Epidémiol. santé anim., 1997, 31-32

STRESS AND THE EPIDEMIOLOGY OF TUBERCULOSIS IN POSSUMS

Lugton I.[1], Pfeiffer D.[2], Jackson R.[2], Morris R.[2]

En Nouvelle-Zélande, le possum Trichosurus vulpecula représente le réservoir de la tuberculose bovine. Les mesures de cortisol d'un échantillon de sérums prélevés sur 900 possums en 5,5 ans pendant une enquête longitudinale représentent la base de ce travail. Huit cent vingt cinq sérums ont été testés en pensant que la mesure du cortisol suite au stress de capture (méthode standardisée) pouvait indiquer le niveau de stress des animaux. Les réponses ont montré un pic en été et un plus petit en hiver. Les taux les plus élevés, au début de l'étude, précèdent le pic épizootique. Ceux que l'on sait être morts de tuberculose ont des taux de cortisol supérieurs à ceux qui ont survécu ou étaient sains. Au niveau répartition géographique, les résultats montrent que la maladie n'est pas liée à l'aspect « stressant » de l'habitat. Des poids faibles précèdent les cas de tuberculose. Les facteurs de stress sont une mauvaise alimentation, le chaud, le froid, l'humidité, qui déclenchent des cas sur des animaux en phase latente. La phase préclinique de la maladie peut durer plusieurs années.

INTRODUCTION

Possums (*Trichosurus vulpecula*) in New Zealand are maintenance hosts for bovine tuberculosis (*M. bovis* infection), and the presence of the disease in wild animal populations poses a serious threat to the success of eradication programmes for tuberculosis in domestic stock. Although the disease has been recognised in possums for over 20 years the epidemiology in this species is still poorly understood. A longitudinal study of tuberculosis in possums was established in 1989 to examine disease behaviour in an infected possum population on a farm in the lower North Island of New Zealand (Pfeiffer, 1994; Jackson, 1995). This investigation has become known as the "Castlepoint" study. One of the hypotheses concerning the epidemiology of tuberculosis in animals, is that environmental stress plays a role in allowing disease progression, and precipitating the death of infected individuals. The current study was initiated to investigate the application of this hypothesis to the free-living possum population of the Castlepoint study. Stress effects on the pathogenesis of tuberculosis may arise from elevation of circulating glucocorticoids, which are known to suppress macrophage function, especially by reducing intracellular destruction of mycobacteria (Cox *et al.*, 1989). As cortisol is the main glucocorticoid secreted by the adrenal gland of possums, and because these animals are considered a corticosteroid sensitive species, this hormone was selected as the most relevant stress parameter to measure in the available stored sera. It was also believed that cortisol released as a response to the acute stress of a standardised trapping regime would provide a guide to the chronic stress levels to which the possums were exposed, as novel stressors superimposed on an underlying stress response will initiate a prolonged burst of cortisol release, the magnitude of which is dependent upon the prevailing background stimulation of the adrenal cortex (Hanlon et al, 1995).

MATERIALS AND METHODS

During the first 5.5 years of this project which commenced in April 1989, over 900 individual possums on the 21 ha site were trapped and examined every 2 months for clinical evidence of tuberculosis. Details on site of capture, number of times caught, length, weight and reproductive and clinical status were recorded. Blood was collected from the majority of possum examined, and the sera retained was stored at -80^{0}C. Through the period from April 1991 till the termination of the first phase of the study in October 1994, 745 heparinised bloods were collected for lymphocyte transformation assay (LTA). At the termination, 428 possums from the study site and surrounds were killed, necropsied and blood sampled. During the study the diagnosis of tuberculosis was made principally by culture of material taken from clinical cases or necropsy samples. A few were also diagnosed tuberculous on the results of gross necropsy, histopathological examination or clinical appearance. An evaluation of disease diagnosis by ELISA on sera, or by the results of LTA, was also conducted (Lugton, 1997). One hundred and ten possums were found tuberculous by either culture, histopathology or clinical examination, and another 78 by were found to have evidence of infection from the blood tests.

Logistic regression analysis was used to examine whether rainfall, or the ratio of average monthly minimum to maximum temperature (data from a weather station 4.5 km distant), body weight and season, had an effect on the point prevalence and incidence of tuberculosis.

Eight hundred and twenty-five sera from 341 possums were assayed (in duplicate) for cortisol concentration using a commercially available radioimmunoassay kit (Coat-A-Count, DPC, Los Angeles). Possum body weight data, and the cortisol measurements (dependent variables) were examined by general linear modelling (GLM) to determine whether tuberculosis infection status, habitat (the site was divided into 4 microhabitats), seasonal and possum characteristics had an influence on the apparent stress levels in the possums, and whether the cortisol

[1] NSW Agriculture, Orange Agricultural Institute, Forest Rd. Orange 2800, Australia.
[2] Department of Veterinary Clinical Sciences, Massey University, Palmerston North, New Zealand.

levels had a significant influence on the epidemiology of tuberculosis. As the data was "messy", in that there were multiple measurements on some individuals taken at varying times, the data was analysed twice. Once with all possible measurements included to improve the power of analyses, and the second time with only a randomly selected subset of the results which included only one measurement from each possum. This second analysis was conducted to show that the results of the first were not unduly biased by the inclusion of the repeated measurements.

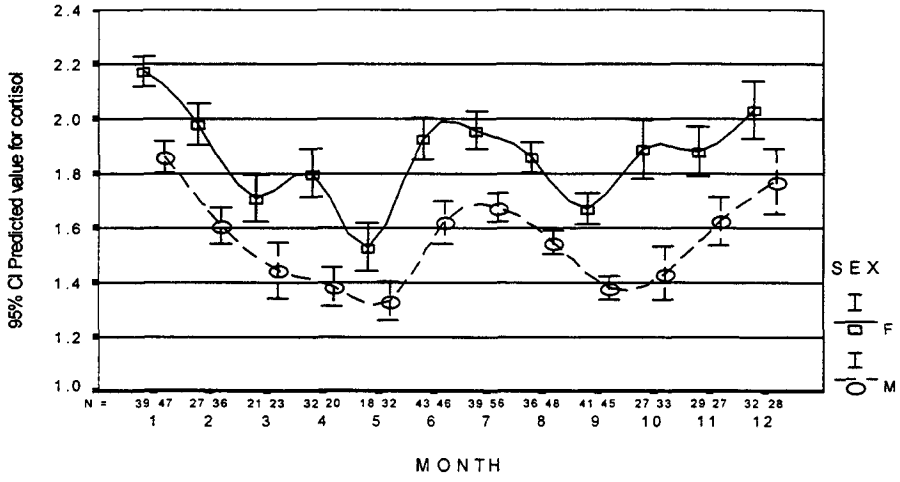
RESULTS

The results of logistic regression analysis of meteorological and other factors which may have been influential on the disease process failed to produce a model in which any confidence could be placed.

The results of GLM analyses were generally comparable whether repeated measures on individuals were, or were not included, suggesting that the inclusion of multiple measurements from some animals did not bias the results unduly. Body weight was significantly affected by site ($p < 0.001$), sex, month, quarter (23 consecutive periods of 3months corresponding to the seasons), length and age. The two sites with the highest estimated marginal mean body weight corresponded to those with a high prevalence of tuberculosis. Plots of predicted body weights derived from the GLM showed that weights at the commencement of the project were low, but rose steadily for the first 2 years, then gradually declined for the next three years, and fell again in the final year. An annual cycle of weight change was found, whereby maximum weight was reached in February, and the lowest weights persisted from May to September, apart from a transient weight increase occurring in August when the possums were gorging on pine catkins. There is a period of rapid weight increase in the period from December to January. Males achieved heavier weights than females, but the annual cycle of variation in weight was similar for both sexes.

There was a significant association between tuberculosis status and cortisol (ug/dl, square root transformed) levels ($p = 0.019$). The estimated marginal mean cortisol values for those with no evidence of disease, those with LTA evidence only, and those with confirmed tuberculosis were 1.61, 1.62 and 1.85 respectively. Contrasts showed that in those which died from tuberculosis, cortisol levels were significantly higher than the other two categories combined and from each other group independently. Plots of serial cortisol measurements on individuals showed no apparent effect of clinical disease elevating cortisol levels.

Figure 1
Plot of the cortisol$^{0.5}$ levels predicted by general linear modeling, showing the variation in both sexes over an annual cycle



Transformed cortisol values were significantly affected by sex, site, month, quarter, number of times captured, weight and length. Although the association with cortisol levels was not strong ($p = 0.031$), estimated marginal means for the four sites showed that the habitats in which the body weights were greatest also had the lowest cortisol levels. Cortisol responses were shown to have a regular seasonal variation (Figure 1), with the highest response in the summer, and a lesser peak in mid-winter. Females showed a minor elevation of stress responses around the two mating/birth periods in autumn and spring. Lactation was not found to be associated with elevated stress responses, and cortisol levels were found to fall significantly in late lactation. The highest cortisol levels occurred early in the study period, coinciding with a period of low body weights, and preceding the most severe outbreak of disease where the point prevalence of disease reached approximately 0.25.

DISCUSSION

The failure to show a statistical relationship between climatic factors and the prevalence and incidence of tuberculosis should not necessarily be taken to mean that climatic factors do not influence the disease process. As in other studies of climatic effects, analyses were limited by low power, the crudity of the dependent variables, and the complexities of the host, agent and environment interactions. Examination of charts depicting the variation in total monthly rainfall versus the long term average monthly rainfall, the average ratio of daily

minimum to maximum temperature versus the long term average, the incidence of new cases and the point prevalence of disease suggested that there may be some biologically plausible associations between climatic events and the pattern of disease. Anecdotal information and meteorological data suggested that the start of the longitudinal study was preceded by a period of drought. This dry period immediately preceded a wet, cool and variable autumn and winter, during which the incidence of tuberculosis climbed rapidly from a low (or zero) base. This was followed by a period of several years where the weather remained mild and stable, around the long term averages. Tuberculosis prevalence during this period initially remained high but gradually declined from the peak prevalence seen in month 5 of the study. A second minor peak in the prevalence occurred following another dry period in 1993, which included a cool and variable winter and spring.

The regular summer peak in cortisol levels (Figure 1) was thought to be caused by heat and/or moisture stress, rather than malnutrition, as the cortisol levels were climbing at a time when body weights were also increasing. The lower winter peak was thought to be caused by cool, wet and variable weather. Although significant microhabitat (site) effects on cortisol levels and body weight were identified, sites with low body weight and high cortisol levels were not associated with an increased prevalence of tuberculosis, which suggested that the clustered distribution of disease observed was independent of the "stressful" nature of the habitat. The higher cortisol stress responses found in possums which died from tuberculosis suggests that these animals were inherently more susceptible to the pathogenic effects of *M. bovis*. There was no evidence to suggest that the infection itself caused a stress response, and in tuberculous humans there is usually a decline in cortisol production commensurate with the severity and chronicity of the infection (Srivastava *et al.*, 1980). High levels of circulating glucocorticoids are thought responsible for tipping the balance between cell-mediated and humoral immune responses in favour of the humoral response, in animals genetically predisposed to high glucocorticoid stress responses (Mason, 1991).

These findings indicate that glucocorticoid assays can be a useful tool for the examination of stressful environmental phenomena and helpful in uncovering aspects of the epidemiology of disease. Major stressful periods involving inadequate nutrition, heat, cold and moisture stress appear to precipitate severe tuberculosis outbreaks, which are believed have their origins in the reactivation of subclinical/latent infection in the susceptible portion of the population (Lugton, 1997). As the period of pre-clinical disease varies substantially, and can be as long as several years, this epidemic of tuberculosis takes several years to subside. Thereafter clinically diseased possums are restricted to areas conducive to transmission of infection.

BIBLIOGRAPHY

Cox J., Knight B., Ivanyi J., 1989. Mechanisms of recrudescence of *Mycobacterium bovis* BCG infection in mice. Infection and Immunity 57, 1719-1724.

Hanlon A., Rhind S., Reid H., Burrels C., Lawrence A., 1995. Effects of repeated changes in group composition on immune response, behaviour, adrenal activity and liveweight gain in farmed red deer yearlings. Applied Animal Behaviour Science 44, 57-64.

Jackson R., 1995. Transmission of Tuberculosis (*Mycobacterium bovis*) by Possums. Unpublished PhD thesis. Massey University, Palmerston North.

Lugton I., 1997. The Contribution of Wild Animals to the Epidemiology of Tuberculosis in New Zealand. Unpublished PhD thesis. Massey University. Palmerston North.

Mason D., 1991. Genetic variation in the stress response: susceptibility to experimental allergic encephalomyelitis and implications for human inflammatory disease. Immunology Today 12, 57-60.

Pfeiffer D., 1994. The Role of a Wildlife Reservoir in the Epidemiology of Bovine Tuberculosis. Unpublished PhD thesis. Massey University, Palmerston North.

Srivastava R., Mukherji P., Bhargava K., Khanna B., 1980. A study of plasma cortisol in pulmonary tuberculosis. Indian Journal of Tuberculosis 27, 3-6.