THE USE OF GENETIC MARKERS TO CLARIFY TUBERCULOSIS EPIDEMIOLOGY

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Traditionally in epidemiological studies, standard diagnostic tests could at best distinguish between susceptible, subclinically and clinically diseased animals. With the advent of molecular techniques it is now possible to reliably differentiate between strains of organisms and show spatial and temporal aspects of transmission among animals using epidemiological marker techniques. This enhances greatly the power of inferences which can be made from field data.

A technique to distinguish strains of _Mycobacterium bovis_ using DNA restriction endonuclease analysis (REA) has been developed in New Zealand (Collins and de Lisle, 1984). It allows restriction patterns of _Mycobacterium bovis_ isolates from cattle, Australian brushtailed possums and other species in various locations to be categorised, so that inferences can be drawn regarding transmission.

MATERIALS AND METHODS

During the first 22 months of a longitudinal field study of a wild possum population with endemic tuberculosis infection (Pfeiffer, 1994), _Mycobacterium bovis_ isolates from possums and three other species sharing the habitat were typed using REA. They were classified according to their similarity with strains which had been described previously by Collins _et al._ (1986).

Information on sex and age class of each of these tuberculous animals had been recorded when the samples for bacteriological examination had been collected. For each of the possums additional temporal and locational information was available depending on how often they were captured and examined during the study period. On detection of tuberculous lesions, possums were equipped with radio transmitters, so that they could be tracked to their den sites regularly, and could be found reliably once they died.

Spatial occurrence of the different strains was displayed and analyzed using the geographic information system software PC-Arc/Info version 3.4D (Environmental Systems Research Institute, Redlands, California, U.S.A.). Locational data, including den site and trap site information for the different possums with the same _Mycobacterium bovis_ strain, was aggregated. Each point location was converted into a Thiessen polygon enclosing the area which potentially had been used by an animal. These polygons were then draped over a triangulated irregular network model derived from a digital terrain model of the study area.

Multiple correspondence analysis (Hoffman and Franke, 1986) was used to create graphical displays of the association between categories of REA types, sex class, age group and season of lesion detection. The space-time interaction between tuberculosis cases was investigated using Mantel's regression approach (Mantel, 1967). Distances and times used in this analysis were based on the time and locations when individual animals were first found with tuberculous lesions.

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RESULTS

Based on a sample of 38 *Mycobacterium bovis* isolates, 4 different REA types were identified within the study area and classified as REA types 4 (n = 14), 4a (n = 21), 4b (n = 1) and 10 (n = 3). These figures include one domestic cattle beast and one feral pig with REA type 4, another domestic cattle beast and feral pig with REA type 4a and a ferret with REA type 10.

One adult female possum with clinical tuberculosis produced a joey (progeny) which was found later with clinical lesions. Both had the same REA strain. Two incidents of sequential den sharing between possums with clinical tuberculosis were recorded, but both times the possums sharing the den were infected with different strains.

Temporal analysis based on cumulative incidence for each of the strains showed that types 4 and 4a dominated at different times during the study period. These dominant strains were also recovered from wild pigs and domestic cattle, collected in each case when the same strain was dominant in possums. Dominant REA type 4a occurred during both winter/spring periods included in the study period, whereas the other dominant strain 4 was most common in the first summer and then disappeared.

Spatial analysis of the presence of the four strains revealed that the two dominant strains occurred in different spatial sub-populations within the study area, which only partially overlapped. The spatial differentiation was associated with preferred sleeping sites of possums, as detected by radiotracking. The two minor strains were isolated from possums which had dispersed into the study site. These animals slept in areas remote from the main sleeping sites, but closer to the feeding sites used by the resident population. These animals died of tuberculosis without their REA types becoming established in the local possum population.

Inspection of the plots produced by multiple correspondence analysis did not reveal any associations between sex and age classes. The plots suggest that REA types 4 and 10 are closely associated and they are more common during spring and summer. REA types 4a and 4b are not associated, but are both more likely to occur during winter.

Analysis of time-space interaction for the major REA types and between them showed that there was evidence of time-space clustering for REA type 4a, but not for type 4 or between the two types.

DISCUSSION

Detailed epidemiological analysis of the data allowed some important inferences to be made with regard to the contribution of various potential transmission paths to the epidemiology of bovine tuberculosis in wild possum populations.

Pseudo-vertical transmission is a very effective transmission path as the case of the mother and joey sharing the REA type demonstrated. If an infected possum joey can survive for a sufficient time, it could be of major importance for maintenance of infection within a population and for spatial spread of infection through infected dispersing juvenile animals.

Using the results of both temporal and spatial analysis two major cycles of transmission were identified within the resident possum population, which occurred in temporal succession and showed only limited spatial overlap. The first cycle was already present at the beginning of the study and disappeared after about 6 months. The second started at about that time and has persisted since.

Dominant REA type 4a was clustered temporally in both winter/spring seasons and spatially as suggested by Mantel's regression analysis. Hence, the two temporal peaks in cumulative incidence of type 4a must have occurred in different locations within the study site. Type 4 was clustered in time, but not as much in space according to time-space regression analysis. This would suggest that there might have been fewer infectious contacts. This finding could explain why type 4 disappeared from the study site.

The two minor strains occurred in tuberculous possums which occupied areas distant from the main sleeping places of the resident possum population, but closer to their feeding sites. It could be concluded that transmission between individuals is likely to be associated with behaviour in the proximity of their sleeping places. Interaction during feeding seems to be less important.

Infection detected in other species was always with an REA type which was present in the possum population prior to its detection in another species, reinforcing evidence from various sources that these other species were spillover hosts.

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