Development of an ELISA for the detection of antibodies to *M. bovis* in ferrets

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Introduction

Increasing evidence of the role ferrets are playing in the spread of tuberculosis, due to infection with *Mycobacterium bovis*, has led to the development of an assay for the detection of mycobacterial antibodies in ferret sera. It is hoped that the ELISA may prove to be of value in epidemiological studies within the ferret population.

Methods

Non-infected ferrets

Sera from seven non-diseased ferrets were used in initial experiments to optimise the ELISA conditions. In further experiments another 23 sera were obtained from animals trapped in areas deemed to be free from infection from *M. bovis*. *Post mortem* studies carried out on each of these animals revealed no visible lesions. These animals were used to determine a cut-off point for each assay and to give an indication of the specificity of the ELISA.

TB-infected ferrets

Initially, from the same trapline, five diseased animals with tuberculosis confirmed by histology and culture of *M. bovis*, were used to optimise assay conditions to obtain maximal differentiation between diseased and non-diseased animals. Included in these five ferrets was one animal with histological results typical of an early case of tuberculosis. A further 32 infected animals were used to determine the sensitivity of the assay.

Enzyme linked immunosorbent assay (ELISA)

Enzyme linked immunosorbent assays were performed based on the technique of Voller *et al.* 1980, as modified by Griffin *et al.* 1994, using separate plates (Maxisorp, Nunc-Immuno) which were coated with purified protein derivative antigens, PPD A and PPD B (CSL, Australia) and MPB 70 (Harboe, and Nagai, 1984). A blocking step was introduced whereby 1% BSA in PBS was added to each well prior to the addition of test sera. Horseradish peroxidase linked Protein A (Amersham) was used for the detection of ferret immunoglobulin. After the addition of the conjugate to the plates the assay was completed according to the method described by Griffin *et al.* (1994).

Interpretation

Each test serum was analysed using three separate plates coated with PPD-A (*a*), PPD-B(*b*) and MPB70(*m*) antigens. A cut-off point for each assay (*On*) was based on two standard deviations (Daniel and Debanne, 1987) above the mean OD value (490nm) for sera from non-lesion animals sourced from tuberculosis-free areas. The OD value for the negative sera (*On*) was subtracted from the OD value for the test sera (*Ot*), then multiplied by 100 to convert to ELISA units.

Bovine 1). $Eb - Ea : \geq 20$
2). $Eb - Ea$: 10 - 20 and $Em \geq 5$

Equivocal $Eb - Ea$: 10 - 20 and $Em < 5$

Negative $Eb - Ea : < 10$

Results and Discussion

In initial experiments, with MPB 70 as antigen, positive results were obtained for all five diseased animals (including one animal typical of an early case of tuberculosis) while all seven sera sourced from tuberculosis-free areas gave negative results. Use of a combination of PPD A and PPD B antigens showed sera from four of the five diseased animals to be positive, while six of the seven non-diseased sera were negative, the remaining two sera yielding equivocal results. Detection of mycobacterial antibodies in a ferret in the early stages of disease suggested that the ELISA may be of value in epidemiological studies in ferrets. The antibody response has been shown to be associated with later stages of tuberculosis disease in other species (Griffin *et al.* 1994) where the ELISA has been shown to be a valuable diagnostic tool. The overall sensitivity and specificity figures for the sera tested are given in Table 1.

Table 1: Sensitivity and specificity obtained from ELISA for the detection of mycobacterial antibodies in ferrets.

	Number of Animals (B-A)	**Number of Animals (MPB 70)**
Bovine	24	12
Equivocal	4	0
Negative	9	25
No. Analysed	37	37
Sensitivity	**65%**	**32%**
Bovine	0	0
Equivocal	3	0
Negative	27	30
No. Analysed	30	30
Specificity	**>98%**	**>98%**

The sensitivity obtained in the initial experiments using MPB 70 as antigen (5/5 diseased animals producing a positive result) did not continue when, in later experiments, sera were sourced from animals from more diverse geographical regions. This is suggestive that the particular disease-producing strain of mycobacteria may be important in determining the type of antibody response induced (Griffin *et al.* 1991). This was further highlighted by a group of five false negative antibody assays obtained from nine diseased animals trapped from the same location. The response to other micro-organisms, such as saprophytic mycobacteria, may also influence the sensitivity and specificity of the ELISA. However, a sensitivity of 65% does compare favourably with pre-skin test (ST) sensitivity figures in other species (Griffin *et al.* 1994).

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