Institution: University of Warwick

Unit of Assessment: A5 – Biological Sciences

Title of case study: A Novel Way to Detect Infection Status of Wildlife likely to have Bovine Tuberculosis ('Badger Infection Forensics')

1. Summary of the impact

A novel, reliable, non-invasive and rapid method has been developed to detect excretion of *Mycobacterium bovis*, the causal agent of bovine tuberculosis (bTB), into the environment ("shedding") by wildlife hosts. This test has been used to establish the efficacy of the bTB vaccine on reducing environmental contamination by shedding of *M. bovis* in the faeces (from January 2010). It has also become an important monitoring tool used by VisaVet (European Veterinary Health Surveillance), targeting bTB in wild boar and red deer (from July 2010) to establish bTB reservoirs and take action to protect the cattle stocks. Farmers will benefit and now be able to monitor environmental contamination by *M. bovis*, which allows them to establish biosecurity best practice.

The method includes both a presence/absence score and a quantitative assay of infectious disease load in faecal matter in the environment. This is the first standard assay to determine environmental contamination, the main route for disease spread to cattle, and allows evaluation of the impacts of vaccination, culling and increased movement of badgers during disease-management strategies. This test also enables precise monitoring of cattle herds infected with bovine tuberculosis (bTB) as it advances from the South West to the North East of England.

2. Underpinning research

Professor Elizabeth Wellington and Dr Orin Courtenay research groups in the School of Life Sciences at the University of Warwick, have researched the microbiology and epidemiology of bovine tuberculosis since 2002 (1). Bovine Tuberculosis is one of the most intractable and challenging problems facing UK scientists today and costs the UK Government £600–700 million per annum. If there is no improvement to the current methods of testing and control in cattle herds, bTB is predicted to cost up to £1 billion in the UK over the next 10 years.

Bovine tuberculosis is caused by *M. bovis*. Although modern practices prevent it from spreading to humans, it causes substantial problems to the cattle farming industry. The incidence of infection seems to be increasing despite attempts at control. Although testing and slaughter programmes managed the incidence of bTB in the UK until around 20 years ago, control and eradication have become more difficult owing to the persistent infection of badgers, which serve as a wildlife reservoir of the disease. Badgers became a protected species in 1973 and are now covered by the Protection of Badgers Act 1992; it is a legal requirement to prove that animals are infected before control measures can be implemented. The incidence of bTB in cattle herds in the UK and the Republic of Ireland has increased from 0.05% of herds in the late 1970s to 2.7% in 2000, leading to serious economic and animal welfare issues. The current level of infection in UK cattle is estimated to be over 10% (Blake and Donnelly 2012). There is controversy over the sources of infection, mechanisms of transmission and the most effective policies to contain the disease. bTB is now spreading rapidly from the Southwest to the North and East of England.

Wellington and collaborator Courtenay started work in 2006 identifying and monitoring sources and risk of transmission by further establishing that the transmission between cattle and badgers is most likely due to environmental contamination. Monitoring viable *M. bovis* in badger faeces on cattle pasture (2-7) and in badger populations, in 2007 established that *M. bovis* can survive for long periods of time after being shed into the environment (1). Progressive infection in badgers is correlated with the likelihood of culture-positive sputum, faecal and urine samples, which are all potential sources of onward transmission with between-sample correlations shown by bacteriology (Delahay *et al.* unpublished data), and quantitative polymerase chain reaction (qPCR) techniques (Travis *et al.* 2013, submitted). The empirical risk of cattle infection from environmentally shed *M. bovis* bacilli is illustrated by infection of naïve calves exposed to pasture previously grazed by excreting cattle or previously seeded with wild-type *M. bovis* (reviewed in (2)) and deer to cattle and deer to deer transmission via excreta and shared food. The most important source of infection to grazing cattle is likely to be the inhalation of aerosolised bacilli following investigation or incidental ingestion of badger excretions and average *M. bovis* loads in badger faecal latrines

measured by qPCR are 10^4 to 10^6 cells per gram, which far exceeds the expected infectious dose (3, 7).
The group took the research further and, in collaboration with Eamonn Gormley at University College Dublin, conducted a randomised trial of the effect of giving oral BCG vaccines to wild badger populations on *M. bovis* loads in faecal matter. The non-invasive molecular assay for bTB shedding in badgers using faecal material was validated by an extensive ring-trial between three laboratories including the Animal Health and Veterinary Laboratories Agency (AHVLA), VisaVet (European Veterinary Pathology Laboratory) and University College London (Mike Taylor).
The test will form part of the current Government's strategy to control bTB in cattle.

University of Warwick Staff

- **Professor Elizabeth Wellington**, Professor of Environmental Microbiology, School of Life Sciences (1987 - present).
- **Dr Orin Courtenay**, Epidemiologist, Reader, School of Life Sciences (2005 – present).

Collaborators

- **Dr Eamonn Gormley**, Veterinary Scientist, Senior Research Associate, School of Veterinary Sciences, University College, Dublin. Supplied badger faeces and tissue and infection statistics for population analysis
- **Dr Glyn Hewinson**, Veterinary Scientist, Animal Health & Veterinary Laboratories Agency (AHVLA), Addlestone, Surry, UK. Collaborated in ring trial as second lab doing testing.
- **Dr Mike Taylor**, Microbiologist, Collaborated in ring trial as third lab doing testing.
- **Professor Luca Dominguez**, Vet, VisaVet Health Surveillance Centre, Madrid, Spain. Collaborated in ring trial as third lab doing testing after Taylor.

3. References to the research

(1) Young, J. S., Gormley, E. and Wellington, E. M. H. (2005). Molecular Detection of *Mycobacterium bovis* and *Mycobacterium bovis* BCG (Pasteur) in Soil. Appl. Environ. Microbiol. 71, 1946-1952. Doi: 10.1128/AEM.71.4.1946-1952.2005

(2) Courtenay O., Wellington E. M. H. (2008). *Mycobacterium bovis* in the environment: Towards our understanding of its biology. Cattle Practice. 16, 122-126.

(3) Courtenay, O.,L.A. Reilly, F.P. Sweeney, V. Hibberd, S. Bryan, A. Ul-Hassan, C. Newman, D.W. Macdonald, R. J. Delahay, G. J. Wilson and E. M. H. Wellington. (2006). Is *Mycobacterium bovis* in the environment important for the persistence of bovine tuberculosis? Biology Letters 2: 460-462. Doi: 10.1098.rsbl.2006.0468

(4) Courtenay O, Reilly LA, Sweeney FP, Macdonald DW, Delahay RJ, Wilson GJ, Cheeseman CL, Keeling MJ & Wellington EMH. (2007). Limitations of targeted badger culling based on the detection of environmental *Mycobacterium bovis.* Vet. Record 161, 817-818. Doi: 10.1136/vr.161.24.817

(5) Sweeney F. P., O. Courtenay, V. Hibberd, R.G. Hewinson, L.A. Reilly, W.H. Gaze and E.M.H. Wellington (2007). Environmental monitoring of *Mycobacterium bovis* in badger faeces and badger sett soil using real time PCR, confirmed by immunoflourescence, immunocapture and cultivation. Appl Environ Microbiol. 73, 7471-7473. Doi: 10.1128/AEM.00978-07

(6) Pontiroli, A., Travis, E. R., Sweeney, F. P., Porter, D., Gaze, W. H., Mason, S., Hibberd, V., Woodbine, K., Holden, J., Moore, S., Courtenay, O., Wellington, E.M.H. (2011). Multi-operator DNA extraction trials lead to improved quantitation of *Mycobacterium bovis* in the environment. PLoS One 6 (3):e17916. Doi: 10.1371.journal.pone.0017916

(7) Travis ER, Gaze WH, Pontiroli A, Sweeney FP, Porter D, Mason S, Keeling MJ, Jones RM, Sawyer J, Aranaz A, Rizaldos EC, Cork J, Delahay RJ, Wilson GJ, Hewinson RG, Courtenay O, Wellington EM. (2011). An inter-laboratory validation of a real time PCR assay to measure host excretion of bacterial pathogens, particularly of *Mycobacterium bovis*. PLoS One. 6(11):e27369. Epub 2011 Nov 14. Doi: 10.1371/journal.pone.0027369

Peer-reviewed grants and awards All grants listed were awarded to either Elizabeth Wellington and/or Orin Courtenay as PIs

- Tuberculosis epidemiology and novel transmission routes in rural Tanzania. NIH International Collaborations in Infectious Disease Research (ICIDR) U01. RFA-AI-09-010 (2010-2014), US$3 mln. Joint with University of California, San Francisco; Sokoine University of Agriculture; Muhimbili Medical Research Center, Tanzania. **PI Wellington** http://projectreporter.nih.gov/project_info_description.cfm?aid=7901697&icde=17402708

- Optimisation of sampling strategies for improving sensitivity of *M. bovis* detection by PCR. DEFRA Bovine Tuberculosis RRD project SE3280 (2012-2014), £380k. **Wellington (PI)** with **Courtenay (Co-I)** http://randd.defra.gov.uk/Default.aspx?Menu=Menu&Module=More&Location=None&Completed=2&ProjectID=18036 - Description
- Intra- and extra-cellular mechanisms affecting the persistence of *Mycobacterium bovis* in the environment: towards molecular surveillance of bovine TB. BBSRC BB/E020925/1 (2007-2011), £797k**. Courtenay (PI)** and **Wellington (Co-I)**, University of Warwick. http://www.bbsrc.ac.uk/pa/grants//AwardDetails.aspx?FundingReference=BB/E020925/1
- Validation and epidemiological application of molecular methods for monitoring *M. bovis* survival and dissemination in the environment. DEFRA SE 3231 (2007-2010), £1.3 mln. **Wellington (PI)** and **Courtenay (Co-I)**, University of Warwick. http://randd.defra.gov.uk/Default.aspx?Menu=Menu&Module=More&Location=None&ProjectID=15327&FromSearch=Y&Publisher=1&SearchText=SE3231&SortString=PrjectCode&SortOrder=ASC&Paging=10 - Description
- The Biology of Environmental *Mycobacterium bovis,* and its significance to the epidemiology of bovine tuberculosis. BBSRC 10668 (2004-2007), £381k. **Courtenay (PI)** with **Wellington (Co-I)**, University of Warwick. http://www.bbsrc.ac.uk/pa/grants/AwardDetails.aspx?FundingReference=BBS/B/08868

4. Details of the impact

a) Health and Welfare.

The Irish government provided exclusive access to samples to enable analysis of the impact of oral vaccination on shedding in a natural population of badger[a]. Data derived from sample analysis were used to determine shedding and infectivity during the vaccine trial. This established the efficacy of the oral vaccine, which could be suitable for distribution in food bait.

The group has trained vets in Tanzania to use the methodology to test shedding in cattle. A letter[b] from the Head of Vet School, Sokoine University of Agriculture confirms that the assay is used to monitor *M. bovis* load in wildlife in the National Parks of Ruaha and Serengeti, and cattle in adjoining areas.

The current UK Government[c] will consider adopting this assay in 2013 as it is unique in providing quantitative, highly specific and sensitive analysis of infection loads (Secretary of State for Environment, Food and Rural Affairs made two visits for detailed discussions and will support its use).

The assay is in use for monitoring faecal shedding in wild boar and cattle, and to detect environmental contamination.

b) Decisions by a regulatory authority have been informed by the research

The assay is in use by VisaVet (European Centre for Veterinary Health, EU Agency) to monitor bTB in wild boar and red deer[d], and in Tanzania by vets monitoring shedding in cattle herds on the border area with the Ruaha wildlife park[b]. Recently DEFRA held a workshop at AHVLA (April 2013) and a group of scientists, including the team from the University of Warwick, participated in a discussion to establish which methods were most specific, sensitive and useful to detection of bTB in wildlife. The molecular assay of the University of Warwick's team was selected as the test of choice to detect shedding and to track environmental contamination. This test will be used by Defra.

c) Public awareness of health risks or health benefits has been raised

Farming cooperatives such as the Conservative Rural Affairs Group in the UK[e] contacted us as a result of media interest following Prof. Wellington's BBC interviews during the past 4 years (latest BBC Midlands, March 2013).

In terms of benefits to wildlife conservation, in addition to the work in Tanzania, the assay has been used to monitor bTB in African buffalo (*Syncerus caffer*) in South Africa in collaboration with the Mammal Research Institute, University of Pretoria; Ezemvelo KZN Wildlife, Mtubatuba; and the Faculty of Health Sciences, Stellenbosch University. The group in Stellenbosch have an interest in monitoring human faeces, particularly in neonates in Stellenbosch hospital. The bTB status of cattle and environmental contamination is a major source of infection in Africa, as raw milk is consumed and this can lead to TB transfer to humans if cattle are infected.

Public awareness of bTB infection in badgers in the UK is acute and the group from the University

of Warwick liaises with the UK Badger Trust to demonstrate how the assay and the team's research are providing important parameter values to improve mathematical models of disease transmission and control. This is particularly relevant at present as culling is taking place alongside vaccination in the UK.

d) Animal Health and welfare has been enhanced

The method benefits animal welfare, as the assay is non-invasive and provides monitoring of disease status in wildlife without the need for trapping, anaesthesia, or any kind of trauma that results from other methods, all of which are invasive.

e) Impacts on public policy and services

- Policy debate has been stimulated and politicians have been made aware of the importance of shedding in relation to infectivity and its control by vaccination. Debate was stimulated in both the National Assembly in Wales (14/02/2007) and English parliament (meetings with Secretary of State for Agriculture 03/05/2006 and with Secretary of State for Environment, Food and Rural Affairs 10/01/2013 and 18/07/2013) about the use of the 'Warwick test' to determine levels of environmental contamination, the extent of faecal shedding and risks presented to cattle.
- There is possibility of new technology being adopted; for example, through Government encouragement of UK farmers to take responsibility for biosecurity. Discussions have focused on the implementation of testing for environmental contamination at the farm scale[c].
- The quality, accessibility, acceptability or cost-effectiveness of a public service is likely to have improved. The assay provides the least expensive method of monitoring bTB in badgers and will contribute to effective bTB control in the future in combination with vaccination and limited strategic culling. Cage trapping is about four times more expensive than the PCR tests.

f) Impacts on practitioners and services

- Training of vets has been influenced by the research; the new methodology has been taught to vets (5) in Tanzania and at the AHVLA in the UK, and staff (4) at VisaVet in Spain. The assay represents an improvement in disease monitoring as current practices for wild life rely on time consuming and more expensive cage trapping, tranquilizing and sampling blood for immune assays.
- Practitioners and professionals have used our research methodology in conducting their work. For example monitoring shedding in African Buffalo, which skin tested positive. Monitoring cattle and goats in Tanzania to detect which herds have a hire prevalence of bTB, current prevalence of shedding is 12% over 6 villages with a range of 24.6%.

5. Sources to corroborate the impact

a. **Person who can be contacted: Veterinary Scientist, Senior Research Associate,** School of Veterinary Sciences, University College, Dublin. Can provide confirmation of the utility of the assay for analysis of the impact of vaccination on shedding by scientists involved in the trial, following Republic of Ireland vaccine trial. (Identifier 1).

b. **Letter of support: Professor of Veterinary Medicine**, (responsible for training vets in Tanzania, centre of excellence, Department of Veterinary Medicine and Public Health at the Faculty of Veterinary Medicine, Sokoine University of Agriculture (the letter emphasises the importance of the technology for farmers, wildlife parks and the health of pastoralists). The Professor is responsible for the Veterinary Public Health Unit at the University. His main activities are to teach, research and outreach activities in the field of Veterinary Public Health, Epidemiology, veterinary laws and policies. (Identifier 2)

c. **Person who can be contacted: Secretary of State** for DEFRA. The Secretary of state gave verbal confirmation of the importance of the technology for controlling disease in badgers. (Identifier 3)

d. **Letter of support: Director, VisaVet,** Spain (the letter confirms the current use of the group's technology). (Identifier 4).

e. Letter of support: **Chairman**, **Conservative Rural Affairs Group**. Verifies the effective of Wellington's method of detecting bovine tuberculosis, which is equal to that of the current skin test. Confirms the benefits of the test over alternatives, in terms of cost and animal welfare. (Identifier 5).