

## A Progress Report of the project to develop a vaccine against avian tuberculosis in wildfowl

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*Techniques for monitoring the efficiency of an anti-tuberculosis vaccine for wildfowl have been developed. These include web testing, lymphocyte transformation test (LTT) and an enzyme linked immunosorbance assay (ELISA).*

*Using these methods a number of trial vaccines have been assessed with a fair degree of success. A vaccine has been found that gives increased LTT and web test responses and will, we hope, therefore increase protection from infection. An optimum dose of this potential vaccine has been found and this should be administered to day-old birds.*

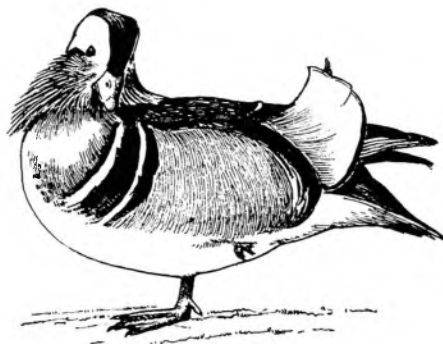
Avian tuberculosis caused by *Mycobacterium avium* has been endemic at The Wildfowl Trust at Slimbridge since 1948 and is now at epidemic proportions accounting for, on average, a third of post-fledgling mortalities. This is a serious problem in itself, but there are also the problems of spreading the disease when birds are translocated to other collections and spreading infection to the wild populations that use the refuges in great numbers. Adult mortality rates as high as 49% in the perching ducks, a particularly susceptible group, have been recorded (Hillgarth & Kear 1981). Indeed, the captive breeding programme of the White-winged Wood Duck *Cairina scutulata*, a species classified as 'vulnerable' in the Red Data Book (King 1978-79), is still seriously affected by deaths from tuberculosis.

During the 2.5 years that this project has been running, considerable progress has been made in developing immunological techniques for assessing a series of potential vaccines. (Precise details of the vaccine are omitted due to possible patent pending.)

### Materials and Methods

#### *Techniques for assessing vaccine efficacy*

- (i) The *in vivo* method of web testing in which tiny doses of reagents prepared from different mycobacteria (the group of bacteria including those that cause tuberculosis) are injected into



the foot web and the size of local responses are measured over a 2-3 day period. Responses indicate immune recognition of the reagents injected.

- (ii) The *in vitro* technique of Lymphocyte Transformation Test (LTT) in which lymphocytes purified from small blood samples are cultured with soluble extracts of mycobacteria. Proliferation of lymphocytes recognising these reagents is measured by the test.
- (iii) An enzyme linked immunosorbance assay (ELISA) which measures the levels of antibodies in the serum to different mycobacteria.

#### *The vaccine studies*

A series of tests have been set up in which groups of ducklings and goslings were vaccinated and control groups were left unvaccinated. The trial vaccines have been given in different forms and doses, and at different ages. After vaccination the test birds and controls were released into the grounds at Slimbridge where infection rates are high.

1936

One hundred and fifty Mallard *Anas platyrhynchos* were vaccinated with one of six potential vaccines at either one day or six weeks old. (Mallard were used due to the comparative ease of raising sufficient num-

bers and their relatively high susceptibility to infection as dabbling ducks.)

1986–87

Half of all White-winged Wood Ducks both present and brought into Slimbridge were vaccinated as adults.

1987

Sixty Mandarins *Aix galericulata* were vaccinated at either one day or six weeks old to find an optimum age for vaccination. (Mandarins were used because of their particular susceptibility as perching ducks).

1988

To find an optimum dose of vaccine in both ducks and geese, 40 Gadwall *Anas strepera* and 40 Ne-ne *Branta sandvicensis* were given one of three doses of vaccine as day-old birds.

1988–89

Half of all birds bred at Slimbridge (and a small percentage of those bred at The Wildfowl Trust Centre at Arundel) were vaccinated.

Every three months, birds from the various vaccine groups are caught, web tested and small blood samples are taken for laboratory analysis.

## Results

### *Techniques for assessing vaccine efficacy*

- (i) Web testing.  
This method has been developed and has shown that local responses vary depending upon vaccine received, vaccinated birds responding more than control birds.
- (ii) LTT.  
Using the culture conditions for duck lymphocytes developed by Higgins & Teoh (1988) and modified by us (Cromie *et al.* 1989), lymphocyte transformation in the presence of mycobacterial antigen has been made to work for the first time. This has proved valuable as a way of showing that immune cells of vaccinated birds

recognise mycobacteria better than do those of control birds.

(iii) ELISA.

This method has been optimised from the method described by Hudson & Hay (1980). The reagents needed are commercially unavailable and suitable ones have only been produced recently. A detailed study of the birds' antibody levels is now being carried out.

### *The vaccine studies*

So far too few of the birds involved in the studies have died to assess directly vaccine efficacy; thus we have discriminated between vaccines on the basis of greater responses in web tests and lymphocyte transformation tests.

In the Mallard study the vaccine giving the best LTT and web test responses was determined and then used in the Mandarin study. This study confirmed the optimum age for vaccination to be one day old.

The results from the Gadwall and Ne-ne study have now helped to determine an optimum dose of vaccine for both ducks and geese.

The results of the study in adult White-winged Wood Ducks have been disappointing with apparently no differences between vaccine and control groups. To date three vaccinated and four control birds have died of tuberculosis.

## Discussion

The project is now at the stage where the vaccine giving the best results has been identified and its optimum dose and age for vaccination have been found. It is hoped that results obtained from web tests, LTT and ELISA are a true reflection of the protection afforded by the vaccine, but this will only be proved in time.

The disappointing results from the White-winged Wood Duck study may be due to the vaccine being ineffective when administered in adult life, as previous exposure to environmental mycobacteria would already have been great, or may be chance as numbers are so small.

Monitoring continues as the project reaches the critical stage where many of the birds approach the age when *M. avium*

infection would be expected. It is the policy to vaccinate half of all birds bred at Slimbridge leaving half as controls. Comparative mortalities due to tuberculosis within the two groups can be assessed over the next few years when birds may begin to develop the disease. There is also a policy to vaccinate all wildfowl sent to and bred at the new

Wildfowl and Wetlands Trust Centre in Llanelli, South Wales.

Avian tuberculosis remains the most devastating and costly bacterial infection of captive birds and, should a successful vaccine be developed, it would be invaluable not only for The Trust but for zoos and private collections around the world.

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