Animal &
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Agency

Developing and delivering an oral contraceptive to control fertility in grey squirrels

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Overview

We provide an update of a five-year project (now in its third year) on the development of an oral contraceptive to control fertility in grey squirrels and on its field delivery. The project was undertaken to move the existing fertility control science to the point that a strategy for the British grey squirrel is available and a product is ready for the registration process.

The research, carried out by the National Wildlife Management Centre (part of the Animal & Plant Health Agency) involves using an existing candidate immunocontraceptive and marrying it with cutting-edge British micro technology to allow these contraceptives to be delivered orally using baits. In parallel, we are designing a grey-squirrel specific bait hopper system and developing methods to quantify bait uptake by grey squirrels.

The project is proceeding extremely well in terms of practical field development of the bait delivery system, despite suffering a few setbacks on the refinement of the contraceptive formulation. Mitigation measures have been put in place and our work has expanded to explore and test other novel contraceptives. We remain confident that this project will make a significant contribution to the development of an oral contraceptive for grey squirrels. Furthermore, our field data and modelling work will provide the first projection of the impact of integrated management methods to reduce grey squirrel numbers. This research has the potential to inform and expand the range of practical applications available to manage other locally overabundant mammal species.

Background and aims

Fertility control is widely advocated as a safe, effective and publicly supported alternative to lethal control. Immunocontraception involves the administration of a vaccine that generates an immune response to proteins essential for reproduction, rendering an individual temporarily infertile. These injectable immunocontraceptive vaccines are increasingly used for wildlife management. For instance, a single dose of GonaCon, which targets the gonadotropin releasing hormone (GnRH), has been shown to induce infertility in both sexes and for several years in many wildlife species that include wild boar, feral goats, badgers and deer.

In contexts where capture, injection and release are not feasible or economically viable, the availability of oral immunocontraceptives would multiply the breadth of field applications, particularly as species-specific oral delivery systems already exist for some wildlife species. Oral contraceptives delivered in baits could reduce population size as well as the rate of population recovery after culling. For this potential to be realised, more research is required to develop oral formulations, to quantify the costs and impact of using fertility control on population size and to address practical issues concerning the selective delivery of contraceptives to the target species.

A Defra-funded project demonstrated that a novel GnRH-based compound, tested as a putative oral contraceptive vaccine, generated an immune response in laboratory rats and reduced fertility in 6 out of 10 animals. This was the world's first ever demonstration that an animal's immune system can be triggered by oral administration of an immunocontraceptive vaccine. This five-year project was originally designed to build on that breakthrough to develop an oral contraceptive to control fertility in grey squirrels and to optimise the delivery of this contraceptive in the field.

Developing an oral contraceptive for grey squirrels involves identifying the effective formulation and the number of doses required to make a squirrel infertile and producing a palatable bait that will not interfere with the effectiveness of the contraceptive. The second strand of the project is focussed on designing a grey-squirrel specific system to deliver contraceptives in baits and on developing methods and tools to quantify bait uptake by populations of grey squirrels.

A third strand of the project, partly funded by Defra, is using mathematical modelling to examine the effects of fertility control, alone or in conjunction with culling, on population size and to compare the effort required by these methods to eradicate grey squirrels.

Specific objectives across the 5 years of this project are:

1. To carry out captive trials with model species (laboratory rats) to test formulations of an oral contraceptive (Y1)
2. To develop a system to identify feeding patterns by individual squirrels (Y1)
3. To conduct pilot field trials to test the feasibility of monitoring bait uptake by squirrels at individual and population level (Y1)
4. To establish a captive breeding colony of grey squirrels to test the effectiveness of oral contraceptives (Y1)
5. To carry out captive trials with model species (rats) to test doses, frequency and bait type (Y2)
6. To conduct pilot field trials to optimise bait uptake by squirrels (Y2)
7. To run pilot field trials, with baits containing placebo contraceptives, to quantify bait uptake by squirrel populations in different contexts (e.g. different times of the year) (Y3)
8. To carry out captive trials with squirrels to test the effectiveness of an oral contraceptive (Y3)
9. To run captive trials with squirrels to test formulations, doses, frequency and bait type (Y4)
10. To refine squirrel-specific methods to deliver oral contraceptives in field trials (Y4)
11. To initiate discussions with regulatory body for assembling a registration package (Y4)
12. To run pilot field trials with oral contraceptives to monitor effects on squirrels populations (Y5)
13. To model the impact of integrated management methods to reduce grey squirrel numbers (Y5)
14. To initiate trials for registration package that will allow a finalized oral contraceptive and a grey squirrel specific delivery mechanism to be manufactured (Y5).

Results of Year 1 and 2

Initial trials confirmed that it was feasible to encapsulate the previously tested oral contraceptive vaccine into sunflower pollen grains (**Objective 1,** see technical appendix). We proceeded to use this formulation in trials with laboratory rats aimed at increasing the efficacy of this vaccine. Rats were either injected or fed the contraceptive that was administered either as a plain vaccine or encapsulated in sunflower pollen grains. The effectiveness of the contraceptive was assessed by quantifying antibodies induced by the vaccine to the Gonadotropin Release Hormone (GnRH) and number of animals rendered infertile. All formulations induced antibodies to GnRH, thus replicating previous results, although the immune response to oral formulations was not sufficient to prevent reproduction. This low response to the vaccine was likely due to the composition of the adjuvant used to boost the animals' immune response to the vaccine. More work was initiated by our US partners at the National Wildlife Research Center (NWRC) to address the composition of the adjuvant. The NWRC experienced delays partly linked to a change of staff and aggravated by the US government shutdown at the beginning of 2019. We are working with our US partners to resolve these issues as quickly as possible. Concomitantly, we are discussing a novel formulation, based on bilosomes (see technical appendix), to deliver the current vaccine with the University of Strathclyde.

We developed a hazelnut paste-based bait to deliver oral contraceptives and tested the palatability of this bait with captive and free-living grey squirrels. We designed a food hopper that could monitor patterns of bait uptake by individual squirrels and we employed the bait marker Rhodamine B to quantify the proportion of squirrels ingesting baits in captive and field trials (**Objective 2 and 3**). We established a captive breeding colony of grey squirrels and developed non-invasive methods to monitor reproduction (cycling and pregnancy) in these animals. This colony will be crucial to establish the effectiveness of an oral contraceptive for grey squirrels (**Objective 4**).

We conducted pilot field trials with baits containing Rhodamine B to optimise bait uptake by squirrels **(Objective 6).** We quantified bait uptake by squirrel populations at different times of the year, with different densities of squirrels and bait hoppers (**Objective 7**). The field trials showed that, by using the current hopper and bait design, we consistently recorded > 75% of bait uptake by grey squirrel populations in small (6-18 ha) woodlands in summer. This is an excellent result as a Defra-funded model indicated that over 70% of the squirrel population must be targeted for fertility control to have the level of population effect required.

As novel candidate contraceptives have become available through the work of other research groups since the start of this project, our work has expanded to explore and test the most promising drugs.We carried out captive trials with squirrels to test the effectiveness of an oral contraceptive commercially available for rodents (**Objective 8)**. These trials showed that this product is not suitable for use in grey squirrels. Internal discussions within NWMC are ongoing to evaluate whether other candidate oral contraceptives (e.g. the cholesterol inhibitor Diazacon) should be tested in grey squirrels.

Captive trials are ongoing to refine and test grey squirrel-specific methods to deliver oral contraceptives by modifying the door of the hopper and by testing bait uptake by target (grey squirrels) and non-target (mainly red squirrels) species (**Objective 10**).

In summary, the project is proceeding extremely well in terms of practical field development of the bait delivery system, despite suffering a few setbacks on the refinement of the vaccine formulation. Mitigation measures have been put in place and novel avenues of research and collaborations are actively pursued to get this part of the project back on track.

Expected benefits

This project will make a significant contribution to the development of an oral contraceptive for grey squirrels. At the end of the five years, we expect to have delivered: 1. an effective, safe, oral contraceptive; 2. a species-specific system (feeder and bait) to deliver this contraceptive, or any other bait-delivered agent, in a field context to grey squirrels only; and 3. a method to monitor bait uptake by local populations of squirrels, that can be used to optimise the delivery of a contraceptive, or other bait-delivered agent, to control grey squirrels. Our field data and model will provide the first projection of the impact of integrated management methods to reduce grey squirrels. The model will quantify the effort and the cost required to use fertility control alone or in conjunction with culling to eradicate local populations of grey squirrels and thus reduce the species' environmental and economic impact.

An oral contraceptive formulation working in grey squirrels would have the potential to be adapted for other mammal species such as the wild boar, for which species-specific feeders already exist. Breakthroughs in this project will expand the range of practical applications of fertility control available to manage wildlife populations that are locally overabundant.

Project management

The project lead for NWMC is Dr Giovanna Massei who sits on the US-based Botstiber International Institute for Wildlife Fertility Control and on the European Group for Zoo Animal Contraception and has more than 15 years of experience in this research area.

The project cost over 5 years is £ 980.000. The majority of the funding covers NWMC staff time; the remaining funding is used for consumables and contribution by collaborators. The projected budget will be revised at the end of year 3.

As several research objectives are inter-dependent, review points are planned into the project every 3-4 months to discuss with the customer progress against objectives.

Scientific Appendix

Further background

A Defra-funded project demonstrated that a novel GnRH-based recombinant vaccine, delivered orally, generated an immune response and caused infertility in 6 out of 10 laboratory rats. The recombinant is called IMX294 and is produced by the French company Osivax. The adjuvant, added to the recombinant to boost the immune response, is produced by the US National Wildlife Research Center and is based on killed *Mycobacterium avium*, used in other veterinary vaccine formulations. This was the first ever demonstration that an animal's immune system can be triggered by oral administration of an immunocontraceptive vaccine. Building on these results, novel formulations are required to develop an oral vaccine that elicits a substantially greater and more persistent immune response in a larger proportion of animals.

A promising approach is an encapsulation technology patented by the UK company Sporomex Ltd. and based on pollen grains and spore shells as vaccine carriers. Pollen grains and spores are emptied of their internal genetic material to obtain sporopollenin exine capsules (SPECs). These capsules can be filled with several drugs to improve the bioavailability and absorption of the drug. Inexpensive and available in a range of sizes, SPECs have been successfully used to encapsulate fats, vitamins, enzymes, flavours, hormones and several other pharmaceuticals. In particular, SPECs have proven successful for the delivery of *in vivo* vaccines. For instance, a trial in the US showed that, when fed to laboratory mice, SPECs filled with a model vaccine elicited an immune response lasting up to 7 months, thus offering a putative mechanism for long-lasting oral vaccination.

Based on previous studies, SPECs of sunflower and club moss were chosen to deliver the contraceptive vaccine being developed by our project. The "largest" portion of the vaccine, called MAF (killed *Mycobacterium avium* fragments) was encapsulated in SPECs of sunflower and club moss. MAF is used as an immunostimulant to increase the effectiveness of vaccines. In the first captive trial, formulations of MAF encapsulated in SPECs were fed to laboratory rats, used as a model species. SPECs were recovered and counted in faeces to establish their passage rate in the days following ingestion. Based on the results of this trial, sunflower SPECs were chosen for further tests with the whole vaccine.

A second vaccine carrier formulation, based on bilosome technology, is actively being explored in collaboration with Dr Ferro's team at the University of Strathclyde. Bilosomes are bile salt stabilised vesicles that act as an envelope to protect their contents through the harsh environment of the gut, thus enabling efficient access to the small intestine. In addition, bilosomes have adjuvant properties in their own right. This technology has proven effective in the delivery of vaccines and can be applied to other biological therapeutics and small molecule drugs. In addition, there is the potential for two other components, xanthan gum and Blue protein to enhance any immunogenic effect of the oral vaccine formulation. Xanthan gum, commonly used in oral drug delivery, is expected to prolong adhesion time on the oral mucosa, potentially increasing vaccine uptake. The Blue protein is a key component of the injectable immunocontraceptive GonaCon and has been proven immunogenic when conjugated to GnRH. Blue protein will be conjugated to IMX294 to further stimulate the immune system.

The laboratory rat is used as a model species, offering efficient study design with limited individual variation under controlled conditions, and the ability to make direct comparisons with previous trials. Initial laboratory trials with SPECs and bilosomes are conducted to confirm the effectiveness of encapsulation and the effect of using adjuvants to increase the immune response to oral contraceptive vaccines. The effectiveness of vaccination is measured by i. quantification of serum anti-GnRH antibody titres; ii. reduced diameter of uterine horns indicating reduced fertility; iii. reproductive output (i.e. number of females cycling giving birth and litter size). These trials will be repeated as soon as the US collaborators have completed the tests with the adjuvant.

The most effective contraceptive will be selected for further trials to assess the dose, number of doses and the frequency of dosing to induce infertility for at least one year. Subsequent trials will be focused on offering rats the most effective formulation in a liquid bait base previously tested for palatability. Further captive trials will test the same bait for grey squirrels, and assess the effectiveness of the dosing schedule developed for rats on squirrels' reproduction.

Field trials using placebo Rhodamine B-treated baits and food hoppers equipped with Passive Integrated Transponder (PIT)-tag readers are conducted to monitor squirrel bait uptake at individual and population level. The hoppers developed in this project are equipped with PIT-tag readers and with a system to monitor the quantity of bait consumed per visit. These hoppers are used in pilot field trials to establish individual patterns of bait uptake and hopper visitation by grey squirrels.

Field trials are also carried out to test whether different densities of bait hoppers (e.g. 1 vs. 3 hoppers per ha) and different spatial configurations of bait hoppers (e.g. single hoppers vs. clusters) affect bait uptake by squirrel populations. The results of these trials will be used to recommend how to optimise fertility control delivery through baits. Pilot field trials will ultimately be carried out in areas with estimated densities of grey squirrels to test the effects of the most effective oral contraceptive at squirrel population level.