Royal Veterinary College
University of London

Summary Minutes: AWERB

Status: Chair approved

Meeting held: 27 July 2021 at 2pm by MS Teams

Present:
Attendees: 9, plus 1 in attendance, 7 by invitation and 6 apologies.

1 **NEW PROJECT LICENCE APPLICATION REVIEW FOR SECONDARY AVAILABILITY AT THE RVC**

The project licence holder applicant was welcomed to the meeting who explained that this project licence application was a reiteration of a number of project licences that had been held previously. This new licence had not changed much as the work was remaining mainly the same. The work involved the evaluation of injections and provided biological reagents that were critical to:

a) The WHO-led decision process for influenza vaccine composition
b) Global influenza vaccine production
c) Quality control testing of influenza vaccines for the global markets.

Two protocols would be carried out at the RVC.

- Red blood cells would be taken from turkeys as these were required for haemagglutination inhibition assays to measure the titre of sera against different viruses. To use alternatives would result in less reliable data and potentially greater volumes of blood from other species.
- Generation of sheep anti-influenza serum reagents for viruses proposed for inclusion in seasonal flu vaccines.

The following comments were raised by AWERB:

- The reference to ARRIVE guidance in relation to experimental design on page 13 of the licence made the licence look dated and it was easy to tell that this had been copied across from a previous licence. It was suggested that reference should be made to NC3Rs experimental guidance.
- The original project licence holder had left about 2 years previously. What experience and background did the applicant have? The applicant was the head of the department that had overall responsibility for influenza; and a virologist with years of experience who was ably supported by the influenza scientists in the team who had inputted into the new project licence to make sure that it met requirements and that it took into account updates that had happened in the influenza area. A new influenza lead had recently been appointed that would take over being the project licence holder once they had received the appropriate training.
- 3Rs: Had there been any developments in this area since the previous project licence was granted that meant it would now be possible to use alternatives to animals? Unfortunately, the prevailing approach to global influenza vaccine manufacture was egg-based. This meant that it was not currently possible to alter the nature and volume of specific reagents required for the successful production and evaluation of candidate vaccine viruses. It was possible that this might change in the future as there was a move towards getting viruses without the full allowance of eggs using reverse genetics. Although there were synthetic systems available as an alternative to

turkey blood, these had not been proven to be as reliable as the haemoglobin assay with fresh blood. Fresh was best as it provided the most reproducible and reliable assays rather than frozen cells or those that had to be transported long distances.

- A query was raised about why the maximum number of uses per bird for one of the protocols was six. If the animals were well trained and not stressed by the sampling procedure it might be better to be able to collect more samples from the animals and use fewer of them? It was explained that these numbers were based on experience. A colony of 10 birds was maintained. Some of the birds would become too unhealthy to be used as they grew too big and had risks of health or leg issues. It had been worked out that 10 was needed to deliver the two samples that were required each week to maintain them throughout the year. No bird was used more than once every 21 days and typically it was at an interval of 42 days. The birds were used on a cyclical basis. In theory one bird could be sampled every 28 days, but because there were 10 of them, they were cycled through. Initially only one or two birds would be used, their health examined and the NACWO would decide if they could be used again.

  Were there any issues experienced from the blood sampling? Sometimes there were problems with the veins and haematoma might occur so either another vein would be used or another bird.

  It was decided that the maximum number of uses should remain the same. After the procedures the birds would be assessed to see whether it was viable to take any more samples from them. If this seemed to be the case, then a project licence amendment could be submitted in the future to increase the number of blood samples taken from one bird so reducing the total number of birds used with no welfare impact. It was something to monitor and refine during the lifetime of the licence.

- The licence mentioned that those birds that did not have their normal health and wellbeing resolved within 24 hours, would be euthanased. It was pointed out that if there was haematoma then bruising would continue a lot longer than that. This section therefore needed to be worded differently to avoid a bird being culled because it still had bruising. After discussion it was agreed that the wording should be amended to say “if it looked like there was no improvement between 48 to 72 hours then NVS advice would be sought”.

- A query was raised about the cumulative stress of repeated blood sampling and injections to the animals. Although these were standard procedures, which the animals could get used to, they could also be a cause of stress to the animals. There was not much detail provided about the extent of restraint that was used. What thought had been given to this and making the situation a more positive one for the animals so they were less likely to get stressed?

  It was explained that when it was known that the animals would have to be handled a lot, time would be spent trying to acclimatise them to being handled. With sheep though, experience had shown that they did not adapt to being trained and could find the process more stressful than the actual blood sampling process. For the birds, if they were handled the right way and by the same people, then they generally remained calm. If an animal was noticed to freak out or showed a clear aversion to the procedure being carried out, then it would not be used again, unless it absolutely had to be.

The project licence applicant was thanked for taking the time to attend AWERB. The project licence would be revised taking into account the comments made and then an updated version of the licence would be circulated for one final check.

2 MINUTES OF PREVIOUS MEETING

The minutes of the meeting held on 14th July 2021 were confirmed as an accurate record.

3 NEW PROJECT LICENCE APPLICATION REVIEW

The project licence holder applicant was welcomed to the meeting. It was explained that the previous licence had expired in 2020, but because of COVID, the submission of the replacement project licence application had been delayed.

The overarching goal of the research program was to improve fertility and reproductive health in mares through a better understanding of reproductive events such as conception and early pregnancy. Early pregnancy loss (miscarriage) was the greatest contributor to the low reproductive outcome of mares but there was very little known about why it happened. This meant that diagnosis of the underlying cause was only possible in approximately 40% of cases making clinical management challenging.

The project licence had a two-pronged approach:

- Understanding the normal process of pregnancy in the mares, as very little was known about normal pregnancy physiology in the horse. This was needed in order to understand the key mechanisms and then how they become dysfunctional in pregnancy loss.
- Studying clinical cases of pregnancy loss alongside normal embryos as required.

There would be 3 aspects looked at:

- The immune response in early pregnancy – understanding the immune cells and the role they played.
- Understanding genetic variants
- Understanding how the placenta forms.

The following AWERB comments were raised:

- There was a query about the blood sampling and giving an indication of how many in vitro experiments could be performed using a single blood sample from a mare, as utilising the blood sample would provide more data, so resulting in fewer animals being used. The project licence holder advised that they did do multiple assays, and they designed these to give as much information from a single venepuncture. This information would be added to the project licence as it was a good 3Rs aspect.
- AWERB discussed whether it was better to have separate protocols or one large one. Currently the licence was set out with separate protocols, however a horse could only progress to the final protocol if they went through each one. There was uncertainty about which approach the Home Office would prefer as there were pros and cons to both approaches. After discussion it was decided that the project licence should remain as it was, but that consideration be given on how to turn the project licence into one long protocol if the Home Office came back to say that was their preference.
- A query was raised about why both ponies and horses required? The intention was to mainly use ponies, however horses were needed as some of the work in relation to genetic variants were more suited to thoroughbreds.
- Was there any difference in pregnancy loss between ponies and horses? There were lots of studies in thoroughbreds but very little had been done in ponies.
- Both the scientific background and the NTS needed to be simplified to make them easier to follow and to understand the bigger picture.
- There was a discussion about severity categories. It was explained that this should be set at what the horse was expected to experience, rather than the most extreme side effect that might happen.

- It was noted that the option of "continued use on other projects" had been included under the section about the fate of the animals. Were there specific projects in mind for the animals to be transferred to? The project licence holder explained that had been included in case there was another project licence that the animal could be transfer too. It was thought though that an animal could only be transferred if there was already another project lined up for the animal to be transferred to, so that authority could be given, otherwise it was just release from the Act. It was agreed that this option would be removed for now, but that it should be revisited if it turned out that an animal would be suitable for another project licence.
- The project licence holder asked for advice on the humane end points. It was explained that these needed to be included when they were directly associated with the regulated procedure Incidental ones should not be included as it was not possible, or practical, to try and account for everything that might happen under the protocol.

The project licence holder was thanked for attending. The project licence would be amended and resubmitted for a final review.

4 MATTERS ARISING

4.1 Item 8: Squeezing of zebrafish (14 July 2021 meeting)
An advice note about when squeezing of zebrafish was appropriate and when it was not was being put together.

4.2 Item 11: Two end of project licence reports (14 July 2021 meeting)
One had now been received. The other one should be completed soon.

4.3 Item 3.2: Rat playpens for improved welfare (9 June 2021 meeting)
Date had been scheduled to discuss the potential options with regards to playpens that could be used for the teaching animals.

4.4 Item 5: ARRIVE compliance report (9 June 2021 meeting)
The survey had been drafted and would be circulated shortly. The aim of the survey was to see if there had been any specific barriers that had prevented ARRIVE guidelines being followed.

4.5 Item 5.4: Camden stables (27 April 2021 meeting)
Estates were looking into what floor covers could be used on top of concrete, so the animals had something soft to stand on.

5 PROJECT LICENCE AMENDMENT
The project licence holder and several colleagues were welcomed to the meeting. It was explained that they were wanting to make some changes to their project licence. These changes included adding a suprachoroidal route of administration as well as updating the range of volumes that could be administered intraocularly (subretinal, intravitreal, suprachoroidal) in rodents. There was also a request for a new protocol involving performing laser-induced lesion generation.

The following queries were raised:

- How much experience did they have with these techniques? It was explained that they were working with a CRO who had established the administration in rats and were willing to share the protocols. They were also in contact with an international academic group who was familiar with the technique. They were also working with their inhouse device team to get an improved device for administration.

  The initial plan was to carry out the technique in cadavers first, by injecting a tracer. There were publications that showed how a tracer would distribute in the back of an eye after a

successful administration, which would then be used as a benchmark to see how successful the administrations were.

- How would the staff be trained to do the administration? The training would be done in cadavers. They would follow the protocol that had been shared with them and then share the findings of the tracer with the CRO as well as doing comparisons with what had been published. That would indicate if the method was working or not. Once they had access to the device, the plan was to get someone over to provide training on the device. The device was a fixed length needle, which should make it much easier to administer. Because it was a fixed depth needle, it should be a relatively easy technique to develop internally and translate quite quickly from development stage.

- It was noted that the intention was to carry out the procedure both in rats and mice. The literature seemed very well characterised in the rats but there was very little about mice? Was the intention to use mice immediately? It was confirmed that the initial plan was to use rats. Depending on how the work progressed, mice would then be included.

- Was it a new injection route that was planned? Or was it a technique that was already established with collaborators that they were looking to transfer across to the RVC? It was confirmed that they would be transferring what had been tested and validated in rats by their CRO and also their collaborators. They would only use mice once they with comfortable with the device.

- If the technique had not been used in mice previously then background information about the new model development would need to be included in the project licence as it would have welfare implications. Would a pilot study be set up? AWERB were advised that it was not a completely novel system as the technique had been done in mice, but there were only a couple of published abstracts available. It was also not a technique that their collaborators had done on mice. AWERB were of the view that it should be classified as a new model development, so any adverse effects and how the technique would be monitored needed to be added to the licence, in order to reassure the Home Office about how the technique was being approach and that proper process and plans were in place.

- For the new devices that were being developed would there be any inhouse validation? That would need to be added as an objective to the licence that developing a new model and new technique and new delivery method.

- Would there be any changes to the animal numbers considering a pilot study would be done before starting a bigger study? It was not thought that these changes would have an impact on animal numbers as less animal studies had been undertaken than originally anticipated, but they would review once the project had started.

- After discussion it was agreed that as the initial focus of the work would be on rats, that reference to mice should be removed for now. If it was decided to include mice in the future then a further amendment would be submitted. This would provide opportunity to discuss and come up with a clear plan on how to introduce this model into the project licence if it was decided that it was needed.

New protocol:

This would involve performing laser-induced lesion generation. The aim was to characterise efficacy of therapeutic vector candidate in animal models of Age-related Macular Degeneration (AMD). It was a translatable model of the disease.

- One of the scientists had used this model before on both mice and rats as part of her PhD. She had also received training on the new set up, which involved image guided assistance. It was a well established model that was seen as a gold standard model for the disease.

- There were well established parameters on the numbers of laser lesions to generate at the back of the eye, the power of the lasers and the size.

AWERB's consensus was that they were happy with the new protocol and that it fitted in with the overall aims of the project licence. The model just needed to be established in house.

The project licence holder and colleagues were thanked for attending the meeting. They would amend the project licence and then submit back to AWERB for a final review.

Summary of main points after PPL Holder and colleagues had left:

- Although they did not have much experience in the new novel laser technique, they were taking a very pragmatic approach about how to proceed with this work by doing it in a careful, well-planned way by working with a CRO and academic collaborators. They would also be carrying out initial pilot projects. It was being used in clinics, but there was very little published work out there in rodents and had not been replicated by very many labs at all, so there would not be many people sufficiently skilled who could come in and teach them, particularly in the current environment. They did need to be clear about how they were doing it, how to proceed on the various steps and this would be small pilot projects on just one or two animals and then repeated on some more animals.

- A query was raised about the anaesthetic plan. It was being done under general anaesthesia so not much detail would be expected, however there could be different approaches. For example one lab could use an anaesthetic that just immobilised an animal but another might use an excellent combination of anaesthetic agents. It was explained that the sub-retinal route involved putting a needle right through the eye and underneath the retina before doing the injection which would cause a retinal detachment, but usually the retina would reattach. This new approach would avoid that problem, as it did not involve putting a needle through the eye. It was therefore actually a refinement if they could get it to work. Doing it in cadavers to show they could get it into the area they needed it to, was a good approach. There was uncertainty though about how reproducible it was: how easy was it to deliver the same quantity, in the same place, in exactly the same manner.

6 MBR ACRES: "CAMP BEAGLE"

AWERB noted the recent protests that were taking place at MBR Acres. There had been some hidden footage of dogs being loaded into crates which had caused an uproar. There had been claims made that the dogs had been scruffed (which was not acceptable as it could cause injury) and were screaming but it was also possible that they were displaying the normal enthusiastic behaviour of beagles and rather than being scruffed, most of the handling was undertaken with hands round the thorax under the forelimbs

The Sunday Telegraph had reported that the Home Office Secretary would be ordering a full review aimed at finding ways to end the use of animals in the development of medicines, but this review had not been confirmed.

7 DATE OF NEXT MEETING

This was scheduled for 8th September at 10am and was the standard agenda items meeting.

8 THANK YOU

As this was the last meeting before the summer break, the Chair thanked AWERB for all their hard work and the time spent on reviewing project licences and contributing to meetings.

Secretary, 5 August 2021