

# USE OF MOLECULAR EPIDEMIOLOGY OF *MYCOBACTERIUM BOVIS* TO STUDY REEMERGENCE OF TUBERCULOSIS IN A WILD BRUSHTAIL POSSUM POPULATION

Corner LA<sup>1</sup>, Lugton I<sup>2</sup>, Collins DM<sup>3</sup> & Morris RS<sup>1</sup>

<sup>1</sup> EpiCentre, Institute Veterinary Animal and Biomedical Sciences, Massey University, Palmerston North, New Zealand.

<sup>2</sup> NSW Agriculture, Orange Agriculture Institute, Orange, New South Wales, Australia, 2800.

<sup>3</sup> Agresearch, Wallaceville Animal Research Centre, Wallaceville, New Zealand

In New Zealand, bovine tuberculosis (TB) is endemic in the wild brushtail possums across a quarter of the country and they are the most significant wildlife reservoir<sup>1</sup>. Control of TB in possum populations is by culling which reduces transmission within the possum population and to livestock<sup>2</sup>. TB in possum populations is clustered in both time and space, so simple population control does not necessarily lead to disease eradication.

When TB reappears after depopulation, it is unclear whether it is due to survival of infected animals, survival of the organism in the environment or the immigration of tuberculous possums. We undertook a study of the emergence of TB after possums were eradicated from a 24 hectare site. The spatial and temporal characteristics of TB as it reemerged were monitored over 40 months.

## Materials and Methods

The 24 hectare study site was as described by Pfeiffer<sup>3</sup> (Fig. 1). The area subjected to depopulation included the entire study site and a 50m to 70m zone around the boundary.

The population was monitored initially at 1 and 2 months after the eradication program, then at approximately two-monthly intervals for 40 months. Each monitoring session consisted of trapping on three consecutive nights. Possums were trapped in cage traps, sedated with ketamine hydrochloride, examined and released. TB was diagnosed by clinical examination for characteristic lesions, draining sinuses and swelling of superficial lymph nodes, and the diagnosis was confirmed by the isolation of *Mycobacterium bovis*. Isolates were subtyped using Restriction Endonuclease Analysis<sup>4</sup>.

## Results

The population, as measured by the number of individuals trapped at each session, built up slowly and reached a maximum of 152 at 28 months (Fig. 2). During the study 28 tuberculous possums were identified; four REA types were isolated from 23 possums and 5 isolates were not available for typing.

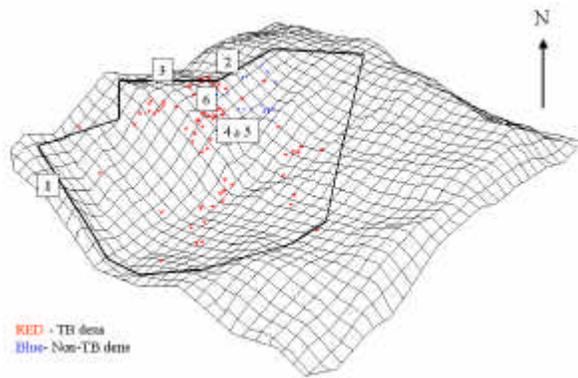


Figure 1. Map of the study site showing where the first 6 tuberculous possums were trapped at the time of diagnosis and the location of the TB hotspots as defined by Pfeiffer et al<sup>5</sup>.

The first cases of TB were recorded at 4 months, then intermittently until 28 months after which the number of cases rose dramatically (Fig 2 & 3). The increased new cases and the increased total number of extant cases continued for the next 10 months. Peak of new cases and total cases occurred at 36 months.

The first four cases of TB were in possums new to the site. The first two cases (Figs 1 and 3), both mature males, were encountered at 4 months; Case 1 (infected with REA type 4B) was trapped only once, on the western boundary. Case 2 (REA type 4) was trapped on the northern boundary. Case 2 had been clinically normal when first trapped at 2 months; it was last seen at 10 months. Case 3, an immature female infected with REA type 41A, was trapped at 6 months on the northern boundary. She had been clinically normal at 2 months and 4 months. The fourth case (REA type 10A), a mature female, was first seen at 9 months and last seen at 11 months.

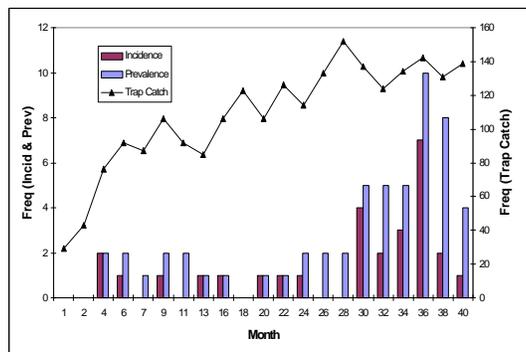


Figure 2. New cases and total number of prevalent cases of TB in brushtail possums, and the number of possums trapped at each monitoring session.

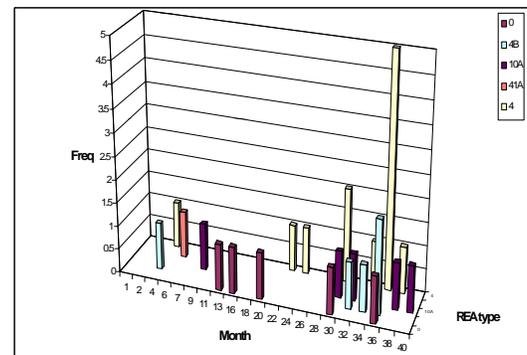


Figure 3. The REA type of the *Mycobacterium bovis* strains isolated from brushtail possums.

The first permanent resident possum found with TB (Case 5) was encountered at 13 months and the second (Case 6) at 16 months; both were mature male possums. The REA types of these strains of *M.bovis* were not determined. Subsequent cases were

seen in possums both new to the site and in those that had been resident for 6 months or more.

Of the 9 possums found to be tuberculous when first trapped on the site, 4 were mature males, 2 were mature females, 2 were immature males and 1 was an immature female. Of the 19 resident possums that became diseased, 11 were mature males, 6 mature females, an immature male and an immature female.

## **Discussion**

TB was continually reintroduced to the site by the migration of tuberculous possums. Three of the first four tuberculous possums were encountered on the periphery of the depopulated area. These mature possums had extended their range into the vacated area. The first tuberculous possum in the centre of the site was an immigrant immature female seen after 6 months. TB in permanent resident possums was not seen until 13 months. Over the length of the study a third of the TB cases were in immigrants, both immature and mature possums.

The number of different *M.bovis* REA types isolated from the immigrant possums showed clearly the constant invasion of the site by tuberculous possums. However, infection was not established in the resident population until about 30 months, although tuberculous possums were on the site for most of the early part of the study. Three of the 4 recognised REA types, plus some strains of undetermined REA type, were involved in the increase at 30 to 38 months. During the period of high incident cases there was an increased number of cases in immigrant possums. This suggests that the factors that influenced the upsurge in TB on the site were also causing an upsurge in TB in the surrounding area, where there had been no population control.

The study showed that after depopulation of an area, TB is reintroduced with migrating possums. It also showed that “hotspots” of TB were related, not to environmental survival, but to some physical feature(s) of the environment that indirectly affected the frequency of infectious interactions between possums.

## **References**

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