Queen's University Research on Bovine Tuberculosis and Badgers

The University has and continues to conduct research into various aspects of bovine tuberculosis, and, in particular, improving ways of detecting the disease in both cattle and badger populations. In addition, important studies on ascertaining how the ecology of the badger in Britain and Ireland differs. Finally, monitoring the NI badger population and factors affecting their numbers has been undertaken since the 1990s**. A short review of this research follows.**

PROJECT 1

InvestNI funded research, Institute of Agri-food and Land Use, Queen's University Belfast to develop new and improved detection methods for exposure of cattle to bovine TB.

Researchers involved:

Professor Chris Elliott, Dr Sharon Doherty, Dr Angela Seaton, IAFLU, School of Biological Sciences, Queen's University Belfast

Collaborator: Dr James McNair, Veterinary Sciences Division, Agri-Food and Biosciences Institute for Northern Ireland, Stormont

Project description:

Current control measures for bovine TB (bTB) rely on the intradermal tuberculin skin test and the Interferon gamma (IFN-γ) test. However, due to the complexity of the disease, both these tests do not correctly identify all infected individuals in the earliest stages of infection. Thus a reservoir of undetected, *M. bovis* infected cattle are present in the NI cattle herds and are a major contributory factor in the persistence of the disease.

A low dose respiratory challenge model was established in cattle that mimicked the typical lung and lymph node lesions found in natural infection. Samples from these animals were examined at regular intervals during the course of the infection to determine the various pathways activated in early stage of bTB. A very intensive study of changes in both gene and protein expression in these samples was undertaken.

Main findings:

We were able to ascertain that several hundred genes and more than 20 proteins had significant alterations in their expression profiles as a direct result of the infection. Pathway analysis of these changes revealed a number of significant pathways including: (1) Cell Mediated Immune Response, (2) Cellular Assembly and Organisation, cellular recognition, (3) Immunological disease, (4) Post translational modification, (5) Molecular transport small molecule biochemistry, and (6) Immune cell trafficking. While a number of these pathways have been implicated previously in tuberculosis infection we have identified potential novel targets which may be beneficial for diagnostic or possibly therapeutics investigation.

Recommendations:

The novel targets identified should be validated in a large field study in NI to determine their effectiveness in the early detection of infected cattle.

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Defra-funded research at the Institute of Agri-food and Land Use Queen's University Belfast to develop new and better detection methods for *Mycobacterium bovis*

PROJECT 2

A two year study to undertake rapid, specific and sensitive detection of *Mycobacterium bovis* infection in animals at slaughter using immunomagnetic separation in combination with phage assay (IMS-phage) has just been completed.

Researchers involved:

Dr Irene Grant, Dr Linda Stewart, IAFLU, School of Biological Sciences, Queen's University Belfast
Collaborators: Dr James McNair and Dr Lyanne McCallan, Veterinary Sciences Division, Agri-Food and Biosciences Institute for Northern Ireland, Stormont

Project description:

A range of *M. bovis*-specific antibodies and peptide ligands were produced to be coated onto paramagnetic beads. The most specific and sensitive coated beads were identified and then used for immunomagnetic separation (IMS) of *M. bovis* from lymph node tissue homogenate. For IMS, the antibody and peptide coated beads are incubated with homogenised lymph node sample and any *M. bovis* cells present bind to the antibody and peptide. When a strong magnet is applied the beads plus any bound *M. bovis* cells can be pulled out of suspension to side of tube and after a couple of washes, to remove residual tissue homogenate, the captured *M. bovis* are amenable to detection by a variety of methods (culture, PCR, ELISA, or phage assay). Our original intention was to couple IMS with a phage-based assay, but in light of early findings when naturally infected lymph nodes were tested, the project ultimately focused on employing IMS in conjunction with PCR (IMS-PCR) and MGIT culture (IMS-MGIT). The performance of the new IMS-based tests to detect *M. bovis* infection was assessed by comparison of IMS-PCR and IMS-MGIT results with statutory TB culture results for 280 bovine lymph node samples collected at slaughter.

Main findings:

- Several novel *M. bovis*-specific monoclonal antibodies and peptides were produced.
- An optimised IMS method for *M. bovis* capture, which employs magnetic beads dually coated with a monoclonal antibody and a peptide, was successfully developed (scientific paper describing this process will be published in May 2012).
- IMS could not be employed with the phage assay to test for viable *M. bovis* in lymph nodes, as originally envisaged, because the captured cells were, apparently, not in a fully viable state at point of capture.
- Instead IMS was employed in conjunction with PCR to provide DNA evidence of *M. bovis* infection in lymph nodes within 48 h of testing, and in conjunction with MGIT culture to detect presence of viable *M. bovis* in lymph nodes. IMS-MGIT culture necessitates up to 8 week incubation period (current statutory TB culture timescale), so does not represent a faster detection method.
- Results of a large-scale survey of 280 lymph nodes (non-visibly lesioned and visibly lesioned, majority from skin test reactor animals) indicated that, together, the IMS-based methods detected around 27% more *M. bovis* infected lymph nodes than current statutory TB culture method.
- Positive IMS-PCR results obtained 48 h post-testing generally translated into positive IMS-MGIT results 8 weeks later, plus a number of additional IMS-MGIT culture positive samples were obtained. These findings suggest that a dual testing approach could permit earlier identification of *M. bovis* infected animals and hence bTB affected herds.

PROJECT 3

An 18 month study to develop and field validate a rapid immunomagnetic separation - lateral flow (IMS-LF) test for detecting *Mycobacterium bovis* infection in badgers and/or badger setts has recently commenced.

Researchers involved:

Dr Irene Grant, Dr Linda Stewart (IAFLU), Prof Ian Montgomery, Dr Neil Reid (*Quercus*), School of Biological Sciences, Queen's University Belfast

Collaborators: Dr Paul Meakin and Dr Jonathan Flint, Forsite Diagnostics Limited, York; Dr Paul (Dez) Delahay and Prof Robbie MacDonald, Food and Environment Research Agency (FERA), Woodchester Park, Gloucestershire

Project description:

Antibodies or peptides generated in course of Defra project SE3262 are being incorporated into a lateral flow device (LFD) test format to provide a rapid field test to detect presence of *M. bovis* in badger faeces. Once the novel *M. bovis*-specific LFD has undergone testing and evaluation in the laboratory for use in conjunction with immunomagnetic separation (IMS), the IMS-LFD test will be taken into the field to assess how it performs as a rapid method of detecting the presence of *M. bovis* in badger faeces collected at setts throughout Northern Ireland. Setts near to bTB affected and bTB unaffected farms will be visited in the course of the study. In the field, a crude IMS will be performed on badger faeces samples and beads applied to the LFD device. An IMS-LFD result will be obtained, photographed, and GPS coordinates recorded at the test site. The residual IMS samples will be returned to QUB to be tested for *M. bovis* by IMS-PCR and IMS-MGIT methods. An evaluation of the performance of the novel IMS-LFD test will be made by comparing field and laboratory results. The final part of the project will involve testing of faeces from badgers of known infection status at the Woodchester research site in Gloucestershire to confirm that the IMS-LFD test is applicable in the GB as well as the NI context.

Evidence on the badger population (*Meles meles*) in Northern Ireland

Researchers involved:
Dr Neil Reid - Centre Manager of *Quercus*, Northern Ireland's Centre for Biodiversity and Conservation Research. Prof W. Ian Montgomery - Professor of Animal Ecology, Queen's University Belfast.

Research Outcomes:

PhD theses by Feore (1994), Sadlier (1999), McCann (2002), George (2011) and Kostka (2011) plus a post-doctoral research project by Reid et al. (2008). Four key publications in international scientific journals are listed as highlights:

1. Feore, S. and **Montgomery, W.I.** (1999) Habitat effects on the spatial ecology of the European badger *Meles meles*. *J. Zool. Lond.* **247**, 537-549.

2. Sadlier, L and **Montgomery I.** (2004) The impact of sett disturbance on badger *Meles meles* numbers: when does protective legislation work? *Biological Conservation*, **119**, 455-462.

3. **Reid, N.**, Etherington, T.R., Wilson, G.J., **Montgomery, W.I.** & McDonald, R.A. (2011) Monitoring and population estimation of the European badger (*Meles meles*) in Northern Ireland. *Wildlife Biology*, 18; 46-57.

4. **Reid, N.**, Wilson, G.J., **Montgomery, W.I.** & McDonald, R.A. (2012) Changes in the prevalence of badger persecution in Northern Ireland. *European Journal of Wildlife Research* 58 (1), 177-183.

A summary of the major findings of this research are detailed below:

PROJECT 4
Badger ecology and epidemiology

- Major aspects of the biology of badgers in Ireland and Great Britain are similar e.g. badgers live in social, territorial groups and are widely distributed across Northern Ireland.
- Differences between Great Britain and Ireland are due to landscape factors e.g. setts and groups smaller; variation in diet between land classes, social groups and at individual level.
- Marginal habitats have larger territories and smaller groups; lowland pastoral areas with occasional woodland have smaller territories with larger groups such that there can be up to 30-fold difference in density.
- Estimation by regular trapping probably underestimates badger numbers by 20%.
- Breeding is seasonal with usually one sow breeding and 2-3 young reaching yearling stage.
- Mature males can cross territorial boundaries; can wander several kilometres from home group.
- Badger territories embrace multiple farms (av. 9); most farms have only one badger social group.
- 40% badgers exposed to pathogen; 14% excrete the pathogen; comparable to other studies; a later study suggested 6% excreting and 2% 'super' excretors (+ve>1 occasion).
- More than 60% farms graze cattle next to neighbours without adequate barriers against cattle-cattle contact.
- Disturbance of setts is associated with smaller groups.
- Badgers show a stress response when trapped and anaesthetised; also elevated cortisol in culture positive badgers; stress is likely to play a role in disease transmission.
- Tb strain types in badger parallel strain types in cattle.

- The chance of badger-cattle contact may be determined by landscape, group and individual variation in behaviour.

PROJECT 5
Current badger population and temporal change

- The number of badger social groups was estimated from a survey during 2007/08 covering 212 x 1km^2 squares throughout Northern Ireland and compared to a similar study conducted during 1990/93.
- Badgers were widespread with 75% of squares containing at least one sett. The mean density of active main setts, which was equivalent to badger social group density, was 0.56 (95%CI 0.46-0.67) active main setts per km^2 during 2007/08.
- Social group density varied significantly among land class groups and counties being highest in Drumlin farmland in County Down.
- The total number of social groups was estimated at 7,600 (95%CI 6,200-9,000) and, not withstanding probable sources of error in estimating social group size, the total abundance of badgers was estimated to be 34,100 (95% CI 26,200-42,000).
- There was no significant change in the badger population from that recorded during 1990/93.
- Sett locations were negatively associated with elevation and positively associated with slope, aspect, soil sand content, the presence of cover, and the area of improved grassland and arable agriculture within 300m of the sett. A model was developed to predict sett locations throughout Northern Ireland at a resolution of 25m.

PROJECT 6
Changes in levels of persecution

- Temporal changes in the prevalence of badger sett disturbance in Northern Ireland were evaluated between 1990/93 to 2007/08 in relation to population status by examining signs of persecution at setts.
- A total of 12.6% of 445 setts surveyed during 1990/93 had been disturbed compared to 4.4% of 653 setts during 2007/08. This was a significant decline (-65%) in the incidence of sett disturbance over the 14-18-year period.
- Most notably, the incidence of digging at badger setts, indicative of local badger baiting activity, declined from 50% to 3.5% of disturbed setts.
- During 1990/93 the most common type of disturbance (50.0%) was “digging at setts”, however, during 2007/08 there was a shift to 72.4% of setts being disturbed by “blocking of sett entrances” indicative of more opportunistic persecution.
- More generally, levels of persecution were associated with large setts in County Down situated in pastoral farming areas. Signs of recent disturbance were significantly more frequent at disused setts suggesting that once disturbed, badgers may vacate a sett indicative of “population perturbation”.
- Implementation of full legislative protection of the badger in Great Britain is thought to have led to increases in badger abundance due to reduced levels of persecution. Conversely, prevalence of badger persecution in Northern Ireland was historically much higher than in Great Britain, and badger abundance remained stable over time despite similar legislative protection.
- The number of badger social groups in Northern Ireland did not differ between the two study periods, suggesting that previously high levels of badger persecution did not limit the number of badger social groups.
- The stability of the badger population in Northern Ireland compared to the growing population in Great Britain cannot be attributed to changes in the prevalence of persecution.

Recommendations Project 4-6

Culling of badgers:

- British and Irish experiences differ. The former find little evidence in favour of culling - any benefit is offset by 'perturbation' i.e. there is a rise in disease around the culled area.
- Benefits are relatively small and may last only a few years.
- Ultimately, this approach is not regarded as cost effective.
- The RoI experience suggests otherwise with a sustained reduction in disease levels in culled areas.
- Differences between GB and RoI are probably due to a combination of the differences in study design and differences in environmental context of the disease. Without a clearly defined, isolated area over which to conduct a cull that is more or less 100% efficient, it is unlikely that any overall benefit in terms of disease control would ensue. Local and national studies suggest that tb in badgers in small, disturbed groups would rise and, hence, make the problem worse.
- **We strongly advise against culling of badgers as a means of controlling bovine TB in its wildlife reservoir.**

Bio-security:

- It has been demonstrated clearly that badgers are a disease reservoir and so keeping them away from places where they might come into contact with cattle is important. Farm buildings are important in this context and relatively cheap measures could be deployed to reduce cattle-badger contact.
- Improved fencing around areas with setts, elevation of water and feeding troughs, use of electric fencing around pasture before cattle are introduced etc. should be routine measures to reduce badger-cattle contact throughout farms.
- Cattle to cattle transmission could be reduced by all round better biosecurity between fields and farms.
- Continued pre- and post-movement testing of cattle is an essential means of reducing disease transmission.
- **Consideration should be given to the deployment of cost effective biosecurity measures for the control of bovine NI. This would require ascertaining the situation 'on the farm'.**

Vaccination of badgers:

- Trials are underway in Great Britain and Ireland using BCG. Results are promising in that BCG reduces incidence of +ve serology by 74% but BCG does not prevent infection.
- Chambers et al (Proc Roy Soc B 2010) suggest 'BCG vaccination of badgers could comprise an important component of a comprehensive programme of measures to control bovine TB in cattle.'
- **Hence, we recommend research on the parameters likely to assist in any such programme of control being initiated in Northern Ireland. For example, factors affecting the status of the disease in the badger population using more advanced test protocols (see Projects 1-3)**.

  Concomitant research is needed to ascertain changes in badger abundance for the purposes of studying disease epidemiology at the local level. Whilst we can be confident that the number of badger social groups has not changed significantly over the last 14-18 years we have little confidence in assessing the change in badger abundance. A large proportion of the variance in badger numbers is accounted by changes in social group

size and not numbers of social groups. Thus, if data are required on the actual numbers of badgers prior to any putative population intervention strategy further research is required to estimate social group size using intensive focal sampling techniques, principally genetic analyses.