Royal Veterinary College
University of London

Minutes: AWERB

Status: Chair approved

Meeting held: Wednesday 14 October 2020 at 10am

Present:
Attendees: 11 plus 2 in attendance and 5 by invitation and 1 apology.

1 WELCOME

The Chair welcomed everyone to the meeting, including an early career researcher who was attending as an observer.

2 PPL PRESENTATION:

One of the AWERB members declared a conflict of interest in this item due to being involved in the research. This member was therefore not involved in any of the discussions.

The project licence holder was welcomed to the meeting. She was applying for a new project licence to replace a previous licence that had now expired. Her previous project licences had been involved in a similar area of work to study the mechanisms in order to try and understand why ponies got endocrinopathic laminitis. It was emphasised that the aim was not to induce laminitis in the animals but to identify potential pharmacologic agents and management interventions that would increase circulating adiponectin concentrations which might reduce the risk of endocrinopathic laminitis in high risk animals.

The following queries/comments were raised:

- **Protocol 3 adverse effects**: There was a contradiction in that for one of the questions the answer given indicated that for one of the steps there could be adverse effects for the animals that would be more than mild and transient; but that in the next question the answer was the likely adverse effects was none. It was explained that when the project licence had originally been reviewed by the NVS there were adverse effects in there but the NVS thought they were so unlikely to happen they should be removed. The project licence had therefore been amended but the answer to the first question had accidentally not been changed to "no".

- **The number of protocols that each animal could go through was queried as the numbers did not add up**. As each animal could go through each protocol, which could occur twice, then the numbers would actually be 8 rather than the specified 5. This needed to be changed for when the Home Office did the harm benefit analysis they would want to look at the possibility of what could happen to an animal over the entirety of the project licence.

- **The project licence stated that a protocol would be carried out first in ponies and then if necessary, carried out in horses. What was the difference between horses and ponies?** It was explained that ponies and horses had a different metabolism. Ponies would be used first, and then the horses, if a comparison was needed due to significant effects of

hyperinsulinaemia, tissue insulin resistance and pasture-induced obesity on circulating adiponectin concentrations being found. This would be clarified in the project licence. It was suggested that a decision tree be included explaining this as that could be useful in relation to the 3Rs as there would be a reduction in the number of animals used, for if a certain criteria were not met in the ponies, then the horses would not be used.

- **Protocol 2: as induced obesity could be repeated in each animal, was there concern over the cumulative effect on the cardiovascular system. Were there any adverse effects from going through the weight change twice?**
  In the wild, horses naturally got fat in the summer and then used these food stores in the winter when food was scarce. Thus, repeated obesity on an annual basis was 'normal' for this species. They were not being fed an unnatural diet to make them gain weight. The pasture was being used which is what they were designed to live on. The aim was to replicate natural weight gain and weight loss in the animals.

- **Inclusion criteria: were animals that had previously had laminitis or other underlying diseases being specifically ruled out?**
  The project licence holder confirmed that they were only interested in healthy ponies. They did not want animals that have previously had laminitis.

- **How was it possible to determine that a healthy pony did not have any underlying laminitis issues?**
  All animals would be assessed to ensure that they had no metabolic abnormalities and no evidence of laminitis prior to inclusion. Non-invasive methods such as endocrine testing and x-raying of their feet would be done.

- **A query was raised whether the protocols on the ponies would be carried out over a period of time, rather than doing them all at the same time as there would not be enough space in the stables.**
  This was confirmed and it was explained that for example for the infusions they would be carried out on each pony individually as it was fairly labour intensive. Previous experience of infusions had shown that ponies did not like to be by themselves, so each pony would also have a buddy to keep them company and happy.

- **For the weight loss, would the animals be stabled for the full 12 weeks?**
  It was explained in previous projects where they had to control weight, they had used bare paddocks, as ponies were generally happier and less stressed in paddocks. It would also act as a method for controlling what the animals ate. The plan was to do something similar for this but it would be judged on the individual animals that were used in the study.

- **Clarity was sought by a lay panel member whether the aim was to induce laminitis in healthy animals in order to then look for treatments?** It was explained that on the contrary, the aim was to ensure the healthy native breed ponies or horses that were being used remained healthy. If they got laminitis, then they would need to leave the study as they were not healthy anymore. The goal was to alter their metabolism either by infusing of insulin or through a systemic administration of corticosteroids. However, the researchers did not want to get to a point that the metabolism had been altered so much that it caused the animals to get laminitis. For example in protocol 2, the ponies would be fed grass to make them fat to see how their metabolism changed from being normal weight to overweight. Some of the ponies would then be put on a diet whereas others would be given supplements

or drugs to see if this had any extra beneficial effect on the animals. If it did, then the long-term aim was to do the same in laminitic animals, however it needed to be done in healthy ponies first to see if there was any benefit.

The project licence holder was thanked for presenting her project licence. She was asked to make the suggested changes and then circulate the project licence to AWERB for one final review.

3 **PPL PRESENTATION: NEW PROJECT LICENCE**

The project licence holder was welcomed to the meeting. One of the scientists that had also reviewed the PPL was also attending the meeting.

A brief background to the research and the aim of the project licence was given. The aim was to assess if Cellogen therapy could be used as a long term treatment for scarred vocal cords and so improve patients' voices and alleviate their suffering. The project licence holder was a throat surgeon and had been involved in several projects over the past 8 years that were aimed at improving the function of the voice and upper airway. He was leading the project as he had been responsible for the pre-clinical investigations to date into the cellogen therapy. The project would involve translational applications with the data being used to support an Investigational Medicinal Product Dossier (IMPD) and clinical trials application to test Cellogen in a first human study.

A wound healing model was to be used – the approach being the same as that which was used in a human. The aim was to create a wound and see how it responded to healing by replacing the scar tissue with healthy type of collagen.

A query was raised about the proposal to run experiments either over 30 or 180 days. Why had this time frame been chosen? It was explained that the aim was to have an environment that sustained the cells and avascularised them. The in vivo model would show to some extent that the cells do survive and were relatively robust. The researchers were interested in the long-term effects where cells influenced the surrounding micro-environment and undergo this remodelling, involving the secretion of matrix proteins and also the preservation of healthy cellogen. When there was scarring this was due to excessive deposition of collagen which became more dense and loses the important elastic proteins. The aim was to reverse this process but this could only be seen at the later time points. The 180 days was one of the requirements by MHRA to see what the effects were as they saw the programme of work as being a critical step towards clinical trials.

A further query was raised about the relative merits of using the same animal as a control versus using separate animals. Was anything being done to investigate that if it was found that there were cells migrating from the scaffold in the animals or difficult to determine what was happening on the contralateral side versus the unilateral side? Were there resources available to do an independent control? It was recognised that it was a new model so that it was not known what was going to be found, however if there were difficulties in interpreting results then it was logical to have an independent control, but was it possible to embark on this? The project licence holder advised that if need be they could revisit how the control group worked. However, he did not think the control group would be a problem as he did not see why there would be a different reaction if using a same animal rather than a different animal. It was pointed out that although AWERB were keen to reduce the number of animals used, if the results did not give a scientifically robust answer, then the animals used would have been wasted. Were they confident that the experimental design would work? Animal numbers should not be reduced at the cost of another animal welfare or adverse effects.

The project licence holder was thanked for attending the AWERB. He was asked to re-word the licence to address the comments received and then to resubmit for a final review.

AWERB discussed the licence further. The consensus was that the work was justified in terms of the overall goal and would be a significant advance. The model needed to be developed properly though although they had obviously thought a lot about the experimental design. What could AWERB

suggest that would refine their approach to improve the welfare? If it worked using the contralateral side as the control then it reduced the numbers, if it did not though, the welfare of the animals would have been compromised too much. Once initial work had been done this needed to be reviewed to see how it was progressing. This was a developmental model, so it should be developed first, before going into more detail. A question was asked that if the first objective was safety, how did they assess that it was not safe? Was there a no-go option that would determine that the study should be stopped? This needed to be followed up on to ensure that there was one.

AWERB's final comments would be fed back to the project licence holder. A copy of the amended project licence and any response when received would be circulated to AWERB for a final decision.

4 **PPL PRESENTATION: SECONDARY AVAILABLITY PROJECT LICENCE APPLICATION**

The project licence holder was welcomed to the meeting. Secondary availability was being sought. It was explained that a scientist who had reviewed the project licence for AWERB was also attending to provide scientific input.

It was explained that the project licence holder was working with an RVC scientist and they were looking to do neurosurgery, involving brain and intra-parenchymal drug delivery through catheters. The work involved actively assessing new neurodegenerative compounds as well as standard and novel chemotherapeutics in their *in vitro* model. Only suitable therapeutics would translate through the pathway towards in vivo assessment.

Could further information be provided about the actual drugs or agents that would be administered to the animals via the Convection Enhanced Delivery (CED) strategy? Did any of these have direct toxic effects? What was known about their toxicological effect when administered intra-parenchymally in other species. How were these drugs chosen? The project licence holder explained that their work followed the lab to clinic strategy and therefore novel compounds (nano particles, novel therapeutics (NA VP), vectors were first assessed in vitro to determine the IC50, then in rodents for toxicity experiments before progressing to the sheep/pig models. It was suggested that this be emphasised in the project licence.

The project licence holder was thanked for attending the meeting. The project licence should be amended based on the comments raised at the meeting that day. The project licence would then need to be circulated to AWERB for a final review.

After the project licence holder had left the meeting, AWERB were asked for their initial thoughts and feelings on the proposed licence work being carried out at the RVC.

The consensus was that although there was concerns about some areas of the project licence research, the work that would be carried out at the College was straight forward and there were no major issues. It was agreed that the College's concerns about the other areas of the project licence should be fed back though so they could be taken on board but that would not impact on the secondary availability related to the epilepsy model.

5 **PROJECT LICENCE AMENDMENT:**

A project licence amendment had been received, which was being reviewed by a sub-group of AWERB, however as the project licence holder was at the meeting, there was opportunity to ask questions about the proposed amendment.

It was explained that the overarching problem with gene therapy research and gene therapy treatments in humans in general was that a large proportion of the human population have pre-formed antibodies and immunity to AAV9 (the main viral vector used to administer the therapeutic transgene). Because of that a large proportion (approximately 20-25% of the population) have pre-immunity to AAV9 and so can't be treated with the current clinical trials or existing gene therapy

treatments. The other issue was that when AAV9 was administered with a gene therapy to a human patient or animal in a matter of a few days they would develop a profound humoral immune response (Immunoglobulin M and then Immunoglobulin G) to the virus. That was expected but because of that it was not possible to re-dose. It was known from work the researchers had already done and also from published work, that some patients have better gene transduction than other patients and some muscles got treated better than other muscles. So this was a huge problem in general. There had been speculation of overcoming this humoral immune response by including treatments with specific drugs, but most felt that any of these approaches were highly unlikely to be beneficial, particularly those whose health was already compromised.

A few months ago a group published a paper where they used a drug which was an enzyme which cleaved IgG to enable redosing of AAV despite an underlying immune response. The drug was currently in human trials and had been tested in rodents and non-human primates. If this enzyme was given intravenously to rodents and then non-human primates, that could reduce the circulating IgG by over 99% so that when the AAV9 was re-administered it was possible to get suitable redosing and demonstrable effect, compared to a control group that didn't get this. This was hugely exciting. It had not been applied in gene therapy work for the condition that the researcher was working on before, so this was the potential next step.

The intention was to do a PK-PD study first (prior immunised with empty capsid vector) and incorporate it into the work being done. Different doses would be tested and the pharmacodynamics examined in laboratory studies.

Advice had been sought from another expert at the College. The expert had advised that immunologically speaking, this strategy was valid, in terms of a short-term depletion of circulating neutralising antibodies directed against AAV. Since the treatment was of relatively short duration of action, he did not foresee any adverse effects in the animals kept in a facility with reasonable biosecurity. In theory the enzyme would degrade all IgGs (including vaccine antibodies) leaving animals open to infection in the short term. However, this risk would be pretty much non-existent in these animals.

A question was asked whether there had been any long lasting impact of the drug in any of the human patients that had been treated? Academic papers had indicated that there had been occasionally some side effects (including with placebo treated patients) such as headaches and nausea, but there had been no long lasting effects reported. IgG in people typically rose again after 3 to 4 days but was back to normal after 2 weeks. That meant there was a therapeutic window in the first 7 days of where the AAV could be re-administered. The published evidence indicated that usually one dose should be enough to get the IgG reduced by 95% but currently in human trials two doses were being used, so that would be the regimen that the researcher's team would follow.

A question was asked whether the drugs was immune specific to AAV or all IgG. It was confirmed that it was all IgG. This meant that it could presumably suppress the immune system to some extent so the animals could become vulnerable to infection, however this was something that could be controlled by increasing the biosecurity around the animals.

A question was asked that for the *in vivo* work, would the standard approach of starting low and then building up be followed. It was confirmed that it would. The plan was to start with lowest dose and then escalate to make sure nothing untoward was seen.

The project licence holder was thanked for attending the meeting. AWERB would review the proposed amendment and make a decision.

After the project licence holder had left, a query was raised whether previous concerns about the project licence, including how many times a bitch could be bred from, been resolved or were there still issues? AWERB were advised that this was still work in progress and there were a lot more positive discussions between the researchers and the technicians. A policy was needed on how

many times a dog could be bred from and by what age should they be rehomed. This was not straight forward as both were linked. A canine reproductive specialist had been identified who was willing to give a talk to the technicians but there would be a charge and a decision was needed on who should cover the costs. It was agreed that the specialist should be booked and a decision then made about who would pay for it. This would then enable a more informed decision to be made.

AWERB's consensus was that this was exciting new science that if successful would add value and improve chances of the AAV form of the treatment being successful. It was felt that it was the right approach to identify the right dose of the drug and to then add it into the relevant protocol. It was important though that AWERB had been made aware and understood what the potential adverse effects of the drugs could be as well as the context of how it was going to be used.

6 NVS REPORT

- Ferret: one of the non-implanted ferrets had developed a mass on their tail which was being monitored. The lump would be removed, given several recovery weeks, then the ferret would be re-started on the study.
- There were a number of animals under treatment with various procedures planned including an eye surgery on one of the dogs. One of the techs would also be involved, to provide her with experience with dog anaesthesia. The long-term aim was to involve her more in that side of the work.

7 NACWO REPORTS

7.1 Camden

- Mice study: the second study had now started following trouble shooting after the first study. The 2nd study had gone well. Once data had been obtained from this study then the next study would be started.

- Zebrafish: there had been a small infestation of bugs in the fish facility. Holes were being plugged and pest control have had a general look around. It did not seem to be affecting fish welfare. The project licence holders had also been informed and asked to look out for signs of ill health with the fish. The ultimate aim however was to move the fish facility into a different area. An area was being refurbished and new equipment was being purchased to house the fish. Once the final plan about the proposed move had been put together it would come to AWERB for information.

- Large animals returned to Camden: two calves and two ponies were back in Camden ready for the anatomy teaching. The animal handling had already started and the animal stewardship was due to start the following week. Changes had been made to the teaching arrangements: the groups were very small, with care taken to ensure that the animals were not overhandled.

  It was noted that one of the calves had slipped in the wet resulting in a wound that the anatomy vet had cleaned up. This was healing well.

7.2 Hawkshead

- Pony: one of the ponies had got really stressed and had been hard to catch. It had therefore been decided to bring him into the yard more often with a buddy in order to be handled and have nice things done so that the pony got acclimatised to being in the yard. Training and acclimatising was so important.

8 FEEDBACK FROM ETHICS AND WELFARE COMMITTEE

The Chair reported that she had provided an update to the previous day's Ethics and Welfare Committee meeting about how the BSUs had been coping during the pandemic. The teams were now back to working their usual rotas however they were all on standby to go back to shift work if required. It had been a very stressful experience for the technicians, both with the physical challenges and also mentally. The current situation was also very unsettling with the possibility of a 2nd wave happening and there being no current end in sight of the end of the stress of the pandemic. The Ethics and Welfare Chair would be writing a personal letter of thanks to the teams but in the interim the Committee had asked that their thanks for all their hard work and dedication be passed onto the teams. AWERB asked that their thanks also be given.

9 MINUTES OF PREVIOUS MEETINGS

The minutes of the meeting held on 15 September 2020 were confirmed as an accurate record.

10 UPDATE ON ACTIONS

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

This item was on hold until lockdown restrictions had eased and the technicians had more time to devote to this.

10.2 Item 5.2: BSU Virtual Tour (May 2020 meeting)

This was on hold due to the pandemic.

11 END OF PROJECT LICENCE REPORT

AWERB noted that an end of project licence report had been submitted.

The project licence holder had commented that they felt the ARRIVE guidelines were not applicable to GWAS type studies. One of the AWERB members suggested that it might be worth asking in future what reporting guidelines people have been using to make sure everything was clear and appropriate (and referenced using https://www.equator-network.org/).

12 PROJECT LICENCES AMENDED BY THE HOME OFFICE

AWERB noted that three project licences had been amended by the Home Office since the previous meeting.

13 ANY OTHER BUSINESS:

13.1 Stake Holder Survey

A request had been received to contribute to a stakeholder survey on the "Evidence of Sentience in Cephalopod Molluscs and Decapod Crustaceans". The survey would be reviewed in more depth to see if there was an appropriate person to respond to this survey.

13.2 DigiGait

AWERB's views on whether DigiGate should be classed as a regulated procedure was sought. A video of the proposed treadmill would be circulated so that AWERB could make a decision.

13.3 **Date of next meeting:**

This was scheduled for 11 November 2020 at 10am.

Secretary, 27 October 2020