Royal Veterinary College
University of London

Summary Minutes: AWERB PPL review meeting

Status: FINAL

Meeting held: 05 April 2023 at 10am via MS Teams and F2, Hawkshead

Present:
13 plus 1 in attendance, 7 by invitation and 12 apologies.

1 WELCOME

An observer was welcomed to the meeting. She had applied to the recent call for new members to join AWERB who had an interest in Animal Welfare Science and Ethics and was attending to get a feel for these meetings.

2 AMENDMENT TO PROJECT LICENCE

The project licence holder (PPLH) and two colleagues were welcomed to the meeting. The PPLH explained that he was looking to make three changes to his project licence to accommodate new areas of respiratory research: Respiratory Syncytial Virus (RSV) and Influenza A and B viruses (IAV) related respiratory diseases such as Influenza and Viral Bronchiolitis.

The changes were:

- The addition of the delivery of viral vectors to the lungs as a method of doing gene expression: this would enable them to use that as either a treatment or as a challenge to develop a specific phenotype.
- To increase the intranasal dose volume for mice from 50µl to 100µl. As Bronchiolitis is a lower respiratory tract infection, a larger dose volume is required to simulate this in mouse models to ensure that the challenge and/or treatment reached the lower tract
- The addition of a new protocol to cover the RSV and IAV work. This would aid the management of the project as it set out the permitted dosing levels and weight loss which was different to the other protocols. It should also aid researchers and technicians to know what adverse effects to look for.

One of the researchers in attendance had prior experience of using an increased intranasal dose volume model, which she summarised for AWERB, including the monitoring that had been done. A key thing to note was that the mice tolerated the increased dose volume and recovery from anaesthesia was quick with no lasting effects.

The following queries/comments were raised by AWERB:

- **Were there any body weight limits for the mice as using 100µl on a 20gm mouse was substantially different in terms of percentage body weight to using a 30gm mouse?**

  The plan was to use 8 to 10 weeks old mice so should weigh just under 30gms. A sentence would be added to the project licence specifying that dosage would be relative to body mass.

- **Instead of dosing a larger volume intranasal to affect the Lower Respiratory Tract (LRT), could intratracheal (IT) or inhalation routes be used, as was done in large animals to achieve a better LRT response when doing Regulatory T Cells?**
  With inhalation it was very hard to control how much each mouse inhaled, so it was not a preferred option for delivering either treatment or infectious material to the lungs because a lot of it ended up on the fur. For some therapies an intratracheal route could be used, however for this virus, as they were modelling how the virus could normally be caught, they needed to include the upper airways, which was not possible through the IT route.

- **Valid justifications for why some experiments would only use animals of a single sex had been given. However, one of the reasons given for not using males was because of their aggression. AWERB recommended that the researchers checked out NC3Rs guidance on minimising aggression in group-housed male mice.**

  In practice was the plan to use both sexes in the experiments or to generally just use female mice? If the latter, consideration should be given to using both sexes as in humans the diseases affected both male and females, so this could help to understand if there were any differences between the sexes.

  Currently just female mice have been used because of the aggression issues. There were no immediate plans to trial using both sexes.

  AWERB pointed out that by designing the experiments to use both sexes, the results might prove to be more interesting to the scientific community, be more reproducible and be more translatable. By only using female mice, it was possible that interesting findings were being missed. Although it was recognised that it was difficult to move away from what has traditionally been done it was something the researchers should consider.

- **In pigs there was quite a big difference in immunology responses between females and males, so only females were used. Did the same apply to this work? Was it important to use both sexes so that could intentionally see the difference or was it more important to use just the one sex for a better relative comparison?**

  For these types of studies, historically females have been used because of issues in relation to housing and to also not skew data. Also most published studies used female mice, presumably to avoid the problems of fighting mice.

  However for infections in knockout lines where they wanted to infect pups and use every animal, then both sexes were used. No differences had been seen though which indicated there was not a strong difference in male and female inbred mouse lines.

- It was noted that in theory the mice could receive a 100µl dose each day for up to 28 days. The licence (including the animal experience section) would be amended to stipulate that only four of those doses could be for 100µl.

The PPLH and his colleagues were thanked for attending the meeting. Once the requested amendments had been made to the project licence it would be circulated for a final review.

After the PPLH and his colleagues had left the meeting, AWERB highlighted the following:

- The project licence needed to specify that dosage would be relative to body mass

- That the researchers need to seriously consider using both sexes for the robustness of this research and to enable the research to have a better translation in a wider context.
- The researchers had justified why they needed the 100µl but this did need to be monitored carefully.
- This was a very well organised group that had good communication lines with the technicians and responded quickly to any issues.

3 NEW PROJECT LICENCE APPLICATION

The project licence holder and two colleagues were welcomed to the meeting. One of the AWERB members declared a conflict of interest as she would be a personal licence holder on the project and had provided significant input into the writing of the project licence.

A brief background to the project was provided. The researchers worked on next generation surgical robots, with which just over 10,000 medical procedures had been completed. Their mission was to transform surgery and to make minimal access surgery available to all that needed it.

The aim of this project was to develop more robotically assisted surgical medical devices, with data collected from device testing being used to inform product development decisions and then used towards regulatory submissions. Having more surgeons able to operate through keyhole surgery would have benefits for patients including less pain, scarring, blood loss and time spent in hospital. Robotics should also make the surgeons more proficient, faster, and more able to do quality surgery through the ergonomics of the actual device. Although robotic surgery was over 20 years old, there were a lot of patients that were not able to access keyhole surgery as existing robotic platforms were far too big, so reducing their utilisation and resulting in higher costs for hospitals.

There were four main objectives to the PPL:

- To assess the safety and performance of proof of the concept devices.
- To evaluate the safety and performance of the instruments
- To evaluate the safety and performance of imaging systems
- To carry out regulatory testing to meet regulatory standards ranging from Medical Device Regulation (MDR) to Food and Drug Administration (FDA).

The following questions were raised by AWERB:

- **Was there a big difference in carrying out the technique directly versus using a robot to manipulate the instruments.**
  Any surgeon that uses the robot had to go through a validated, standardised training process for the robot itself (currently consisting of a 3 day training programme). It was important that surgeons knew how to use the system, as well as having the relevant surgical skills to do the operation.

- **How many surgeons would be involved in this work?**
  There would be three surgeons involved. They were fully trained on using the robot as well as involved in full time clinical practice (so carrying out operations on a day-to-day basis).

- **The licence mentioned that regulatory guidelines required that pigs be kept for up to 28 days, but that animals could be kept for up to a maximum of 35 days. Why the extra days?**
  Up to 35 days had been included, for although the majority of the work would be under the regulatory study, not all of it would be. It was also to cover practical issues such as days falling on weekends and bank holidays and also staff sickness. If it was not possible to

run a study on a particular day, it could be done the following day without encountering a technical infringement.

- **In order to evaluate the robotics equipment, procedures would be carried out to remove organs. Could there be any adverse effects from removing these organs that needed to be considered?**
  For the cholecystectomy and the removal of the gall bladder, there should not be any long term issues. Hysterectomy had obvious adverse impacts on fertility. There had also been complications in humans with hysterectomies that have been carried out laparoscopically and robotically. From a safety point of view, it was therefore important to prove that hysterectomies could be carried out safely using the system.

- **There have been reports in other studies involving laparoscopies, that there was a chance of bloating and cramps. Had that been noticed in pigs?**
  For those animals that have received a robotic laparoscopy, the vets looking after the recovery animals, have noticed that the pigs seemed to be in less pain and were up and about faster than those pigs that have had a standard laparoscopy. It was not known for sure why that was, but it could be due to being less trauma to the area.

- **The licence mentioned that animals would be housed to minimise wound interference from other animals. Did that mean they would be singly housed?**
  All pigs have to be singly housed following a procedure to prevent injuries inflicted by cage mates and increased aggression. Studies have proved that pigs tolerate being singly housed so long as they can see, hear and smell other pigs in the same room.

  The licence mentioned that there was a possibility of postoperative internal bleeding. How would this be detected?
  For immediate bleeding, this would be fairly obvious as it would be seen during the surgery and the animal would be euthanised immediately, unless it was possible to gain control of it straight away.

  For early bleeding, as the arteries were very small and the pigs would have healthy blood vessels, the likelihood of this happening was extremely unlikely. It had to be included though as haemorrhages were an issue with humans. If there was going to be a haemorrhage it was likely to be immediate, especially with what was being tested.

  It was also extremely unlikely to get a late bleed from advanced energy devices, however one of the reasons they were doing the study was to check this.

  In terms of detecting internal bleeds in the post operative period, pigs showed if they were in discomfort by becoming quiet and also their skin colour changes. A veterinary post operative monitoring form was used to record heart rate that was taken initially every two hours and then decreased to twice a day. Capillary refill times were also taken. If there were any changes then the pig would have an abdominal ultrasound scan and any fluid or changes reported to the surgeons. A short description of how internal bleeding would be identified would be added to the project licence.

  The licence mentioned the possibility of thermal bowel injuries caused by electrosurgical instruments. How would an injury in a pig manifest? Could this happen in humans too?
  Yes these injuries could happen in humans and was one of the biggest problems with the energy devices. This was one of the main reasons they were carrying out recovery

surgeries as they needed to demonstrate to the regulators that the energy was being focused where it was required with no inadvertent energy transmission to other organs.

If an injury did happen, the pigs would develop mild peritonitis and show some signs of inflammation. Their heart rate would also go up and temperature might change. These signs would all be picked up through the clinical monitoring. This information would be included in the adverse effects.

- **The licence mentioned that in relation to randomising test items, this would be done by selecting the devices in no given order, which was not actually randomisation. It was important to have a proper randomisation strategy in place.**
  The PPL would be amended to include reference to using an appropriate random generator tool.

The PPLH and colleagues were thanked for attending the meeting. They were asked to make the requested changes to the project licence and then resubmit for a final review.

The overall consensus from AWERB was that this was a well written project licence.

4 AMENDMENT TO PROJECT LICENCE

An amendment to a project licence had been received. The project licence holder (PPLH) was welcomed to the meeting. The PPLH explained that he had recently read a study that involved the researchers anaesthetising an animal before injecting therapeutics and cells straight into the kidney without surgery. Currently an echo guided injection was used which was very expensive, took a long time and required specific training.

The PPLH therefore wanted to test this technique, for if it worked it would enable them to get a treatment directly into the kidney. The proposal was to test the technique in wild type animals and once validated, carry it out in experimental animals. The technique had been tested on cadavers and did seem to work.

The PPLH also wanted to change the frequency which the mice were weighed after tail vein injections from 3 days to 7 days. This request was because it had been noted that the animals got stressed from the frequent weighing.

The following queries were raised:

- **Were there any benefits welfare wise from the new technique?**
  If the technique worked, it would be a quicker and safer way of doing it.

- **Would the technique be carried out in one kidney or both?**
  The NVS had advised to initially just test in one kidney in case there were any adverse effects. It was also important that the technique was carried out on animals that did not have any underlying conditions.

- **Could there be any adverse effects from the new technique?**
  The paper did not mention any adverse effects, but the animals would be monitored very carefully.

- **Have ultrasound guided kidney injections already been done?**
  Yes, this had been done elsewhere. It was a very slow technique though and expensive as specialist equipment was needed to do the echo guiding.

- **The paper mentioned a very patchy distribution of treatment. Would that be an issue? Also, although there were no close up images, it was likely there would be a lot of inflammation as a result of the damage done by the needle. Would these be looked at, as they were both potentially adverse effects?**
  Yes, the plan was to try the technique and then do a full histology. They had a targeted treatment so it was hoped that once it was in the kidney it would target the place they wanted it to go. They would be using GFP, as well as the histology. If the technique resulted in too much trauma then it could not be used. This information would be added to the project licence so that it was clear a full examination was intended.

- **Was there justification to reduce body weighing from 3 days to 7 days?**
  This would only apply to those animals where tail vein injecting was done. Their experience showed that there was not generally great variation in body weight and the weighing caused the animals stress. Animals that had surgery, would be weighed daily for the first 3 days after the procedure. **A statement would be added to the PPL to explain that this amendment request was based on the researchers knowledge of the model and animals.**

The PPLH was thanked for attending the meeting. He would make the requested adjustments and then the licence would be circulated for a final review.

AWERB confirmed they were supportive of the proposed amendment but that it needed to be made clear that as this was a very new technique it would be a pilot study that would be very carefully monitored and only a small number of animals would be used to see if the technique worked.

5 MINUTES

The minutes of the AWERB meeting held on 8 March 2023 were confirmed as an accurate record.

6 DATE OF NEXT MEETING

This was scheduled for 26th April 2023. It was a standard agenda items meeting.

Secretary
9 May 2023