Home Office

NON-TECHNICAL SUMMARY

Studies to investigate the development of immunity during the prenatal and neonatal period in the bovine calf

Project duration

5 years 0 months

Project purpose

- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants
  - (iii) Improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes
  - (ii) Assessment, detection, regulation or modification of physiological conditions in man, animals or plants

Key words

Calf health, Immunity, Passive transfer

Animal types	Life stages
Cattle	adult, pregnant, neonate, juvenile

Retrospective assessment

> The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

To understand the interplay between calf and maternal factors during immune development. To investigate how these factors affect calf health, performance and survival. To improve the diagnosis of inadequate transfer of immunity (known as failure of passive transfer) after birth.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

There is an urgent need to improve the health and welfare of calves reared from commercial dairy farm origins in the United Kingdom. The occurrence of disease in this population is a result of an interplay between their environment, exposure to pathogens, and their immunity. With regards to the latter, due to the structure of the bovine placenta, immune cells (including antibodies) are unable to pass from mother to calf during pregnancy. The calf is therefore born without a fully functional immune system, and relies upon absorbing immune components including antibodies within the first milk produced by its mother, known as colostrum. These immune components provide protection, or “passive immunity" until the calf’s own immune system is fully functional. If this is insufficient, the calf will be at a greater risk of succumbing to disease until it is able to produce its own antibodies, from around 5-6 weeks of age. This was recently evidenced in a study of a large dairy herd located in America. The authors demonstrated that, when compared to calves with excellent levels of passive immunity, inferior values were associated with greater risk of diarrhoea and pneumonia. This is highly important since within UK dairy herds, inadequate transfer of passive immunity is highly prevalent, with over 20% of calves having poor transfer of passive immunity.

Many tests are available for the measurement of antibody concentrations in the calf. One of the cheapest and most accessible methods is called refractometry. A small portable device called a refractometer is used to estimate the protein concentration within the blood and the result is used to estimate antibody concentrations. Although it is used very commonly, this method is only accurate in calves aged between 1-7 days of age. In older calves, measurement is rarely performed. This represents a challenge for producers who buy calves over a week old, since they are unable to quickly assess if stock have sufficient antibody-derived immunity. There is an urgent need to improve cost effective, fast tools for the measurement of antibody concentrations in calves aged over a week old and to produce guidelines regarding "normal" concentrations and their association with risk of disease.

This would provide stakeholders with improved guidance regarding the purchase, management and subsequent rearing of their stock in ways that optimise antibody-derived immunity.

Calfhood immunity is not only provided by antibodies. In fact, the development of immunity begins during pregnancy and continues during the neonatal period. Gene expression analysis of neonatal calves has identified deficiencies in specific immune components. What is relatively unknown is the importance of these immune deficiencies on the development of disease. Does under- or over-expression of specific genes or other immune components result in a greater risk of disease or death, and how does the timing interact with disease risk? What are the impacts of colostrum management and maternal health during pregnancy on gene expression in circulating immune cells in neonatal calves, and the function of immune components, and how does this impact their health and growth outcomes?

What outputs do you think you will see at the end of this project?

- Results will provide data regarding antibody concentration measured during early life and the disease risk.
- Results will also provide information regarding the development of calf-derived immunity and its association with health and performance
- Results will inform the accuracy and precision of a unique test for the measurement of antibodies in calves prior to weaning. It is a lateral flow test (similar to many at-home COVID tests), whereby a drop of solution is added to the testing device and the appearance of red line(s) across the test window allow the interpretation of results. The test can be performed quickly and easily on-farm by the farmer, and results collected rapidly. Although lateral flow tests for the measurement of specific antibodies in calves already exist, this test is unique because unlike those currently available it is semi-quantitative. This means that the results provided are on a scale, rather than just "pass" or "fail", and this allows its' use in calves aged over a week old. If effective, this could provide farmers and vets with a low-cost tool to measure immunity in calves during this period.
- Publications: Results will be published in peer-reviewed literature as well as the farming press, presented at scientific and industry conferences nationally and internationally.
- Study outcomes will be discussed at internal seminar meetings of the institution

Who or what will benefit from these outputs, and how?

The proposal should provide a net benefit towards promoting calf health and welfare.

Animals: Diagnosis of low antibody concentrations could inform improved management of high-risk individuals, to reduce their risk of disease. Results will also inform calf producers about current standards of rearing, and if improvements are required. This feedback-loop will allow subsequent cohorts to be managed better, improving disease resilience and reducing the risk of poor health and mortality whilst improving calf performance (such as growth, production and reproduction parameters). The health outcomes in calves could also be improved by gaining a better understanding of how the

health of the mother during pregnancy may alter both the development of the calf's own immune system and the quality of the antibodies the mother delivers via her colostrum.

Veterinary surgeons and Farmers: Results will provide information regarding thresholds for measurement of antibody concentrations in calves aged older than a week of age, and risk of disease. In clinical veterinary practice, this might be especially useful to veterinary surgeons both for the diagnosis of failed transfer of passive immunity at the individual and herd level. Understanding of the dynamics of antibody concentrations in older calves in response to disease is crucial: these calves may not produce an appreciable quantity of their own antibodies during the preweaning period, hence if they encounter disease are antibodies "used up"? If so, this will impact on how stakeholders interpret antibody concentrations when performed on older calves. An understanding of the dynamics of antibody concentrations will also inform the use of a calf-side lateral flow test for the measurement of immunoglobulins in calves prior to weaning. Pilot studies have already shown good accuracy when performed in calves aged between 1 and 14 days of age, but no data are currently available to show its usefulness in older calves.

How will you look to maximise the outputs of this work?

Findings will be shared with the veterinary and farming communities via presentation at scientific meetings and veterinary clinical clubs, and publication in open-access journals. We will utilise the social media to disseminate key findings. This project represents a collaboration between academia and veterinary clinicians, which will be useful for the practical utilisation of the findings.

Species and numbers of animals expected to be used

- Cattle: 1100 (500 calves and 600 mothers (cows))

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

We intend to use calves reared on commercial farms from birth until weaning, as well as their mothers. Our research specifically pertains to calf development during pregnancy and during the neonatal and preweaning periods. On a typical commercial farm weaning (defined as the transition to a 100% solid-food diet) occurs at around 8-10 weeks of age, although occasionally calves may be weaned slightly older, and it is from birth until this age range that we intend to study. The management of calves and their risk of disease is different based upon whether they are going to be kept on their farm of birth as a replacement dairy cow or sold onwards. This project seeks to follow both populations. Initially, it will monitor a population of replacement female dairy calves on a single dairy farm. Thereafter, a population of calves born on a dairy farm but destined for beef production will be monitored.

Typically, what will be done to an animal used in your project?

This study will be performed on commercial farming facilities and on this basis animals enrolled will be kept according to normal commercial husbandry conditions. Two types of facility will be used, commercial dairy farms and commercial calf rearing organisations.

For each (pregnant) mother, blood will be collected at approximately two timepoints from either the tail vein or the neck vein: Around the time of pregnancy diagnosis and during mid gestation. Colostrum taken as part of routine herd management will also be collected at the first milking after calving.

Sampling of each calf will be performed at a maximum frequency of every 5 (+/- 2) days, with a maximum of 1% blood volume collected at a single sampling (~27mls for a 45kg calf), for a maximum of 12 weeks, although we expect 8 samplings to be the most common. The volume of each sample will be considerably less than maximum permitted volume suggested by the NC3Rs guidance. On each occasion samples will be collected from the neck vein, whilst a subset of samples will additionally be collected via nose pricks. Nose prick samples involve the collection of a very small volume of blood a similar manner to the collection method performed in humans for the measurement of blood sugar concentrations in diabetics. This is the recommended sample collection route for the lateral flow device to be tested and is becoming a standard method of collection in clinical veterinary practice. At every sampling visit the health of the calf will be assessed using a published scoring system, which considers faecal consistency, discharge from the eyes and nose, temperature, ear and head position, and presence or absence of a cough.

Beef calves will be enrolled into the study upon arrival at a commercial calf rearing facility, aged between 1 and 4 weeks old. For some of these animals we expect to be able to gain information from prior diagnostics performed routinely on the farm of birth (under the Veterinary Surgeons Act). Where possible these will be used to minimise further sampling. Repeated sampling will be performed approximately weekly from arrival until weaning. The first sample will be collected within 7 days of arrival. This is essential, since this is the period during which immune responses are likely to be crucially affected by transportation and mixing. Collection of this sample is therefore required to develop our understanding of how these management practices affect immunity and the occurrence of disease. Using this sampling strategy, the maximum number of blood samples collected from a single calf is 11, although we expect 5-7 samplings to be the most common.

What are the expected impacts and/or adverse effects for the animals during your project?

The only harm we expect during this project is that associated with the collection of blood. In all cases of blood collection, we expect animals will experience a mild, transient discomfort associated with the passage of the needle or prick device through the skin.

Very rarely there is the potential for short lasting adverse effects (minutes to days) associated with the collection of blood which may include bruising under the skin, and even more rarely inflammation of the vein. In the event that these occur, and it is necessary to take further samples as part of the procedure, another sampling site will be used where possible. Thereafter, the animal will not be used again until an improvement is seen.

Animals may also encounter stress from handling. This is unavoidable, but will be minimised by ensuring that animals are handled by trained operators in a manner that avoids prolonged handling and minimises stress, where possible in their housing environment or appropriate handling facilities.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

Calves: 100% mild

Cows: 100% mild

What will happen to animals at the end of this project?

- Kept alive
- Rehomed

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

Animal models are required for the study of immune development and response to disease in a commercial farming environment. It is not possible to replicate the complex interactions between the animal, the environment and disease pathogens within a test tube or using other laboratory techniques alone. Furthermore, by performing this study on animals within a commercial environment, the results of this project will be highly applicable to calf-rearers, which will maximise the impact of the study outcomes and their relevance.

Which non-animal alternatives did you consider for use in this project?

A literature search using the search engines Pubmed and Agricola were used following established guidelines (Fund for the Replacement of Animals in Medical Experiments, FRAME) to research the alternatives to animal models for the study of passive transfer. No suitable alternatives were found by this method.

Why were they not suitable?

The study of passive transfer from mother to calf cannot be replicated sufficiently through the use of either cell lines or organoids, especially given that the underlying physiology is multi-organ and not limited to a specific tissue or cell type. Study of maternal effects are not possible via these methods. Validation of a lateral flow device for antibody measurement requires animal disease models.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

1. We followed the PREPARE* guidelines regarding experimental design, which provide clarity on how to ensure this stage of our research is optimised.

2. In accordance with our study objectives and hypotheses we conducted three pilot studies to determine:

- The agreement of the novel diagnostic test with a "gold standard", when performed on leftover blood from samples that had been collected for other reasons. This allowed us to estimate the expected accuracy of the novel test.

- The current levels of passive immunity within herds at present.

- The current levels of disease in calves between birth and weaning in UK dairy herds as reported in recent literature.

This allowed us to produce an estimation of the minimal number of animals required to be enrolled in order to fulfil the study objectives.

3. We considered and took into account the risk of potential losses-to-follow up, either due to departure from the farm or death as well as the clinically-relevant effect size we should seek to measure.

4. We took into account our interest in considering two discretely managed populations exposed to different risk factors for naturally occurring disease: replacement dairy calves and calves destined for beef production reared at commercial facilities.

5. We considered recent, similar publications within the same field.

6. We sought advice from colleagues (including a biostatistician) experienced in the design of animal studies regarding sample size determination.

*Planning Research and Experimental Procedures on Animals: Recommendations for Excellence. https://norecopa.no/prepare

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

- Experimental design was refined using the Planning Research and Experimental Procedures on Animals: Recommendations for Excellence (PREPARE) guidelines as well as the NC3R's Experimental Design Assistant. These guidelines can be accessed using the following links: https://norecopa.no/prepare and https://eda.nc3rs.org.uk/

- Sample sizes were estimated using standard power analyses based upon our pilot data.
- Taking into account the results of a pilot study (unpublished) of 16 farms within Southern England, and through collaboration with a veterinary practice, we considered the effect of farm selection on risk of losses to follow up and prevalence of disease to ensure selection of an appropriate farm that meets the requirements for this project.
- We will use residual blood samples wherever possible, already routinely collected under the Veterinary Surgeons Act (VSA) for the purpose of measuring mother metabolic and health status pre-calving, and for measurement of calf health and immune status (with permission from our Clinical Research and Ethics Review Board).

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

- Data previous research was used to produce a more accurate estimation of the minimum number of animals necessary.
- We sought and found collaboration from colleagues who could use samples collected from the same protocols outlined to avoid the requirement for future studies with similar design, and to ensure that wastage from collected samples is minimised.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

Pregnant bovine dams will be enrolled and blood samples taken from either the tail or neck vein to study their health, metabolic and immune status. Optimisation of the sampling process will be through efficient, fast sampling by experienced operators in appropriate handling facilities to minimise the duration of possible distress.

In the calf population we will seek to minimise distress during blood collection by using animal-specific restraint such as a halter, locking yoke or with an assistant holding the animal.

During the study no specific treatments will be withheld and animals will be kept under the same animal husbandry conditions as calves on the premises that are not being studied. Sampled animals will be monitored for signs of adverse effects during visits.

Why can’t you use animals that are less sentient?

The study outcomes of the project are only relevant to cattle, and are specific to their immune development during pregnancy and during the neonatal period. Due to its complexity, animal models are the only available method and there are no less sentient species that can be used for this research.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

Animals will be handled by trained operators in a manner that avoids prolonged handling and minimises stress, either in their housing environment or using appropriate handling facilities. Study animals will be monitored for signs of adverse effects associated with venepuncture during subsequent visits. Risks will be minimised by use of a sterile needle for each collection of the smallest practical gauge. In circumstances where the collection site is dirty it will be cleaned prior to sampling

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

Published practical guidelines are available to us to ensure that the design, collection of data and reporting of our scientific findings are clear, repeatable, and legitimate. These guidelines are variable depending on the type of study which we report. Since this project licence covers a number of potential publications, there are various guidelines which we will follow. For each guideline mentioned, further information is accessible via the hyperlinks provided.

For example, during the experimental planning and design stage we have followed the PREPARE guidelines (provided by Norecopa), which provide clarity on how to ensure this stage of our research is optimised.

During the project reporting stage of the project, we will follow the ARRIVE 2.0 guidelines. We will also consider the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines and, for comparison of diagnostic tests, the Studies of Diagnostic Accuracy (STARD) guidelines when writing for publication to ensure transparent experimental design and clear reporting. Each one of these guidelines provides the authors with specific advice relevant to the study design which we intend to report.

https://norecopa.no/prepare/

https://arriveguidelines.org/arrive-guidelines

https://www.strobe-statement.org/

https://www.equator-network.org/reporting-guidelines/stard/

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

This institution is active in the dissemination of NC3Rs and 3Rs relevant news and training via a newsletter and regular group meetings, which I will participate in.