Evaluation of Immunological Tests for Detection of Tuberculosis in Badgers

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Introduction

In areas where there are tuberculosis problems affecting a number of herds, the involvement of infected wildlife in the introduction of Mycobacterium bovis infection into herds act as a constraint to eradication of the disease (Gormley and Collins, 2000). Clinical and post-mortem studies on badgers suggest that infection commonly spreads within social groups via the respiratory tract or by bite wounds between groups (Pritchard et al., 1986). The route of infection is primarily respiratory, with more than 40% of lesions most often being found in the thoracic cavity (bronchial and mediastinal lymph nodes and lung tissue). Although a number of potential transmission routes have been suggested, the precise mechanisms by which badgers transmit tuberculosis to cattle are still unclear. Evidence from a longitudinal study of natural M. bovis infection in badgers has indicated that individual badgers can intermittently shed bacilli over a number of years (Newell et al., 1997). Upon infection with M. bovis, the specific outcome depends on a number of factors relating to both animal host and pathogen. The infection can lead to rapid progression to disease or to the development of a protective immune response. Alternatively, it has been suggested that the infection can be contained in a 'latent' phase, the duration of which depends on many factors including the age and immunocompetence of the host. Wildlife hosts such as the badger appear to be highly susceptible to infection with M. bovis. Following infection, they exhibit a disseminated disease with high bacillary load and multiple lesions. Unlike other species, which die within a few months post-infection, the infected badger appears to survive for longer periods, thus increasing the probability of transmission. Whether this longevity is due to a stronger directed immune response, or is evidence of a 'latency' phase, is unknown.

Protection against infection/disease can be considered in two stages, an early and rapid innate resistance, followed by an acquired 'protective' immune response. It has long been established that the development of protective immunity to tuberculosis is mediated through a specific T lymphocyte-mediated immune response (CMI) in which T lymphocytes (a set of white blood cells that are involved in fighting tuberculosis) and macrophages (another set of blood cell involved in fighting infections) ultimately control infection by inhibiting growth of the tuberculosis mycobacteria (Orme *et al.*, 1993). Thus, by measuring T lymphocyte activity it is possible to indirectly evaluate the capacity of the host to fight the disease (Carpenter *et al.*, 1997).

One of the major constraints to eradicating tuberculosis in badgers has been the general lack of tests for rapid and specific diagnosis of tuberculosis. Presumptive diagnosis of tuberculosis in badgers is made at *post-mortem* by examination for gross lesions followed by histology/pathology. However, if the animal is at the earliest stage of infection these tests may be unreliable and may not detect the infection. The most sensitive and specific test is the 'culture' test where lymph nodes and/or other tissues are cultured for the presence of M. bovis. Although this is considered the 'gold standard' test, its main disadvantage is that it can take up to three months to obtain a result. As with histology it is also contingent on the bovine tubercle bacillus being present in the cultured tissue. Until recently there

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were few reagents for evaluating badger immune responses directed against tuberculosis. An ELISA system (Brock Test) has since been developed in Britain which measures antibody to a protein that is a major target of the antibody response in *M. bovis* infected badgers (Goodger *et al.*, 1994). In general however, the sensitivity of this test is low and only works best when the animal is in the advanced state of disease. A lymphocyte transformation assay (LTA) has also been developed using bovine and avian tuberculin as the source of antigens to detect cell mediated (CMI) responses in infected badgers (Dalley *et al.*, 1999). This test holds considerable promise as it allows for the detection of *M. bovis* infection at a very early stage.

The Study

As a comparative investigation, the objective of the study was to obtain blood samples from badgers in areas with a persistent tuberculosis problem in order to measure and evaluate blood responses to tuberculosis infection. The aims were two fold:

- 1. Determine if the lymphocyte transformation assay (LTA) and the Brock test are sensitive and reliable tests for detection of tuberculosis in badgers.
- 2. Relate the immunological responses of each animal to the pathology observed at *post-mortem* and with culture for *M. bovis*.

Methods

Blood samples were taken by heart puncture immediately following death of the badger. The samples were brought to the Large Animal Clinical Laboratory, fractionated using standard procedures and the blood lymphocytes purified. The responses of the lymphocytes were measured following culture in the presence of the test antigens PPD-bovine and PPD-avian. Serum samples were sent to the Veterinary Laboratories Agency (Weybridge, UK) for the Brock test. Tag numbers were forwarded to Abbotstown who received the badgers and carried out detailed *post-mortem* analysis. The lesion status was then recorded and the information collated with the immunology results. The tuberculosis status of representative animals was confirmed by bacteriological culture from lymph nodes and suspect lesions.





Results and Discussion

Figure 1 summarises the results obtained with conventional pathology and blood-based tests for the 36 badgers sampled. Seven badgers were found to be positive by all tests, demonstrating a positive correlation between conventional tests and blood-based tests. It is likely that these badgers would have the highest potential for transmission of tuberculosis. Regarding the remaining animals a spectrum of results were obtained with the combined tests. For example, six badgers were positive by Brock test and LTA, but were negative by pathological testing. One badger was positive by the Brock test and pathology but not by the LTA. Two animals were negative by conventional testing and LTA but were positive by the Brock test. A further seven animals were negative by conventional testing and by the Brock test but were positive by LTA. It is likely that the particular response profile of each of these badgers reflects their post-*M.bovis* exposure status at the time of sampling. Finally, 13 out of the 36 animals tested were negative by all tests.

Our results indicate that the level of lymphocyte activity is positively correlated with numbers and severity of lesions in infected badgers. Thus, it appears that the LTA can be used to rapidly and readily identify both infected and diseased animals. It is also appears to detect infected animals that are otherwise negative by gross *post-mortem* examination and pathology. If this can be verified, then the LTA positive results may indicate that the prevalence of disease in a population may be higher that that suggested by conventional diagnostic techniques alone. Knowledge of these responses will help our understanding of immune parameters associated with disease progression and protective immunity and can be exploited in the development of a vaccine suitable for use in badgers.

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