## FIELD TRIAL WORK FOR THE LYMPHOCYTE TRANSFORMATION ASSAY

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Field trial work to explore the possibility of using the Lymphocyte Transformation Assay to study the immunological response of deer to tuberculosis and to perhaps be able to define the Tb status of deer started in June 1985 after Dr Colin McIntosh of the Invermay Research Centre suggested a co-operative project between the Animal Health Division of M.A.F. and Dr Frank Griffin of the Otago University Microbiology Department.

Blood samples were collected from "reactor" deer which had been identified by practitioners during routine Tb testing and forwarded to Otago University for analysis. The results were sufficiently encouraging to warrant further work and an opportunity came with the discovery of a herd close to Invercargill which had a high incidence of Tb.

The herd was bought by the project and testing started in September 1985.

The previous testing history of this herd (The Invercargill Herd) is as follows:

## INVERCARGILL HERD

DATE June 1982	NUMBER TESTED 59	NUMBER REACTORS Nil
October 1983	Hind died	Tb lesions & +ve culture
January 1984	30	1 lesions a veccurrate
February 1984	29	4
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	26	2
March 1984	30	Ni 1
	60	2
April 1984	24	Nil
May 1984	64	Nil
February 1985	26	4
	28	4
	32	6
	20	16
March 1985	15	6
	44	10
April 1985	47	16
June 1985	77	22
August 1985	50	8
	41	14
September 1985	25	1
		TOTAL <u>117</u>

The protocol in the Invercargill herd was for all animals to be skin tested using the Comparative Cervical test, blood samples taken for the Lymphocyte Transformation assay and analysis of other blood parameters, and all animals to be euthanased and autopsied. Samples of any lesions found were submitted to Invermay Animal Health Laboratory for histological examination and a limited amount of culture was also done.

Using the Lymphocyte Transformation assay a survey of the Tb status of all the deer in the herd was done. The routine after this was for 8 animals to be blood sampled and the CC test applied on Mondays, the test read and a further blood sample taken the following Thursday and the animals euthanased and autopsied the same day.

This routine was followed for all the "interesting" animals identified at the initial survey.

The remainder were slaughtered at the Kennington D.S.P. and examined during routine post mortem.

A total of 95 animals were tested and post mortemed of which 36 were fawns, 4 were adult stags and the remaining 55 yearling and adult hinds.

Tb lesions were found in 2 of the fawns, 2 of the stags and 28 of the hinds.

The last animals were autopsied in July 1986. In December 1986 the farm was restocked with yearling stags which were Tb tested using the single intradermal test in March 1987.

All were negative to this test. These animals will be slaughtered during the next few months and post-mortemed at the D.S.P.

In August 1985 another herd with an interesting Tb history came to our attention. The Tb testing history of this herd (the Walmahaka Herd) is as follows:

# WAIMAHAKA HERD

DATE	NUMBER TESTED	NUMBER REACTORS
May 1982	23	Nil
March 1983	38	Nil
November 1983	6	Nil
March 1984	37	Nil
May 1985	59	3
	196	7
August 1985	236	47

It was decided that the objective in this herd would be to try and salvage a clean herd using all tests currently available including the Lymphocyte Transformation assay and indicators from other blood measurements.

On the basis of skin tests and Lymphocyte Transformation essays the herd has been divided into a mob of "clean" animals, a mob of "infected" animals and a mob of "equivocal" animals. The clean mob is kept isolated from the other two mobs. The infected mob is to be slaughtered and some have already been slaughtered. The major testing effort is in the equivocal mob to try and better define the Tb status of each animal before deciding its fate.

The fawns from the "clean" hinds born in 1985 have been transferred to other properties and so far have remained clear to skin tests and blood tests. A similar procedure has been followed for the fawns born in 1986.

#### COMMENTS ON PROCEDURES:

The skin tests have been applied in the way described in the booklet "Tuberculin Testing of Deer".

Blood samples are taken from the jugular vein using 18 G. 1" needles into heparanised vaccutainers and EDTA vaccutainers. A minimum of 2  $\times$  10 ml heparanised and 1  $\times$  10 ml EDTA tubes are taken from each deer. At times we have taken extra tubes for further testing.

In the Invercargill herd restraint was achieved by using an air operated crush. This was satisfactory but slow as the animals were difficult to get into the crush. Some aggressive animals required tranquilisers to allow handling. We used 2% Rompun.

In the Waimahaka herd restraint is by holding the animals against the wall of a pen. This works very well and up to 25 - 30 animals an hour can be bled this way.

### FUTURE:

Further work is continuing in the Waimahaka herd.

I see the Lymphocyte Transformation assay as a useful ancillary test which in no way replaces the current skin tests but provides another way of determining the Tb status of an individual deer or groups of deer.

In a Tb infected herd the Lymphocyte Traqnsformation assay can help to identify the false negative animals to the skin test.

It can also be used to clarify the Tb situation in herds where testing and history leaves some doubt as to the true status of the animals.