that light in an incubator has a positive effect on chicken embryo development, but further work was needed to determine what wavelength of light was the most beneficial.

The following queries were raised:

- **A list of paper references had been provided with the application. Some of these papers suggest that green light does not have any affect, so why use?**
  There was conflicting data regarding the impact of wave length of light. For this project they would be using two small scale industry standard incubators, enabling them to exactly replicate conditions of very large industry incubators, which laboratory standard incubators were not able to do.

- **Would the research have any welfare consequences by causing suffering or distress to the chick embryos and the chicks?**
  The embryos would experience natural incubation (as much as can be possible in an incubator) and also be subject to standard incubator controls to carefully regulate humidity levels and so should hatch naturally with a healthy status. There would be no forced hatching. For the culling, this would only be carried out by skilled and experienced individuals.

- **Was there any evidence in the literature that treating modern broiler embryos like jungle fowl embryos, results in an improved quality of life?**
  In terms of grow out performance, there have been a limited number of studies. Within the embryological state there is an improved hatch percentage; there have also been studies, mostly with incandescent light on brain lateralisation which has a positive impact; and green light has recently been shown to do that too.

- **Has there been any research on the welfare perspective of chickens and whether they know how they were being treated?**
  There was no known research in this area. It was therefore not known if there would be positive welfare indicators for this project. One of the longer term core objectives was to look at welfare behaviour measurements.

- **There were two incubators for this project. The experiments would involve white light versus green light, but there was no dark control. This would make it difficult to determine if the experiments have worked, as it would only be possible to compare the two treatments to each other and not to the current standard practice of incubating eggs in the dark.**
  As they only had two incubators, they had to choose what to focus on. Design wise it was not possible to partition one of the incubators to provide half light/half dark. Although not ideal, as they already had a lot of data on dark controls from previous research, they had opted to go for white and green lights.

- **There was concern about the numbers of embryonated eggs that were required for the study to fill the incubator. Could smaller incubators be used?**
  The challenge was that these were bespoke industry standard incubators. The technicalities of getting an incubator to behave like an industry standard incubator limits the miniaturisation that

was possible. They had made the incubators as small as they could effectively go.

- **In that case as there were all these eggs available, could the experiments be designed so that more samples and data could be obtained?**
  The incubators will not operate once more than 30% of the eggs have been removed as the CO2 levels get impacted which are critical for an optimal hatch. The numbers of embryos in the incubator are absolutely critical for the right level of heat and CO2. Although new eggs could be substituted to replace eggs that are taken out, this would require a 3rd incubator which although did not have to be a commercial incubator, would result in a lot of variability which might have an impact.

- **Could the chicks or eggs be used in other studies?**
  This would be investigated.

- **How were the sampling timepoints selected? Was there likely to be more/less variation at some timepoints than others?**
  With a non-commercial incubator there would be much more variation up to day 10. Beyond that it should standardise out and it should be known by day 14 if there was synchronization.

- **Could a third incubator be provided so solving both the experimental design question and the issue with the wastage?**
  It would take up to 3 months to create an additional incubator and due to funding, this project had to be completed before then.

- **Could the project be changed from white versus green lights to white versus dark light?**
  The pilot was very time limited. Although the project could be changed it would limit understanding of which was the better light source. More data was needed for the green light to confirm preliminary findings and to follow up aspects in relation to LED lights.

The researchers were thanked for attending the meeting. There were several areas that still needed further work particular in relation to wastage and what can be done with the surplus eggs and chicks to be used in a responsible way that would benefit science or teaching. AWERB needed to be able to ethically justify the numbers of eggs and chicks required.

4 TEACHING ANIMALS:

A summary paper providing the background to concerns raised about keeping teaching cattle in Camden had been circulated. AWERB's focus was on the welfare of the animals and assessing whether the benefits of providing these teaching animals in Camden outweighs the associated harms.

The following was agreed:

- Information to be obtained about how many students attend the teaching sessions.
- The Student Body to be approached to ask if any formal complaints have been received about Camden not being a suitable environment to keep the calves
- A separate meeting to be held to discuss what training and support could be provided.
- With the upcoming major building works, there would be a review of what was needed to safe guard the animals
- The concerns raised would be discussed with Anatomy so that solutions can be identified.

5 **MINUTES**

The minutes from meeting held on 25 June were confirmed as an accurate record.

6 MATTERS ARISING

6.1 Dog Unit: update on the pilot study

Following discussions held at the previous AWERB, about the pilot study taking place, the project licence holder would be attending the next meeting to discuss the questions raised by AWERB and what refinements/changes could be introduced.

6.2 Dates of next meeting:

This would be 6th August.

Secretary
11 October 2024