Home Office

NON-TECHNICAL SUMMARY

Production of antisera and immune cells in fish

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (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

Key words

No answer provided

Animal types	Life stages
All fish species	Juvenile, Adult

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?

The aim of this project is to generate antisera and immune cells from fish for research and diagnostic purposes.

This will support and improve the maintenance of aquatic biosecurity for farmed and wild fish as part of compliance with national, EU and international legislation regarding aquatic disease control.

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?

With the decline in wild fish stocks, aquaculture, the fastest growing food-producing sector globally, is increasingly critical to future global food security. Infectious diseases causing significant losses to farmed fish continue to be a major constraint impacting on both economic resources and animal welfare. The sera generated under this project will be used in characterisation and improved understanding of aquatic diseases. The sera will be used in the development of diagnostic assays and in the regulatory testing of starting materials feeding into vaccine development for serious diseases of cultured and wild fish.

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

A successful epidemiology programme and accurate diagnostics are essential to prevent the introduction and control the spread of aquatic disease. The outputs from the project will improve our ability to detect and identify pathogens, to support UK aquatic biosecurity and comply with international legislation on aquatic disease. The availability of specific immunoreagents is vital to development, validation and implementation of tests designed to trace and control any spread of infection. Availability of reagents for pathogens present in the UK is also of considerable importance to follow the development or introduction of new strains of significant bacterial and viral pathogens that are defined by serotype.

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

The principal output will be evidence for policy and regulation. The improvement of our current serological tests and subsequent use in refined epidemiological surveys will aid the development of policy and regulations at both national and international levels, particularly in relation to the risk of exotic or emerging pathogens to native species in the wild and farmed environments. Outputs are typically in the form of advice reports, conference proceedings and peer-reviewed papers.

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

Improved serological assays will be made public and may be suitable for commercialisation and uptake by the wider scientific/public community.

Additional benefits may be realised by use of these sera in fundamental and applied (translational) microbiological research and development of effective treatments.

Species and numbers of animals expected to be used

- Other fish:
  - All fish species: 400

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.

Rabbits and mice are often the standard laboratory animals used for the generation of polyclonal and monoclonal pathogen-specific antibodies. However, in recent years a greater understanding of humoral responses in fish has highlighted major differences in Ig isotypes and performance. For this reason, for the planned reseach into fish diseases, mamalian antibodies are inadequate. Therefore, after confirming materials are not available via other research groups or commercially, this licence seeks authority to produce autologous immune sera in fish to improve non-lethal immunological tests.

When possible, specific pathogen free fish will be used for the antisera production under this License. Atlantic salmon, rainbow trout and common carp are the most relevant for UK aquaculture industry. The laboratory has more than 50 years of combined husbandry experience with these species. Variation in response between animals is minimised by size grading, and prior group holding in common conditions.

We will preferentially use these species for the generation of control sera for the development of immunological-based diagnostic tests. However, wild caught-fish will be used in the absence of commercially available stocks (e.g. European eel).

To generate positive immune sera, the fish need to be capable of generating a strong measurable humoral response. The first appearance of IgM in lymphocytes varies considerably among fish species. The use of larvae for immunization is not an alternative for the generation of positive sera as they lack a full developed humoral system. Furthermore, the likely yield of serum from such immature forms would be inadequately small. Thus, this license seeks authority for the use of juvenile and adult fish for the collection of large amounts of control sera.

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

Use of fish will be solely for the production of polyclonal antisera and immune cells.

Fish under procedure will receive a maximum of 5 injections of the antigen and adjuvant mix with an interval of aproximatelly 500 DD between immunisations; and blood samples might be taken to assess

the immunoglobulin titres with a maximum volume of blood permitted of 0.4% of body weight per sample, and no more than 15% of total blood volume over any four-week period before the final blood harvest.

The antigen may be used with adjuvants. The adjuvant programme will be selected depending on the study, with as minimal local reaction as possible whilst remaining effective. Freund's Complete Adjuvant (FCA) can be used only on the primary injection. For a fish weighing more than 50 g, the maximum volume injected per immunization by IM is 100 µL of the antigen/adjuvant emulsion in each of two sites. The maximum volume by IP is 100 µL of the emulsion in one site. Stable emulsions should be used with no more that 50% FCA mixed with antigen in aqueous solution. The antigen/adjuvant mixture will not exceed 100 µL in fish <50g or 200 µL in fish >50g. Alternative mineral oil vaccinations will also be considered (e.g. SEPPIC Montanide ISA) where evidence of reduced tissue reaction is provided. General anaesthesia with recovery will be administrated for the immunisation injection.

Immunisation by the oral/immersion route is preferred when fish size (i.e. small fish ≤20g) and antigen nature allows it. Antigen will be administrated either by bath or as a food additive.

Marking (optional) can take place together with pre-immunisation blood sample. May be carried out by visible implant elastomers (VIE), passive integrated transponder (PIT) tags or suitable alternative methods. Marking can be repeated if loss of the tag is observed alongside booster immunisation or blood sampling. The most appropriate tag will be chosen depending upon the fish species and animal size.

The procedure described above will follow a full formal Study Plan. These Study Plans are developed following PREPARE guidelines (Smith et al., 2018) and require the consideration and approval of the AWERB and the Project Licence holder, before any experimental work commences. Copies of all approved Study Plans are filed in the Experimental Facility records area, by the Study Director, and in the electronic archive where they are readily available.

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

Bath immunisation in fish may cause loss of appetite for a short time.

Intramuscular (IM) or intraperitoneal (IP) immunised fish may develop lesions in the site of injection due the use of oily based adjuvants, especially when using Freund's Complete Adjuvant (FCA).

In IP injected fish, the most common type of reaction is a localised fibrinous peritonitis in the area adjacent to the injection site. Fibrinous strands typically form adhesions between the ventral body wall, spleen and pyloric ceca which may lead to mild and moderate lesions. IM immunisations are less common in fish, as the oil adjuvants can lead to the development of granulomatous inflammatory infiltrates. The IP route of injection is preferred over IM, except when using DNA-plasmids for the immunisation, where the IM route of infection has been shown to be more efficient.

Adverse effects due to the nature of the antigen:

- Anaphylactic shock- Unlikely. It will be avoided by suministrating the smaller amount of antigen/adjuvant possible and by following the recommended doses of immunisation.

• Inactivated pathogen used as immunogens may cause an infection – Unlikely, pathogens will be inactivated. When possible, antigen inactivation will be confirmed *in vitro* prior to immunisation (e.g. by growth in cell culture).

Adverse effects due to the nature of the adjuvant:

• Granulomas caused by infection or adjuvant – Antigen will normally be combined with an adjuvant in order to enhance the immune response. Adjuvants will be chosen to stimulate the required antibody response whilst minimising local tissue damage. Selection will depend upon the route of administration, the nature of the antigen and previous experience. In order to minimise any adverse effects of immunisation, the sites of injection will be well separated, and regular inspection will be made. If any fish show persistent or extensive lesions or signs of distress, the monitoring frequency will be increased. The advice of the NVS and the NACWO will be taken on whether the animal needs to be humanely killed by S1M or the non-S1M method as follows: surgical anaesthesia followed by the collection of blood sufficient to result in exsanguination, followed by removal or destruction of the brain.

Table 1. List of possible adverse effects, likelihood, estimated duration, controls and humane endpoints.

Adverse effects (What could go wrong)		Indicators (How we will recognise it)	Checks (How we will monitor it)	Estimated duration	Likelihood	Controls (How we will prevent or ameliorate harms)	Humane endpoints
Procedure-related	Injuries from anaesthesia, vaccination and bleeding procedures	Skin lesions, bruising/scale loss	Visual checks	< 24 hr	Likely- max: 40% of IM injected fish; less than 10% of IP injected fish	Appropriate procedures, training and skilled operators	Individual euthanasia if open/haemorrhagic lesions.
	Systemic allergic reaction (anaphylaxis)	Abnormal behaviour (extreme ventilation, unusual positioning, loss of equilibrium)	visual checks	< 4 hr	Unlikely	Correct dosage and use of adjuvants	Individual / population euthanasia if abnormal fish behaviour persists
	Side effects of vaccine components (adjuvants)	Lesions at injection site, temporary reduced appetite	Visual checks	< 3 days	Unlikely	Good hygiene during vaccination preparation and procedures, controlled dosage type of adjuvants, increased frequency of monitoring	Individual euthanasia if open/haemorrhagic lesions, or prolonged inappetence leading to emaciation
	Pathogenic disease (incomplete antigen inactivation)	Clinical signs of illness	Visual checks	< 2 days	Unlikely	Confirm effective inactivation, short term temperature manipulation outside of permissive temperatures	Individual euthanasia at confirmation of pathogen related clinical sign >mild. Population euthanasia if therapeutic treatments unsuccessful/ unavailable.
Not procedure-related	Aggressive interactions and cannibalism	Physical injuries to fins, eyes, skin. Observations of aggression/ cannibalism	Visual checks	< 3 days	Unlikely	Grading for similar size; Ensuring appropriate feed ration; Maintain environmental conditions (e.g. water flow, fish density) known to minimise risk; Tank enrichment	Individual euthanasia if injuries considered > mild (e.g. open / haemorrhagic lesions).
	Poor water quality	Abnormal fish behaviour	Visual checks + routine and responsive water quality monitoring;	< 2 days	Unlikely	Stock at and maintain fish biomass within appropriate loading rate (kg/m^3/L, i.e. biomass per inflow) for temperature and fish size. Increase water/air inflow rates.	Individual / population euthanasia if abnormal fish behaviour persists after mitigation actions taken.
	Pathogenic disease (unrelated to vaccine antigen)	Clinical signs of ill-health	Visual checks	< 2 days	Unlikely	Prior health screen samples; use of in house spf fish, prevention of pathogen presence - via prior quarantine, prophylactic and therapeutic treatments as advised by NVS, biosecure working practices and facility; maintenance of tank hygiene	Individual euthanasia at confirmation of pathogen related clinical sign >mild. Population euthanasia if therapeutic treatments unsuccessful/ unavailable, or >25% of population affected.
	Non-pathogenic disease	Clinical signs of ill-health; poor performance	Visual checks; Growth monitoring	< 3 days	Unlikley	Checks on performance during quarantine and acclimation. Provision of complete commercial diets. Obtaining fish from proven reliable suppliers.	Individual euthanasia at confirmation of clinical sign > mild. Population euthanasia if >10% of population affected.

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)?

- 40% of IM immunised fish may reach MODERATE in recognition that the occasional use of Freund's Complete Adjuvant (FCA) may induce localised granulomas.
- 10% of IP immunised fish may reach MODERATE due the development of moderate lesions associated with adhesions in the ventral body wall.
- 60% of IM and 90% of IP immunised fish should not experience severity above MILD.
- 100% of fish immunised sorely by the oral/immersion route should not experience severity above MILD.
- 100% of fish used to maintain the stock density will not experience severity above SUB-THRESHOLD.

What will happen to animals used in this project?

- Killed

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?

This license aims to improve non-lethal immunological tests currently used in epidemiological surveys. In contrast to molecular tests, which inform the presence or absence of a given pathogen, serological tests detect previous pathogen exposure and are needed for the designation of disease-free areas for trade purposes (visit OIE http://www.oie.int/animal-health-in-the-world/official-disease-status/) and inform on pathogen introduction and epidemiological surveys.

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

In vitro culture will be used to produce the antigen (i.e. use of susceptible fish cell lines for culturing virus). *In vitro* methods will also be used to confirm antigen inactivation prior to immunisation.

Alternative methods for polyclonal antibody production have been considered, such as recombinant antibody phage display. However, due the nature of the autologous antisera reference test material required and neutralizing capability of the polyclonal antibody, the use of animals is required. During this licence, advances in neutralising antisera production using non-animal platforms will be closely followed, as well as attendance at specialised workshops and networking on non-animal-based antibody production.

Why were they not suitable?

Suitable synthetic non-animal alternatives do not currently produce antibodies with the range of specificity and affinity required in the assays these sera will be used for. For serological test development known positive controlled sera from the host species (fish species) are required.

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?

Power calculations are not needed for this license. The number of animals used in each assay will be the minimum required to produce the volume of antiserum needed and will depend on the species chosen and the antisera titres.

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

Control fish (non-immunised fish) will not be used in this licence as the immune status of each individual animal will be determined in the pre-immunisation blood sample. However, fish stocking densities and population size are necessary considerations to ensure the expression of normal feeding and social (e.g. schooling) behaviour and to minimise aggressive behaviour. The minimun number of fish per tank will depend on the fish species, animal size, and the species needs following advice from fish husbandry experts. Experimental facilities offer a range of flexible tank sizes to minimise animal numbers while enabling basic social behaviours. Taking into account individual variation in response, a maximun of 400 fish over 5 years is expected to maintain stock density and produce the volume of antiserum required. Depending on the titre of the antisera, a single batch of antiserum appropriately stored will last for many years and may be shared with other laboratories in the same research field. Heterologous antisera against a small number of fish pathogens and anti-species immunoglobulins are commercially available. Where these have been demonstrated to be suitable for our purposes, these will be purchased.

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

Every effort is made to reduce numbers of fish used to a minimum, incorporating animal husbandry expert advice on fish social needs and AWERB scrutiny on each study plan. Additionally, within the constraints of available tanks sizes and appropriate animal numbers required for acceptable stocking densities and with respect to individual variation in immune response, large fish will used to generate larger and longer lasting volumes of sera from fewer animals.

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.

Specific pathogen-free (SPF) common carp, Atlantic salmon and rainbow trout will be used for the antisera production under this License. Those species are the most relevant for UK aquaculture industry. The laboratory has more than 50 years of husbandry experience with these species. Variation in response between animals is minimised by size grading, and prior group holding in common conditions. Consideration is given to providing an appropriate environment, including enrichment such as shading and/or refuges, during experiments. Stock density, water current and conspecifics that promote social behaviour, plus nutritionally complete diets are carefully considered throughout the animal holding. Fish are stocked into experimental tanks by staff experienced in fish handling and are routinely acclimated after stocking prior to study initiation.

The adjuvant selected for the antigen emulsion will be the one that causes least discomfort but elicits the desired immune response. The selection of the adjuvant will be carefully justified in a Study Plan after discussion with the NACWO, NVS and the Licence Holder. Consideration will also be given to the route of administration when deciding the fish species and size. When possible, immunisation by immersion/oral route will be selected, to minimize the severity of the procedure (i.e. immunisation injections will not be needed).

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

Rabbits and mice are often the standard laboratory animals used for the generation of polyclonal and monoclonal pathogen-specific antibodies. However:

- Fish are less sentient than mammals, as they do not have the extensive cerebral cortex seen in the forebrain of mammals.
- Major differences in Ig isotypes and performance within lower and higher vertebrates exist. For this reason, for the planned reseach into fish diseases, mamalian antibodies are inadequate.
- The use of fish larvae for immunisation is not an alternative for the generation of positive sera as they lack a full developed humoral system. Furthermore, the likely yield of serum from such immature forms would be inadequately small.

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

Where possible, we will administer antigen by the mildest possible route - e.g. orally where possible, and only use parenteral routes where there is scientific evidence that the oral or immersion routes would be unsuccessful. Intraperitoneal (IP) injections will preferred to intramuscular (IM).

Changes to the diet prior to oral immunisation, to mitigate any adverse impacts on feeding (e.g. palatability adaptation) will be discussed with the NACWO and NVS and stated in the Study Plan.

General anaesthesia will be bath administrated prior to IP or IM injection and prior to blood sampling. Experienced PIL holders will conduct the anaesthesia following standard operating procedures.

Fish under procedure will be monitored via direct visual checks of condition and behaviour by both husbandry staff (during feeding and tank cleaning) and experienced PILs (at least daily) using standardised in-house scores sheets. The frequency of direct PIL checks will increase if adverse effects are present. If clinical signs associated with suspected infection with pathogen in use is observed (see Table 1) the animal will be schedule 1 method (S1M) terminated or killed by the humane non-S1M as follows: surgical anaesthesia followed of collection of blood sufficient to result in exsanguination followed by removal or destrucion of the brain.

Direct visual checks will be accompanied by video observations using underwater cameras mounted within tanks. Water temperatures are recorded automatically and maintained at ±0.2°C. Fish are normally fed at least once per day by hand. Hand-feeding means that fish feeding behaviour (a good indication of fish welfare) is better observed than if mechanically fed. Water replacement is normally maintained at 5 changes per day. Call out alarms are installed at the Weymouth facility which activates if pre-set parameters are exceeded.

Consideration is given to providing an appropriate environment, including enrichment such as shading and/or refuges, during experiments. Stock density, water current and conspecifics that promote social behaviour, plus nutritionally complete diets are carefully considered throughout the animal holding. Fish are stocked into experimental tanks by staff experienced in fish handling and are routinely acclimated after stocking prior to study initiation.

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

Protocols used will follow the Home Office guidance: “Antibody Production, Principles for Protocols of Minimal Severity”. Fish blood samples will follow the guidance from Canada Department for Fisheries and Oceans, Canadian Council on Animal Care, September 2004. 4.0 Blood sampling of Finfish.1-15.

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

During this licence, advances in neutralising antisera production using non-animal platforms will be closely followed, as well as attendance at specialised workshops and networking on non-animal-based antibody production.