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THE EPIDEMIOLOGY OF BOVINE TUBERCULOSIS IN THE COMMON BRUSHTAIL POSSUM

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In New Zealand endemic *Mycobacterium bovis* infection in feral populations of Australian brush-tailed possums (*Trichosurus vulpecula* Kerr) is considered an important reservoir for repeated episodes of tuberculosis infection in cattle. It has gradually been accepted by disease control authorities that in areas where possum tuberculosis is endemic, eradication of tuberculosis is not possible. After years of intensive possum population control programs it has also been recognized that a better understanding of the epidemiology of the disease in possums and the interaction between cattle and possum populations is needed. In April 1989 a longitudinal study of a possum population with endemic tuberculosis was initiated.

METHODS:

The study site is located on a sheep/cattle farm in the southern part of the north island of New Zealand. It occupies about 21 hectares within a 40 hectare paddock. Possums are captured in cage traps on a trapping grid comprising 295 traps. Captured animals are permanently identified on first capture. They are anaesthetized and clinically examined on recapture, at minimum intervals of two months. An animal is categorized as suspected of tuberculosis infection if any enlarged superficial lymphnodes or open lesions are found. In suspect cases fluid is collected from open or closed lesions and submitted for laboratory examination to confirm the presence of *Mycobacterium bovis*. Every animal suspected of tuberculosis infection is radiotagged, in part to ensure that the carcass is found once the animal dies. Where possible, possums which have died during the course of the study have been subjected to post-mortem examination for pathological evidence of tuberculosis. Ecological population parameter estimation methods were used to calculate population size, survival between visits, number of births and immigrants. [1] Den sites of possums were located using radio telemetry. Home range estimates were calculated based on information on trap and den locations using the computer software TELEM88. [2] The convex polygon, the 95% ellipse and the harmonic mean (30%, 60% and 90% contours) method were used. [3] A center point of each home range was calculated using the arithmetic mean method. A mean activity radius was estimated as the average distance of each animal location from the arithmetic center. Estimates were compared between adult sex classes for animals with at least 40 locations available using the t-test and the Mann-Whitney test. [4] Incidence of tuberculosis infection was monitored in a group of about 20 - 30 beef cattle, kept principally on the study site. A geographic information system (GIS) has been used to map the study area. Locations of trap and den sites were digitised to identify spatial patterns in the distribution of tuberculous possums compared with non-tuberculous animals. The spatial interpolation method "kriging" was used to interpolate between point measures such as tuberculosis prevalence at a given trap location. Thiessen polygons were generated from a triangulated irregular network to determine areas influenced by a particular point location. Distances between all point locations such as den sites and centers of activity were calculated using the GIS software. Measurements were compared using the t-test and Mann-Whitney test. The Kaplan-Meier product limit estimator was used to estimate cumulative failure probabilities. Cox proportional hazard regression analysis was used to estimate hazard rates adjusted for covariates and their interactions. Statistical association with cumulative incidence for climatic variables was examined for lags of up to two months. Variables which were considered potential risk factors were included in a multivariate analysis using stepwise logistic regression. Measurements for the most important climatic variables were analyzed retrospectively to determine how representative they were with respect to long term trends, cyclical and seasonal patterns. Monthly climatological data collected since 1972 was used for this comparison.

RESULTS:

Between April 1989 and January 1991 the catch effort totalled 26779 trap catch units over 108 trap nights, with an average catch success of 0.21. 378 individual possums were captured and tagged, or were found untagged within the study area. 41% of possums were females. During a period of 12 months 23% of possums were classified as sexually immature. Major birth peaks with up to 90% of adult females carrying a pouch young were observed in April 1989 and 1990. Minor birth peaks occurred in October 1989 and 1990. Pouch young spent on average between 145 and 197 days with their mothers. 33% of joeys disappeared after independence from their mother. Jolly- Seber estimates of survival probability between successive visits and population size averaged 0.91 (95% confidence interval 0.90 - 0.92) and 144.7 (95% confidence interval 133 - 157) respectively. Immigration and births averaged 11.8 (95% confidence interval 10.1 - 13.6) animals per visit. Based on the convex polygon method home ranges averaged 1.82 (s=1.03) hectares for adult females and 3.27 (s=2.58) for adult males. The sign of the difference between adult sex classes was consistent for the 3 different home range estimators (convex polygon, 95% ellipse and harmonic mean). During the study period 39 possums were found with tuberculous lesions and subsequently confirmed as infected with Mycobacterium bovis by bacteriological examination. *Mycobacterium bovis* was cultured from 5 out of a total of 7 possum carcasses which were found untagged on the pasture in the study area. Period prevalence was 0.11 and monthly prevalence averaged 0.08 (range 0.022 - 0.179). 4% of immatures and 17% of sexually mature possums were found to be tuberculous. Cumulative monthly incidence reached peaks in July 1989 with 0.14 (N=28), in November 1989 with 0.11 (N=61) and in October 1990 with 0.07 (N=54). Except for the first field visit tuberculous possums were always present over the study period. Physical and gross pathological examination of possums showed that 79% of tuberculosis cases had lesions associated with the axillary lymphcenter. Exudate was weeping from lesions of 39% of animals having superficial lymphnodes involved in the disease process. Adult female possums frequently had lesions with open sinuses. In 50% of cases the lung was involved in the disease process. Survival probabilities for lesioned possums were 0.37 (95% CI: 0.2 - 0.55) for a period of 12 months from study entry. Survival estimates for the apparently noninfected population were based on confirmed deaths and possums which had disappeared. Hence, they would include emigration. This cross section of the population had a survival probability of 0.96 (95% CI: 0.88 - 0.98) for confirmed deaths and 0.51 (95% CI: 0.37 - 0.64) for deaths and disappearances over a period of 12 months of follow up. Using Cox regression, tuberculosis infection status, sexual maturity and weight loss were identified as important covariates with regard to survival of infected and apparently noninfected possums. The hazard rate ratio for infected possums compared with disappearances controlling for the other factors in the Cox model was 1.82 (95% CI: 1.02 - 3.2; p = 0.04). The Kaplan-Meier product limit estimator was used to model the time from lesion detection until death. Tuberculous possums had a 50% chance to survive for a period of 2 months after leson detection. Sexual maturity was identified as a statistically significant covariate. The hazard risk ratio was 0.23 (95% CI: 0.01 - 0.81; p = 0.022) for sexually immature tuberculous possums compared with mature possums using Cox regression. The most important climatic factors related to cumulative incidence were "Ratio of Monthly Average Daily Minimum and Maximum Temperature" and "Total Monthly Rainfall", both at a lag of 1 month. Compared with the distribution of values over the period 1972 to 1990, ratios between minimum and maximum daily temperatures in March, June, July 1989 and April, September 1990 were just above the 10th percentile of the longterm distribution. Total monthly rainfall was above the 75th percentile in May 1989 and below the 10th in July and December 1990. The most important population parameters with respect to cumulative incidence were identified as "Average Body Weight in Adult Males (lag1)" and "Survival between Visits (lag1)". Arithmetic distances between centers of activity of individual possums and the nearest tuberculous possum were statistically significantly greater for non- tuberculous possums compared with tuberculous animals. The average distance from individual den locations and the nearest den which had been used by a tuberculous possum was statistically significantly greater for sites which had not been used by tuberculous possums. Cumulative catch location data showed a concentration of tuberculous possums in terms of proportion of total cumulative captures in a single part of the study area. This area did not coincide with areas of highest total captures. The temporal and spatial distribution of areas which were utilized by tuberculous possums shifted within the study area between autumn 1989 and summer 1991. The area where most den sites were concentrated which had been used by tuberculous possums remained relatively stable in space and time. Den site sharing occurred infrequently, mainly between mothers and pouch young. Possums used on average 3.22 dens. *Mycobacterium bovis* was recovered from a pile of three decomposed possum carcasses in a den site with the radiotagged occupant resting on top of them. The majority of den sites were located in flax bushes. In a mob of 31 cattle which were kept in or in the vicinity of the study paddock 15 reactors to the tuberculin test were identified between summer 1989 and autumn 1990.

DISCUSSION:

Based on information from a number of large scale surveys tuberculosis prevalence in possums in New Zealand has been found to average around 2%. This study shows that there is considerable variation in point prevalence in possum populations in time and space even within an area of as small as 21 hectares. Disease information collected during this study was based on the detection of lesions through physical examination or post mortem examination. Therefore, the estimates of disease frequency are likely to be underestimates of true infection within the population. Results of the multivariate analysis suggest that the risk of infection is increased during months with large daily fluctuations in temperature and increased total rainfall. Also, risk of infection increased with loss of body condition and higher survival probabilities. The latter factor is probably a surrogate variable for unidentified extraneous factors (e.g behaviour patterns), because peaks in survival during the spring months coincided with peaks in cumulative incidence. The climatic information available suggests that the winter 1989 was unusually wet with large daily temperature fluctuations. Conditions were closer to normal in winter 1990. This explains why a peak in tuberculosis incidence occurred in winter 1989, but not in winter 1990. A rise in incidence was observed in spring 1989 and 1990. During spring and late summer possums have their mating seasons. This probably results in increased opportunities for transmission and a stress-induced reduction in immunological resistance to infection. Paths for transmission of *Mycobacterium bovis* infection between possums can be broadly categorized in patterns which are static and dynamic in a spatio-temporal sense. As long as an infectious possum is in medium to good condition, it is able to use a number of den sites and utilize an area which allows extensive direct and indirect contact with other possums. Towards the terminal stages of the disease the sick possum will gradually turn into a "static" point source of infection. The period of maximum activity would probably coincide with the highest risk of transmission to other individuals in the population. This period is likely to be extended when environmental conditions are favourable (e.g. during mild winters, summer). The results of this study show that the maximum number of infectious cases would have occurred during periods of adverse weather conditions. But these animals only survived for 1-2 months. When environmental conditions were more favourable, incidence was lower. But these animals presented a risk for infection as a spatially dynamic source of infection for up to 9 months.

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