
Minutes: AWERB minutes: Standing Agenda items meeting

Status: Final

Meeting held: Tuesday 21 June 2022 at 11am via MS Teams

Present:

16 plus 1 in attendance, 3 by invitation and 9 apologies

1 **PROJECT LICENCE AMENDMENT DISCUSSION**

A project licence amendment request had been received. The PPL Holder explained that they were wanting to submit a new protocol for this project licence to enable the researchers to assess the metabolomic responses in circulating blood to a single acute exercise testing session in some dogs (from any breed) using a cross-sectional design and comparing them to those in saliva. For this proof of concept study they were hypothesising that the metabolomic responses in circulating blood to a single acute exercise testing session would be reflected in the saliva. The data from this pilot study would inform the design of larger studies that would be aimed at answering how did changes in metabolomic signatures differ by breed in response to acute exercise; and did trade-offs between physiological functions become more extreme for dogs engaging in chronic high-endurance exercise compared to those seen for acute exercise.

The selected dogs for the project would need to be considered generally healthy by their owners; not be on medication and be able to exercise without difficulty. These dogs would be assessed by a veterinary surgeon to make sure they had no cardiovascular issues or any observed lameness. Before undertaking the exercise they would need to have been fasted and rested for at least 12 hours.

Immediately after the exercise, 3 ml of blood would be collected from a superficial vein and 1 to 1.5 ml of saliva. This would be followed by a clinical examination by a vet, before the dog was then signed off.

The following queries were raised by AWERB:

- **How many times would an attempt to take blood be made, if not successful on the first attempt?**
No more than 3 times. **Action: Project licence to be amended to indicate up to how many attempts to collect a blood sample would be done. It was recommended that it should be a maximum of two attempts.**
- **Would that be from the same vein?**
Current thinking was to prepare the left and right jugular veins, so if the first vein did not work, then the other one could be tried.
- **Sometimes after a blood sample there could be a slight dermatitis or clipper rash. Would anything be done to prevent that or to monitor for this afterwards?**

Although it was not a common occurrence, the protocol had been amended to state that the area would be cleaned and topical creams applied if required. Advice on monitoring would be given to the owners.

- **Would blood samples be taken both before and after exercise; likewise with the saliva samples?**

Originally the plan was for samples to be taken before and after exercise, however it was decided that this would make it more intensive for the dog owners, as well as overall cost and time intensive. It had therefore been decided that instead a cross sectional design would be done with a saliva sample taken before exercise, right after exercise and a hour after exercise (to measure recovery) to see if there was a correlation. Although the blood sample was the “gold” standard, the saliva was a good biofluid to use for exercise metabolism. **Action: Licence to be amended to make this clear.**
- **If blood samples or saliva samples were not taken before the exercise, although the inclusion criteria indicated that only those animals in a general good health would be used, could there be anything happening in the dog that was not observable, that could affect the blood and saliva?**

Yes, there were a lot of factors that might affect the metabolism, but it was impossible to check these without taking a full blood sample. Instead, a general health check would be done to make sure there was nothing untoward going on and that the dog did not have any specific disease as well as making sure that the protocol was safe for them. So, for example if a dog was in stage 3 of heart disease, then they should not be running around after a ball in case something happened to them.
- **It was not generally standard to use EMLA cream when taking blood samples from dogs. It was only usually used if it was known that a dog was nervous of needles. Why therefore was it proposed to use the cream as standard? If a dog did require EMLA cream then maybe it was not a suitable animal to have on this study?**

Using EMLA cream would enhance the welfare of the dogs that were having the procedure. There had been a previous study done elsewhere which showed that dogs that had received EMLA cream had a better reaction to intravenous catheters being placed. Reassurance was given that if a dog showed signs of being stressed by having a blood sample taken despite having been given EMLA cream, then they would not be used for the study.
- **How would the dogs be recruited for the study?**

This would be done in several ways:

 - Staff asked whether they have healthy dogs that could be used for the study
 - Social media call
- It was pointed out that an additional question needed to be added to the eligibility check to make sure that the animals used had not previously been on another Home Office project licence study (particularly with using staff dogs as they might have been volunteered for other studies too) as there was no reuse of animals allowed on the project licence.
- **The licence stated that the dogs would be fasted for 12 hours before taking part in the study. Why was that and did it have to be 12 hours?**

Previous studies on humans had found that fasting for less than 4 hours resulted in a very different metabolomic response than fasting for 13 hours or more (the effect of fasting duration in dogs had not yet been examined). Based on this it had been decided that participants in the study should have been fasted for at least 12 hours prior to sample collection for metabolomics. This was to ensure that the levels were as close to 0 as possible.
- **What was expected to change after 12 to 13 hours in the blood sample?**

Many things would have changed: different proteins and different lipid profiles, which were important from an exercise metabolism point of view. The aim was to make the fasting as consistent as possible.

- **Could the fasting be done for just up to 4 hours so that the dogs did not have to fast so long?**
The problem was that no standardised diet was being given, so it would be difficult to work out whether any effects were actually due to the food that had been eaten. Most dogs received their final meal between 5pm and 7pm, so if the exercise was undertaken at 9am the next day and the dogs fed immediately afterwards, this would probably fit in with when they would eat anyway. **Action: it was recommended that the licence be amended to indicate that the fasting would be done overnight to make it clear that it would not have too much of a physiological loss on these dogs.**

- **The aim of the study seemed to be whether it was possible to use saliva rather than blood to pick up the metabolomic changes? If that was the case, did it matter whether the dogs have been fasted as it was blood and saliva that was being compared, so it should not matter what had happened to the dog beforehand?**

Although this was correct, as the metabolites pass differently in the blood versus saliva after eating, this might mean there would be some metabolites that were higher in blood compared to saliva, as they diffused a different way. The aim was to remove this variable as otherwise if there was a difference it would not be known whether it was because saliva was not a good biofluid or because the dog had eaten a lot of carbs or fats. It was the act of metabolism (breaking the food down after eating) that took a lot of time, which then went through the blood and saliva. The effect of exercise would not supersede the effect of eating. Metabolism was affected by the time of day, so all the studies needed to be at the same time every day in the morning, in the same season, so there was no effect on seasonality or circadian rhythm. Checks would also need to be done to see if there were dogs that were entire as it might not be possible to include those.

If the aim had been to do the study with no confounders, then these would not have been included. However for this study, the same dog was its own control. As the dogs were on different diets and so would metabolise the food differently, then they needed to be fasted, to make the factors consistent between the dogs.

- **It was argued though that if the saliva did not reflect what was in the blood, then it did not matter what had happened to the dog before that. It seemed that the question that was being considered was whether a saliva sample could be taken in place of a blood sample to measure what was happening in the blood. If that was the case, it would not matter whether exercise was done or not if the saliva did not reflect what was in the blood.**

Saliva however was a very good biofluid for a lot of things such as drug studies, oral microbiomes and for seeing if a dietary supplement made any difference in how much calculus was built this. This however had not been investigated in dogs, specifically in relation to exercise. If the comparison between blood and saliva was not looked at as purely as possible then it would not be possible to say if it was good for exercise or not. In human studies, such as for endurance exercise events where a lot of samples were taken, then saliva samples were used rather than blood samples. However as dogs were a different species, it was not known whether this would be the same.

- **When recruiting the dogs for the study, what steps would be taken to ensure that the majority of the samples were not from the same breed, or from breeds that were closely related to each other, such as cockapoos as that might result in artificial genetic clustering?**

The aim was to get a representation of different ages, sex and different breeds. There was no intention to exclude specific breeds (unless for health-related reasons). However, it was recognised that this might require a larger study to confirm that it worked across all breeds, sexes and ages etc.

- **In the exercise section it mentioned that dogs would be allocated to different levels of exercise. What was meant by that, as there was only one tennis ball throwing being done?**
This related to the number of times that the ball was thrown and how long the exercise went on for. Some breeds were more inclined to run after the ball for a longer time period than others.
- **Would the type of dogs chosen be biased towards the breed and their likelihood to chase the ball for longer?**
The aim was to have a variety of breeds and not just have those dogs that were more inclined to run after the ball for a longer time.

The PPL Holder and colleague were thanked for attending the meeting. A summary of the comments made so far on the licence would be provided to them after the meeting so they could amend the protocol accordingly and it be circulated for a final review.

After the PPL Holder had left the meeting, AWERB discussed further the concerns that had been raised about fasting the dogs and whether fasting was needed, and also the design concept from a scientific perspective. If the correlation was likely to be so sensitive that in an ideal world the dogs would have been on the same diet and ate at the same time, in order to have a correlation, then that was a concern. It was also pointed out that if the intention was to have the same time period between having eaten and doing the exercise, then this was defeated by having the dogs doing different amounts of exercises as well as using different breeds and age of dogs.

A quick pubmed search on human studies that looked at metabolomics was done which AWERB noted indicated that fasting was needed, as it seemed that unstimulated pre breakfast samples were more correlated with blood than unstarved samples. An overnight 12-hour starvation period for the dogs, particularly if it was done overnight, should not be too stressful, as dogs would not normally eat during the night and a single blood sample should be fine. The study should probably therefore be allowed, so long as those results were presented before they went on from there.

Some AWERB members felt that the question that really needed to be asked was whether this technique could be used as a replacement for undertaking blood sampling as it would be less invasive, rather than whether exercise affected what was being measured in the blood. However, other AWERB members felt that the exercise element was a crucial part of the study as the question they were asking was whether blood and saliva were mirrored when exercise was undertaken.

It was agreed that the additional points that had been made would be put to the researchers for them to respond to.

2 **NEW PROJECT LICENCE FOR DMD DOG COLONY**

AWERB reviewed the draft project licence for the DMD dog colony. The licence was a much simplified version of the previous licence.

The following points were noted:

- The Natural History study was now pretty much complete. The publications that were coming out of that had looked at sample size requirements for particular assays. There were some assays that appeared to be very robust in terms of differentiating between the dystrophic and non-dystrophic and if they got an effect size of for example 25% shift towards the non-dystrophic state, then would end up with very small n values for some of the assays. A lot of the future plans and the current clinical trial were based on these Natural History studies comparing the dystrophic and the non-dystrophic dogs. **Action: It was suggested that this explanation should be added to the licence as it also provided a justification for the numbers.**
- Several protocols had been removed:
 - The Natural History protocol had been removed completely;

- A previously added protocol to allow spaying of dogs when on oestrus had also been removed as it had not been used and would be an easy one to re-add if it was required.
- Several protocols that related separately to intravenous, intramuscular and subcutaneous injections of the same agents, had been combined into one

The following queries were raised:

- **Clarity was needed in relation to continued use and reuse of animals.** The PPL Holder had put in the licence that animals coming onto one of the protocols would be classed as “continued use”. What was actually meant was that animals would be transferred from the current licence to the new licence.
- **The most up to date rehoming figures needed to be provided**, which was 120 dogs rehomed since 2016.
- **When assessing severity, the licence made reference to some animals being “subthreshold”:** this should only be used to refer to actual severity. To assess the actual severity, the whole experience of the animal needed to be considered.
- **Animal Experience mentioned that the maximum number of litters would be 6 litters per bitch. Was this acceptable to AWERB? And should there also be an upper age limit for breeding bitches?** It was pointed out by one of the NVS that mating and giving birth was not a regulated procedure, so a bitch that had a litter was not regulated. What made it regulated was when the pups had a genetic alteration and that would be under this protocol so come under A(SP)A. If AWERB wanted a limit adding to the number of litters that a bitch could have then this would need to be placed elsewhere in the licence. If there was any kind of manipulation that was linked with the breeding of the animals to generate pups then that might need to be its own protocol. What was needed was a good dichotomy of what was where and what was happening to whom and that should then be structured according to the protocols. **There would be a separate discussion between the PPL Holder and the NVS about what was required and to clarify any confusion that might have arisen from earlier discussions held.**
- **Blood sampling:** the licence mentioned that pups would be subjected to genetic analysis from either a venous blood sample and/or a cheek swab. **If a cheek swab could be taken, then why would a blood sample also be needed?** It was explained that the standard was to do a buccal swab and that was what all the routine genotyping was done on. The blood sample was required as a safeguard in case additional samples were needed to confirm that the genotyping was there. Also, the blood sample could be used to check CK levels, which was an indicator of whether dystrophy was there or not. The combination of the two would confirm the genetic diagnosis. It was suggested that if the PPL Holder wanted to fit blood samples into one step, then he should write down for each type of situation why the blood sample was being taken. This would then make it a lot easier to read and to understand.
- **Section: How will you minimise the harms associated with these phenotypes: the licence mentioned that insufflation would be provided:** occasionally when the pups were born they had difficulty taking their first few breaths and had respiratory problems. What was being found on the ground was that sometimes interaction was needed with the puppies to help them take their first few breaths such as giving them a little bit of oxygen, however there was a difference between doing this and carrying out insufflation. It was felt that using the wording insufflation was too strong as it suggested active ventilation or positive pressure to the dog which was not appropriate for the licence. Also oxygen supplementation should only be provided for a few seconds at birth, as an environmental supplementation, not for up to 48 hours postnatally as that could be detrimental. The oxygen that was provided was through a medical oxygen tank that was held in front of the pup’s nose – no mask or nasal cannula was stuck over or up the pup’s nose. **Action: the wording in the licence should be amended and carefully worded to reflect the actual actions that were taken when providing the additional oxygen to the pups.**

- **Humane endpoints:** The licence referenced for several potential endpoints that the *dogs* “... will be subject to veterinary examination/advice and treated/managed appropriately or euthanased depending on advice” – It was argued though that if some of these signs were being seen then veterinary advice was not needed as it was clear the humane endpoint had been reached. She suggested that there should be a move away “from going to get a vet for their advice”, when the humane end points have clearly been reached. The NVS agreed that the current approach effectively meant a delay in making a decision. It was important that there were clear endpoints written in the licence, which all who worked with the dogs were aware of.
- **“Moderate or severe apparent lameness or weakness”:** the licence mentioned that if the dog did not respond to treatment within 2 weeks then the dog should be terminated. It was felt that this was too long. It was explained that with muscular dystrophy it was possible to get acute necrosis in a bunch of muscle fibres in a particular muscle, which might cause acute lameness. These generally regenerated fairly rapidly within 2 to 3 weeks, so was a part of the Natural History of the disease. In humans, when this happened, in most cases movement would be restricted for a bit but would then improve. If lameness happened in a dog it was not necessarily then a downward path for the dog, as in many cases there would be a degree of regeneration of the muscle that would overcome the problem and therefore the lameness would improve.
- **There was a debate about how it was possible to tell if a dog was in pain.** This had caused a lot of discussion previously. If there was a doubt whether a dog was in pain, was it better to err on the side of caution and assume that they were? However, that would mean removing the dog from the study and if they were not actually in pain, were valuable study dogs being removed too early? It was suggested that the emphasis should be on how lame the dog was – although lameness might not necessarily cause pain it would cause discomfort and possibly stress to the dogs in that they were not able to move properly. So, should the cut-off be in relation to that? The technicians that were with the dogs every day would be the best people to determine whether a dog was in pain or suffering as they would be used to their general behaviours.
- It was suggested that the questions should be:
 - Was the dog in pain and if it was could that pain be alleviated? If the pain could not be alleviated, how long was acceptable to keep the dog in that state?
 - If the dog was lame but showing no signs of pain, how long was acceptable for the dog to remain lame and not show signs of improvement?

It was agreed that for the way forward, AWERB should continue to review the project licence and to add their comments to it by 28th June. The comments would then be consolidated, and a meeting convened with the project licence holder to go through them.

3 MINUTES

AWERB would review the draft minutes from the 8 June 2022 meeting and advise if they had any queries.

4 MATTERS ARISING

4.1 Research Policies

The list of research policies have been circulated for AWERB members to indicate which ones they would like to take the lead on reviewing.

5 DATE OF NEXT MEETING

This was scheduled for 05 July 2022. It would be a standard agenda items meeting.