R7048 – Development of a genetically marked rinderpest vaccine
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Executive summary

- This project aimed to develop a vaccine against the rinderpest virus in cattle. The vaccine was genetically engineered to give the virus a marker gene in its genome.
- This would enable animal health workers, using Enzyme-Linked Immunosorbent Assays (ELISAs), to determine whether an animal has been vaccinated or is suffering from the disease.
- The marker allows differentiation between infected and vaccinated animals and, thus, the continuation of vaccination against Peste des Petits ruminants (PPR) in areas declared 'rinderpest-free'. It is of particular use in areas of civil unrest where control of animal movement is difficult.
- The use of a marked rinderpest vaccine allows ring vaccination to contain rinderpest outbreaks and assist in the monitoring of sanitary cordons.
- So far two versions of the rinderpest virus genome with a green fluorescent protein (GFP) gene inserted has been successfully produced and authenticated.
- An ELISA test has been developed and validated (fine-tuned) to detect GFP antibodies. This test should also work for the detection of antibodies generated by the live rinderpest virus, which will eventually be involved.

Project dates: April 1997 – March 2000

Background
As with the human disease smallpox, scientists believe it is possible to eradicate global rinderpest in cattle. The Global Rinderpest Eradication Programme (GREP) has had a major impact on disease incidence, and rinderpest has been eliminated in many African and Asian countries. Cessation of rinderpest vaccination is an essential step in the eradication process, but this will necessarily be followed by a rise in the percentage of susceptible animals. More animals will be at risk of contracting rinderpest and a rapid response to any disease incursion is essential. Routine serum surveillance is also essential to the eradication programme.

One of the stumbling blocks to eradication is that it is not currently possible to differentiate between vaccinated and
infected animals. Current rinderpest vaccines cannot be used in a country where rinderpest has been eliminated and that country wishes to declare itself 'rinderpest-free'. Peste des Petits ruminants (PPR) virus, related to rinderpest, is also found in many parts of the world where rinderpest is endemic. Rinderpest vaccine is routinely used for vaccinating small sheep and goats against PPR.

Therefore a genetically marked vaccine that helps animal health workers differentiate animals with natural infection from vaccinated animals would be of enormous benefit in control programmes in Asia and Africa.

Reverse genetics technology can be used to produce genetically marked vaccines by altering or inserting genes at will at specific sites in the virus, although none of these manipulations can be carried out using the RNA genome of the virus itself.

The use of a marked rinderpest vaccine would allow ring vaccination to contain any rinderpest outbreaks and assist in the monitoring of sanitary cordons, or immune belts.

**Objectives**
The aims of the project were to:

- Insert suitable foreign genes into the rinderpest virus (RPV) genome to act as genetic markers of the vaccine
- Develop specific ELISAs to measure the humoral antibody response to the marker gene(s), and assess the usefulness of different viruses as vaccines.

**Highlights**
The researchers successfully produced and authenticated two versions of the RPV genome, each with a green fluorescent protein (GFP) gene inserted. These two versions of the genome allow GFP to be expressed either internally in the cell or as a secreted protein. They also developed and validated an ELISA test to detect the GFP antibodies in cattle induced by immunisation with the purified protein. This test could also be used to detect antibodies in infected animals generated by the live rinderpest virus.

Work to produce other affordable and cost-effective tests for use by governments and farmers in developing countries is also underway.

The researchers genetically engineered viruses that were shown to function efficiently as vaccines; they were able to protect cattle against challenge by highly pathogenic strains of rinderpest, and goats against the antigenically related PPR virus. All animals were fully protected from clinical disease, indicating that the insertion of the GFP gene did not affect the ability of the virus to act as a vaccine. The serology test to detect antibodies to the GFP marker showed clearly that a humoral response to GFP was only achieved when the GFP was produced in a membrane-
anchored form. A second vaccine marked with the HA protein gene from influenza virus gave the best humoral antibody response to the marker protein in cattle and was also highly effective in protecting cattle from virulent rinderpest challenge. This is the most promising candidate marked vaccine for cattle.

No evidence could be found that the marker proteins were incorporated into the virus envelope. This is an important safety point for using the marked vaccines widely in the field.

**Impact**
Reverse genetics technology has enabled the production of two new genetically marked vaccines for RPV, which is a major cause of death in cattle in affected areas of the world (i.e. parts of eastern Africa and southern Asia). Using a membrane-anchored GFP as a marker generated a good antibody response, while a much stronger antibody response was generated to the influenza HA marker protein.

The positive impact of this research will be best seen in areas where vaccination is carried out in a sporadic manner such as those suffering civil unrest and war, as in southern Sudan and Somalia.

Livestock farmers in these areas are under immense strain, and any help scientists can give them to improve disease control will have a positive impact upon their livelihoods. The use of genetically marked vaccines could greatly help with the control measures in that it would enable animal health workers to distinguish those animals carrying infection from those that have been vaccinated. This will make it possible to determine the status of rinderpest in any area and take the appropriate action.

**Dissemination**
This type of research is extremely important to multinational organisations such as the Food and Agriculture Organization of the United Nations (FAO), the International Atomic Energy Agency (IAEA) and the Pan-African Rinderpest Campaign (PARC), which are all involved in co-ordinating programmes whose aim is the global eradication of animal diseases such as rinderpest.

Discussions with the Chief Vet in Kenya have lead to an official request seeking approval from the Kenyan Genetic Manipulation Committee to test the marked vaccines on Kenyan cattle.

**Related projects**
- **R4661** – Using sheep and goat pox vaccines to control rinderpest, PPR, bluetongue and Foot and Mouth diseases
- **R5033** – Field trials of the capripox/rinderpest recombinant virus
- **R6557** – Field trialling of the capripox/rinderpest recombinant virus
- **R5504** – Inducing immune responses
R7362 – Developing a cheap and effective pen-side test that differentiates between vaccinated animals and those infected by the rinderpest virus

**Selected publications**

