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Review

Vaccination against tuberculosis in badgers and cattle: an overview of the challenges, developments and current research priorities in Great Britain

OPEN ACCESS

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Bovine tuberculosis (TB) is a significant threat to the cattle industry in England and Wales. It is widely acknowledged that a combination of measures targeting both cattle and wildlife will be required to eradicate bovine TB or reduce its prevalence until European official freedom status is achieved. Vaccination of cattle and/or badgers could contribute to bovine TB control in Great Britain, although there are significant gaps in our knowledge regarding the impact that vaccination would actually have on bovine TB incidence. Laboratory studies have demonstrated that vaccination with BCG can reduce the progression and severity of TB in both badgers and cattle. This is encouraging in terms of the prospect of a sustained vaccination programme achieving reductions in disease prevalence; however, developing vaccines for tackling the problem of bovine TB is challenging, time-consuming and resource-intensive, as this review article sets out to explain.

'BOVINE tuberculosis (TB) is one of the most complex animal health problems that the farming industry in Great Britain faces today'. This was the view of the Chief Veterinary Officer in 2006 (Reynolds 2006) and, despite advances in our understanding of the disease and its epidemiology, this view still stands. The disease picture varies considerably within Great Britain; it is endemic and spreading in parts of England and Wales while Scotland has been officially TB-free since 2009 and only sees rare sporadic cases from imported cattle (Abernethy and others 2013). Despite regional differences, annual fluctuations and different ways of presenting the data, the overall picture of bovine TB incidence in Great Britain is that it has been on the increase since the early 1980s, although there is evidence that the increase may have plateaued in the last couple of years (Blake and Donnelly 2014). This implies that current TB control measures are slowing but not reversing the spread of disease. In addition, the significant financial and emotional impact bovine TB has on farmers and the cost to government in control (bovine TB has cost the taxpayer £500 million in England alone in the past 10 years [Defra 2014a]) means tackling this disease is a major animal health priority for government. Finally, its complex (and sometimes controversial) epidemiology, recently reviewed by Godfray and others (2013), means only a comprehensive, multifaceted eradication programme is likely to have a significant impact on infection levels. Readers are encouraged to refer to the review by Godfray and colleagues, which provides an excellent understanding of the natural science evidence base relevant to the control of bovine TB in Great Britain, including vaccination.

This review focuses on one component of the English and Welsh eradication programmes; namely vaccination. Vaccination of cattle and/or badgers could contribute to TB control in Great Britain (Delahay and others 2003, Wilson and others 2011). The aim of this review is to set out current knowledge and experience regarding the development and application of vaccines against bovine TB for badgers and cattle. We highlight the most important gaps in our knowledge, where empirical data are lacking, and some of the more significant challenges to implementating vaccination.

The aim of vaccination is to stimulate an immune response in the vaccinated animal, such that it is either resistant to infection or, if infection occurs, it is less susceptible to clinical disease and less likely to spread infection. Even a vaccine that only partially protects animals to the extent that they are less infectious to other animals over their lifetime may still eventually reduce disease prevalence in the population. There are significant gaps in our knowledge regarding the impact that the vaccination of either badgers or cattle could have in practice. For example, there is a lack of empirical data on the effect of vaccinating badgers with the licensed vaccine (BadgerBCG) on TB incidence in cattle.

At present, the vaccine agent for tackling TB in both cattle and badgers is Bacille Calmette-Guérin (BCG), a live attenuated strain of

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Mycobacterium bovis. This has been given to people as a TB vaccine since 1927 and is one of the most widely used of all human vaccines. The BCG strain used in the badger and cattle experiments in the UK is BCG Danish strain 1331 produced by the Statens Serum Institut in Copenhagen, Denmark, which is the strain licensed for human vaccination in the UK. For simplicity, we will refer to this strain from now on as BCG, although not all of the experiments referenced in this review use this strain.

The nature of protective immunity to mycobacterial infection is complex and still only partly understood. Pathogenic mycobacteria evade and exploit the immune system of the host to their advantage (Raja 2004), meaning that infected animals do not readily clear the infection. As a result of this evasion of the immune system, BCG does not induce full protective immunity in all individuals. Thus, a proportion of vaccinated humans and animals can still be infected and develop disease (Barreto and others 2006, Dye 2013, Waters and others 2012). However, laboratory studies have demonstrated that vaccination with BCG can reduce the progression and severity of TB and the excretion of *M bovis* in both badgers and cattle (Buddle and others 1995, Lesellier and others 2011).

Vaccination of badgers

In areas with a reservoir of infection in badgers, the goal of badger vaccination is to reduce the pressure of infection from badgers to cattle such that transmission between the two is eliminated or significantly reduced. A vaccine has the potential to achieve that goal either through preventing infection altogether or by reducing the infectiousness of vaccinated badgers that become infected with *M bovis*. Badger vaccination could also have a role in protecting uninfected badger populations at risk of disease spread, for example, in the face of advancing disease at the edge of bovine TB endemic areas. Both scenarios are novel, as vaccination has, so far, not been used extensively to control chronic bacterial infections such as TB in wildlife (Blancou and others 2009). The only available TB vaccine for badgers (BadgerBCG) was licensed by the UK competent authority the Veterinary Medicines Directorate (VMD) in 2010, following 10 years of studies carried out by the AHVLA (formerly the Veterinary Laboratories Agency [VLA] and the National Wildlife Management Centre of the Food and Environment Research Agency, now also part of AHVLA). It is an injectable vaccine with a Limited Marketing Authorisation and is currently available for use by vets and trained lay vaccinators under prescription from a veterinary surgeon. For more information see Brown and others (2013). Licensing of BadgerBCG required evidence of vaccine safety and efficacy, obtained from laboratory and field studies, but the duration of immunity from BadgerBCG remains unknown.

The value of a vaccination campaign can be assessed in three main ways, each providing a different measure of success (Blancou and others 2009). These are: quantification of vaccine uptake; assessment of the immune response in vaccinated individuals; and evaluation of the epidemiological consequences of vaccination. In the case of BCG vaccination where the vaccine is used as a national disease control tool, the principal interest is in the epidemiological consequences in cattle and badgers, although this is the hardest measure to assess. The likely impact of badger vaccination on TB incidence in cattle is poorly understood. Modelling studies have provided predictions of the effects of vaccination relative to other interventions (Smith and others 2012); however, in the absence of the necessary field data, it is not known whether mathematical predictions will be borne out in reality. Measuring this empirically and accurately would involve monitoring cattle TB incidence in areas where badgers were vaccinated and in other areas where they were not, ideally within a large randomised and controlled field experiment with an appropriately structured sampling framework.

Protective effect of badger vaccination

Our understanding of the effects of vaccination on badger immune responses is derived from laboratory and field studies. Laboratory studies with captive badgers supported the claim that the vaccination of badgers by injection with BCG significantly reduces the number and severity of lesions of tuberculosis caused by *M bovis* (Lesellier and others 2011). The protection afforded to badgers by BCG in experimental challenge models such as these is rarely complete (defined as the absence of visible pathology and the isolation of *M bovis* from tissues), most likely because of the relatively high infection doses used in experimental studies in order to generate reproducible levels of infection. Hence the protection afforded in experimental challenge models may not reflect the level of protection afforded against 'natural challenge' in the wild, where animals may be exposed to lower numbers of virulent bacteria (see experimental evidence for BCG in cattle later in this review). The results of a four-year field study of BCG in wild badgers were consistent with the direct protective effect of BCG observed in experimental studies. Individual badgers that initially tested negative to a panel of diagnostic tests and were presumed uninfected were significantly less likely to subsequently test positive to serological and immunological tests for TB following vaccination, compared to non-vaccinated control animals (Chambers and others 2011, Carter and others 2012). The risk of yielding a positive result was reduced by 54 per cent using a combination of diagnostic tests ('triple test') to detect infection (bacterial culture for *M bovis*, the Brock (TB) Stat-Pak serological test and an interferon-gamma (IFN-γ) test based on the use of specific *M bovis* antigens ESAT-6 and CFP-10). When test results were restricted to culture and Stat-Pak, risk was reduced by 76 per cent, consistent with an additional impact of vaccination in the prevention of disease progression in vaccinated animals that still became infected (Carter and others 2012). The 'triple test' represents the most sensitive panel of tests available to detect infection in a live vaccinated animal, whereas positive Stat-Pak and culture results are better indicators of more advanced infection.

Although the BadgerBCG field study was relatively small-scale and designed primarily as a field safety study, it also demonstrated an indirect beneficial effect of vaccination; evidence of the herd immunity effect of vaccination, whereby unvaccinated individuals are indirectly protected as the vaccine prevents circulation of an infectious agent in susceptible populations by increasing the prevalence of immunity (see review by Kim and others [2011]). In this case, non-vaccinated cubs captured in vaccinated social groups were significantly less likely to test positive to TB when more members of their group had been previously vaccinated. When more than a third of the social group had been previously vaccinated, the risk of non-vaccinated cubs testing positive by culture, Stat-Pak or the IFN-γ test was reduced by 79 per cent (Carter and others 2012). The most plausible explanation for this result is that vaccination had reduced the rate of transmission more effectively in social groups where a higher proportion of animals had been vaccinated during the four-year study. The indirect protective effect conferred to non-vaccinated cubs living in such groups was evident after the point that they had emerged from the sett, that is, at the point at which they could be caught and vaccinated themselves.

There is no evidence of either a beneficial or detrimental effect of BCG in infected badgers. Assuming widespread annual deployment, the beneficial effects of vaccination should accrue over time as the proportion of the population vaccinated increases and animals with pre-existing infection die off naturally. There is no empirical evidence on the optimal size or duration for a badger vaccination programme. Benefits will start to accrue from the onset of immunity and most badgers (whether infected with TB or not) are expected to die off within five years (Wilkinson and others 2000).

Field delivery of an injectable badger vaccine

BadgerBCG has been deployed in an area of Gloucestershire in each of the four years since it was licensed in 2010, as part of the five-year Defra-funded Badger Vaccine Deployment Project (BVDP) (Defra 2014b). The BVDP aims to increase knowledge of the practicalities and costs of deploying injectable BCG, train lay badger vaccinators and build confidence in the principle of badger vaccination. It was not designed to estimate the impact of badger vaccination on the incidence of TB breakdowns in cattle herds. Up until the end of 2013, 182 lay vaccinators from a range of organisations had been trained on the bespoke training course built into the BVDP. The project has provided an understanding of what is logistically possible in terms of injectable vaccine delivery. During the four-month field season in 2013, 834 badgers were vaccinated over an area of approximately 90 km^2 of

farmland, encompassing around 100 farm premises. This was carried out by a core team of five trapper/vaccinators. Nationally, BadgerBCG is being deployed under three main models: government agency-led (accounting for the largest share); voluntary and community sector organisations (with a degree of government support); and commercial operators. Combining deployment under all three models, a total of 2781 badgers were vaccinated in 2013 by 15 organisations. A total of 6788 badger BCG doses were delivered in England and Wales between 2010 and 2013 inclusive.

The single largest vaccination project to date was that initiated in 2012 by the Welsh Government. In the first year of this five-year project the Welsh Government vaccinated 1424 badgers over 241 km^2 of land in west Wales at a cost of approximately £945,000 (Government 2013). In the second year (2013), 1352 badgers were vaccinated over 258 km^2 at a cost of approximately £927,000 (Government 2014). The cost of injectable vaccination has been estimated to be between £2000 to £4000 per km^2, depending on a wide range of factors including the type of organisation delivering the work and environmental factors such as badger density and landscape characteristics. Incorporating emerging models of deployment into economic analyses will be useful as the costs (and benefits) associated with a government agency-led scheme may differ from a stakeholder initiative incorporating voluntary staffing input.

The proportion of the badger population that receives and is protected by vaccination will influence the rate at which the incidence of disease changes in badgers (Wilkinson and others 2004). Estimations of the proportion of the badger population that is trapped are not built into current vaccination projects and therefore this remains a knowledge gap. Estimated trapping efficacy in triplets during the Randomised Badger Culling Trial (RBCT) varied from 35 per cent to 85 per cent (Smith and Cheeseman 2007). However, this is likely to have used a different pattern of trapping over the area and therefore may not be directly comparable.

Oral vaccination

The potential of oral vaccination for controlling diseases where there is a wildlife reservoir is well illustrated by rabies control, where oral vaccination of wildlife has successfully controlled the disease across large parts of Europe and North America (Brochier and others 1991, Slate and others 2005). However, there are important differences between vaccination against rabies and TB, not least the degree of protective immunity afforded by vaccination and the type of vaccine used. The efficacy of oral BCG has been demonstrated in cattle, brushtail possums (*Trichosurus vulpecula*) (Cross and others 2009), wild boar (*Sus scrofa*) (Ballesteros and others 2009) and white-tailed deer (*Odocoileus virginianus*) (Nol and others 2008), as well as badgers (Murphy and others 2014); each following experimental infection with *M bovis* of captive animals, but also against natural infection in wild possums (Tompkins and others 2009). However, the dose for oral administration of vaccination is likely to be higher than that given parenterally because BCG is killed and degraded in the gut and uptake is relatively inefficient (Mortatti and others 1987). Experimental studies in possums have suggested that in order to generate immunity it is necessary for oral BCG to retain viability up until the point of delivery to the intestine (Buddle and others 2006). This has been facilitated through the formulation of BCG in a lipid matrix that provides a stable storage and delivery vehicle (that protects the live attenuated bacillus during passage through the stomach) (Cross and others 2009). Recent success using heat-inactivated *M bovis* to experimentally vaccinate wild boar orally has increased the number of candidate oral vaccines for TB (Garrido and others 2011, Beltran-Beck and others 2014).

Since 2005, Defra and the Welsh Government have funded research into the development of an oral vaccine for badgers. Candidate vaccine baits for badgers have been identified and are being evaluated for palatability and efficacy (degree of protection afforded to badgers that consume a vaccine bait), but the formulation of the vaccine itself is only one element. Linked to this is the need for a practical deployment strategy that will maximise uptake among the target badger population and, as far as possible, minimise consumption by other wildlife species or cattle. Uptake of candidate vaccine baits (not containing BCG) among badgers has been measured for a range of deployment scenarios (for example, spring versus summer deployments, above ground versus below ground, etc) in different populations by adding a harmless biomarker to baits, as used successfully for wild boar (Ballesteros and others 2013). Following bait feeding, badgers are captured and bait uptake rate (proportion of captured badgers that consumed bait) assessed from the presence or absence of the biomarker in blood taken from anaesthetised animals. This work has produced some encouraging results, but further research on both vaccine efficacy and bait deployment needs to be concluded before a final candidate vaccine is ready for licensing.

Current research suggests that baits will need to be deployed at all active badger setts in target areas to maximise bait uptake rates (S. Carter, unpublished results). This relies on the locations of active setts to be known or surveys to be carried out in order to identify them. Badgers in bovine TB endemic areas of the UK tend to live in relatively discrete, contiguous group territories that contain, on average, one main sett where the majority of the social group spend most of its time (Neal and Cheeseman 1996, Roper 2010), with groups also having additional outlying setts. It is possible that considerably less bait could be deployed at the smaller outlying setts, although in practise it is difficult to predict the number of badgers in residence in a sett from field signs (Wilson and others 2003) and, because of this, current research has focused on targeting all active setts.

An oral vaccine will only be a viable control tool if the vaccine bait and associated deployment costs are relatively inexpensive, or less than that for injectable vaccination. The cost of a vaccine and the number of baits deployed at a sett, as well as its efficacy, are likely to be key factors in determining the cost effectiveness of oral vaccine deployment. Too few baits will result in an ineffective strategy and too many will make it economically unviable. The number of baits deployed is likely to represent a compromise between maximising uptake and minimising cost. Deployment costs may be reduced by pre-baiting for a number of days with bait that does not contain the vaccine formulation. This may increase vaccine bait uptake by more 'neophobic' badgers, by habituating them to the novel food source (Delahay and others 2003).

The safety of oral vaccine baits in non-target species must also be considered before an oral badger vaccine can be licensed for use (Blancou and others 2009). Additionally, consumption of baits by non-target species has the potential to adversely affect uptake rates by badgers, and hence vaccine efficacy, especially if the bait used is attractive to a wide range of species. A range of wild species, particularly rodents, may be exposed to bait deployed at badger setts, but there is little evidence from ongoing work with candidate baits to suggest that uptake by non-target species will detrimentally affect the uptake of vaccine baits by badgers (S. Carter, unpublished results). Consumption of an oral vaccine for badgers by cattle needs to be avoided as the ingestion of large quantities of BCG can sensitise cattle to the current tuberculin skin test (Buddle and others 2005). Current research indicates that deployment of bait down setts is the most likely delivery method. This approach would substantially reduce the risk of exposure to cattle and other livestock.

Research priorities

The current focus is on generating data that will allow submission of at least a Limited Marketing Authorisation application to the VMD for an effective, value-for-money oral BCG vaccine. Although oral vaccination of badgers has been demonstrated to give protection experimentally (Corner and others 2010, Murphy and others 2014), it needs to be shown that oral vaccination provides consistent levels of protection, and the minimum dose of BCG needed to provide protection is yet to be defined. As BCG is currently the largest component of the cost of the oral vaccine, this is essential work for reducing the overall cost of the oral product. A programme of field research aimed at determining the uptake by wild badgers of different numbers of biomarked bait is also required.

Vaccination of cattle

Vaccination of cattle against bovine TB could reduce the prevalence, incidence and spread of the disease in the cattle population, reducing

the number, duration and severity of breakdowns. The ability to provide these benefits would be dependent on the effectiveness of a vaccination programme in terms of the vaccine used, the way in which it was deployed, and on the performance of a compatible diagnostic test.

BCG was first demonstrated to be an efficacious vaccine against TB in cattle in 1911, as reviewed in Waters and others (2012). Extensive work has been carried out since to optimise the dose and route of administration of BCG vaccine to cattle. Despite ongoing work to develop more efficacious vaccines, BCG remains the best candidate vaccine for use in the field in the short to medium term.

Protection from BCG in experimental infection studies

The level of protection afforded by vaccination with BCG has been tested in studies where vaccinated cattle were experimentally challenged with relatively high doses (1 to 5 x 10^3 cfu) of *M bovis* administered through the endobronchial route. Experimental challenge carried out in this way results in highly reproducible pathology solely in the lower respiratory tract, which is reflective of the pathology seen in the majority of infected cattle in Great Britain and in other developed countries. As with badger vaccination, the protection afforded to cattle by BCG vaccination in experimental challenge models is assessed by comparing the visible histopathological and microbiological consequences of infection between control and vaccinated animals. However, due to the relatively high infection dose in this experimental infection model, BCG rarely induces 'full' protection (defined as absence of visible pathology and ability to isolate *M bovis* from tissues) but is mainly measured by reductions in visible and microscopic pathology as well as bacterial burden. Since 2005, the AHVLA and its collaborators have carried out a series of experiments comprising 80 vaccinated and 64 control animals in total. Animals vaccinated with two different BCG doses, as early as five days of age and up to nine months old, and challenged with *M bovis* between three and 12 months after vaccination were protected, as demonstrated by a significant reduction of pathology and bacterial loads. Furthermore, differences were observed in the proportion of animals that presented with no visible lesions (NVL) between the BCG-vaccinated and unvaccinated control animals (29 of 80 vaccinated animals compared to two of 64 controls). Some of these NVL animals were also culture-negative, indicating that BCG vaccination in cattle can confer complete protection even in this very stringent challenge model (see also the description of the field experiment results below). The onset of immunity has been demonstrated to be as soon as 25 days after vaccination. The levels of protection calculated from the median reduction of visible pathology scores in vaccinated and control animals in these studies was around 76 per cent (range 50 to 100 per cent). Therefore, as with BCG in other species, it provides cattle a spectrum of protection; some cattle will be fully protected, some cattle will exhibit reduced pathology, and some cattle will not be protected (for reasons we do not understand). When BCG Pasteur strain was delivered to neonatal or very young calves (under six weeks old) it was at least as effective as in older animals (Buddle and others 2003, Hope and others 2005).

Protection of cattle by BCG vaccination in this model, while unchanged between three and 12 months after vaccination, fell to below a statistically significant level when animals were challenged 24 months after vaccination in an additional experiment. The duration of immunity based on these experiments employing a severe experimental challenge protocol is therefore a minimum of one year, and cattle would likely require annual revaccination with BCG based on these data alone (Thom and others 2012), although it is possible that the duration of immunity in natural transmission settings may be longer.

Protection with BCG in natural transmission settings

To provide information about the performance of BCG in a natural transmission setting, the AHVLA conducted a limited pilot study with collaborators in Ethiopia. Thirteen Holstein-Friesian calves obtained from a source of cattle with a bovine TB incidence below 1 per cent were vaccinated with BCG between one and 15 days old. They were introduced into an infected herd containing a large proportion of skin test reactor cattle, between 25 and 96 days after vaccination, along with 14 control calves. Vaccinated and control animals were slaughtered between 12 and 23 months later and examined by postmortem examination and mycobacterial culture (Ameni and others 2010). Visible pathology was significantly reduced in vaccinated cattle. More importantly, 69 per cent of vaccinated animals presented with NVL and were culture-negative compared to 21 per cent of cattle in the control group. This equates to a level of protection against detectable infection, rather than simply a reduction of disease, of 61 per cent. When the condemnation rate at meat inspection was used as an additional measure, 73 per cent of the control calves would have been condemned compared to 23 per cent of the vaccinated calves (68 per cent protection). Although this was a relatively small pilot experiment, these data suggest that the capability of BCG to fully protect against natural infection in the field is higher than in experimental infection studies where a more stringent, high dose infection protocol is employed, although this hypothesis has yet to be confirmed in future experiments.

As the exposure times of vaccinated calves to the infected donor herd varied between 12 and 23 months, this study also provided information on the duration of immunity under field conditions. Animals that were in contact for 23 months were as protected as animals that were in contact for only 10 months, suggesting that the duration of infection under these field exposure conditions is longer than the 12-month minimum duration of infection determined from the experimental studies quoted above. However, as in any field experiment, one cannot guarantee that the infection pressure was constant during the 23 months of experimentation. The findings of this study need to be substantiated, but are consistent with a contemporary study in Mexico where cattle were vaccinated with a different strain of BCG (Lopez-Valencia and others 2010).

Diagnostic test for use in BCG-vaccinated cattle

Vaccination with BCG sensitises cattle to tuberculin-based diagnostic tests, including the single intradermal comparative cervical skin-test (SICCT) that forms the basis of the UK test and slaughter policy for bovine TB. However, while 80 per cent of vaccinated but uninfected cattle test positive to the SICCT six months after vaccination, sensitisation wanes to approximately 10 per cent at nine months after vaccination (Whelan and others 2011). This sensitisation is the reason a diagnostic test is needed, which will allow accurate detection of infected cattle among the vaccinated animals (a so-called DIVA test; differentiate infected from vaccinated animals) and so allow use of a BCG-based cattle vaccine for bovine TB disease control alongside a test and slaughter programme (Vordermeier and others 2011b).

The primary candidate DIVA test is based on the IFN-γ test platform, but is modified from the original tuberculin-based IFN-γ test that has been used since 2006 in Great Britain to increase the sensitivity of testing in some TB herd breakdowns. Instead of tuberculin, the DIVA IFN-γ test exploits antigens that are present in *M bovis* but whose genes were deleted in BCG during its attenuation, namely the ESAT-6 and CFP-10 antigens, reviewed in Vordermeier and others (2011b). However, this version of the test is not as sensitive as the SICCT. Inclusion of the Rv3615c antigen (which is present in the BCG genome but is not secreted by BCG) has improved the sensitivity of the DIVA test, because it detects a cohort of infected animals that escape detection using ESAT-6 and CFP-10 antigens (Sidders and others 2008). The sensitivity of the IFN-γ DIVA test using the CFP-10, ESAT-6 and Rv3615c antigens was estimated in 75 BCG-vaccinated *M bovis*-infected animals and 179 BCG-vaccinated non-infected animals, giving estimates of test sensitivity and specificity of 96.0 per cent (88.77 to 99.17) and 95.53 per cent (91.38 to 98.05), respectively (Table 1). These data also highlight the poor performance of the conventional IFN-γ test in BCG vaccinated animals, which showed a test specificity of 71.51 per cent (64.36 to 78.01). The performance of the DIVA reagents was also evaluated in naturally infected skin-test reactor animals and non-infected control animals. The test sensitivity was 90.43 per cent (83.54 to 95.12) while specificity was 98.70 per cent (97.55 to 99.40). The effect of repeat BCG vaccination on the specificity of the DIVA IFN-γ test has been investigated in BCG-vaccinated non-infected animals and had no impact on the specificity

Table 1: Performance of the interferon-gamma DIVA test

	DIVA[a]	B-A[b]
BCG vaccinated/experimentally infected		
Number positive/total	72/75	70/75
Sensitivity (per cent)	96.03	93.33
95 per cent confidence interval	88.77–99.17	85.15–97.8
BCG vaccinated/not infected		
Number positive/total	8/179	51/179
Specificity (per cent)	95.53	71.51
95 per cent confidence interval	91.38–98.05	64.36–78.01
Field reactors		
Number positive/total	104/115	110/115
Sensitivity (per cent)	90.43	95.65
95 per cent confidence interval	83.54–95.12	90.15–98.57
Controls		
Number positive/total	9/691	10/691
Specificity (per cent)	98.70	98.55
95 per cent confidence interval	97.55–99.40	97.35-99.30

[a] Using the DIVA antigens ESAT-6/CFP-10 and/or Rv3615c, [b] Using bovine and avian tuberculins

of the DIVA IFN-γ test. Constraints on the use of cattle TB vaccines in the EU in the field mean that, to date, field trials using BCG vaccine have not been possible in the UK. The performance of the vaccine and DIVA test under UK field conditions is therefore unknown and remains to be determined.

Recent progress has also been made on using the DIVA antigens in a skin test format (Whelan and others 2010). A protein and peptide cocktail derived from ESAT-6, CFP-10 and Rv3615c allowed differentiation between *M bovis*-infected and BCG-vaccinated cattle when used as a DIVA skin test reagent. Addition of the antigen Rv3020c improved the diagnostic sensitivity still further without compromising specificity in the face of BCG or Johne's disease vaccination (Jones and others 2012). To date, the skin test DIVA has been used in fewer animals than the IFN-γ DIVA test but it offers advantages as it could be easier to deploy and offer a better balance between sensitivity and specificity than the IFN-γ test platform.

Research priorities

The highest TB vaccine research priority for Defra and the Welsh Government is the design of field trials of the cattle vaccine. As stated above, development of the vaccine and the DIVA test has, to date, only been possible in the laboratory in the UK, as the use of vaccination against *M bovis* in cattle is explicitly forbidden in EU legislation. In January 2013, the European Commission outlined a tentative timetable for the use of TB vaccination in cattle in the UK and EU (EU 2014). Included in this are large scale field trials of vaccine efficacy and DIVA test validation. In December 2013, the European Food Standards Authority (EFSA) published its opinion to the EU Commission on the requirements of these field trials (EFSA Panel on Animal Health and Welfare 2013). Defra has recently commissioned the design of these field trials taking into account EFSA's opinion, and this work is due to conclude in summer 2014.

BCG is not yet licensed as a vaccine for use in cattle. In 2012, a Marketing Authorisation application was submitted by the AHVLA to the VMD for assessment. However, the initial assessment has highlighted requirements for additional data that are required before the application can progress. These include further safety studies, which are underway. The results from these studies will be one of the factors that determine whether field trials can be carried out.

After the design process, the field trials themselves could follow. This is subject to agreement on the field trial design by the European Commission and granting of an Animal Test Certificate (ATC) by the VMD, which enables a field trial to be carried out in the UK, as well as a cost-benefit assessment. It is too early to know how many cattle herds would be needed in these trials but the scale of the research programme is likely to be considerable, spanning the different TB risk areas in England and Wales.

Ongoing research aims to improve the sensitivity of the IFN-γ DIVA test (Vordermeier and others 2011a, 2011b) and to seek alternative cytokine/chemokine or other alternative biomarkers that could be used to replace, or be used in conjunction with, the IFN-γ DIVA test. This work also aims to develop biomarkers that correlate with protection. The definition of such biomarkers would accelerate vaccine development as their use as surrogates of protection could reduce the need for lengthy and costly experimental challenge experiments. Several such markers have been identified, including quantification of 'central memory cells' (Vordermeier and others 2009) and production of certain cytokines such as IL-22 that predict vaccine efficacy when measured after vaccination but before *M bovis* infection (Bhuju and others 2012). In addition, work is ongoing to further develop the DIVA skin test.

Research is also underway to develop vaccines that offer higher levels of protection against TB than vaccination with BCG alone. While no single vaccine currently offers equal or superior performance to BCG, when used in combination with BCG, several offer enhanced protection, for example, recombinant human adenovirus-vectored mycobacterial antigens (Dean and others 2014), reviewed in Buddle and others (2011). Further assessment of this adenovirus-based strategy as well as development of other approaches should result in vaccine protocols that impart better protection than with BCG alone, and could prolong the duration of immunity. A longer-term research goal is the development of vaccines that do not sensitise cattle to tuberculin-based diagnostic tests. This would allow the SICCT and conventional IFN-γ test to be used alongside vaccination.

Conclusions

Vaccination of both badgers and cattle are potentially important components of what is necessarily a comprehensive, multifaceted eradication programme. However, the difficulties in achieving wider use of the currently available injectable badger vaccine and deployment of a cattle vaccine and oral badger vaccine are sizeable and will take time to overcome.

Field data suggest that BCG vaccination provides a similar spectrum of protection in badgers as well as cattle and other species whereby some individuals are fully protected, some are partially protected by having reduced disease, and the remainder are afforded no protection at all, but it is not possible to attribute precise figures to these categories. For BCG vaccination of both badgers and cattle, there is currently no direct experimental evidence of reduced transmission of TB to and between cattle. Computer modelling has indicated that sustained badger vaccination campaigns could be beneficial in lowering TB incidence in cattle (Smith and others 2012), but empirical data are lacking. To be able to quantify this contribution would require additional data from a large-scale field trial.

We still know relatively little about how oral vaccination against *M bovis* actually works in any species, how it can be optimised, and how applicable results in one species are to other species. Until the results of ongoing research into an oral badger vaccine are known we cannot be certain of the timescale within which an effective and affordable oral badger vaccine may be available. As of now, no products are ready for licensing.

Despite the undoubted progress that has been made towards the vaccination of cattle against TB, widespread benefit will not be realised until the European Commission permits wide scale use of a vaccine and associated DIVA test. Vaccination of cattle is currently prohibited under EU legislation because of the incomplete protection offered by BCG together with the sensitisation of vaccinated animals to the tuberculin skin test (the primary test prescribed under EU legislation for defining the TB status of cattle and cattle herds). Defra and the Welsh Government is working with the European Commission to enable vaccination of cattle to be conducted in UK field trials, and the current redrafting of the EU Animal Health Legislation potentially offers the opportunity to change the legislation on the use of cattle TB vaccines in the medium to long term.

The field trials of cattle vaccine offer significant benefits through better understanding of vaccine efficacy and DIVA test characteristics, but also face substantial challenges relating to legal and practical delivery. Given the likely large scale of the vaccine field trial, they will need to be supported by a strong cost-benefit case. The ultimate endpoint of using BCG in cattle without trade restrictions may not be achieved before 2023. This process will also need to involve adoption of the DIVA test as a trade test by the World Organisation for Animal Health (OIE).

Use of vaccine in badgers or cattle requires buy-in from key stakeholders such as veterinarians and farmers. Acceptance is influenced by economic considerations and social attitudes, such as perceptions surrounding vaccination. Social research, carried out as part of the BVDP, suggests that farmers are cautious about the ability of badger vaccination to reduce the incidence of TB in cattle and the vast majority of farmers do not think it is their responsibility to pay for badger vaccination (Enticott and others 2012). However, around half of the farmers interviewed thought that vaccinating badgers was a good thing to do. In addition, a study of farmer attitudes towards TB control measures suggested that cattle vaccination is the most accepted TB control measure (Bennett and Cooke 2005). Research has also shown that cattle farmers have a substantial willingness to pay for a TB cattle vaccine (Bennett and Balcombe 2012), but this is dependent on its effectiveness and cost. To build on this, any cattle vaccine field trials should have a substantial social research component to further understand the drivers for acceptance by vets and farmers, as this is crucial for a successful vaccine policy.

Despite the obstacles presented, much progress has been made on the development of TB vaccines. The ongoing drive to eradicate TB in England and Wales means we need to use all the disease control measures available, and therefore the need to bring effective and cost-effective vaccines to market that can be used to tackle the problem of bovine TB in England and Wales remains an urgent one.

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References

ABERNETHY, D. A., UPTON, P., HIGGINS, I. M., MCGRATH, G., GOODCHILD, A. V., ROLFE, S. J., BROUGHAN, J. M. & OTHERS (2013) Bovine tuberculosis trends in the UK and the Republic of Ireland, 1995-2010. *Veterinary Record* doi: 10.1136/vr.100969

AMENI, G., VORDERMEIER, M., ASEFFA, A., YOUNG, D. & HEWINSON, R. (2010) Field evaluation of the efficacy of Bacille Calmette Guerin (BCG) against bovine tuberculosis in neonatal calves in Ethiopia. *Clinical and Vaccine Immunology* **17**, 1533-1538

BALLESTEROS, C., GARRIDO, J. M., VICENTE, J., ROMERO, B., GALINDO, R. C., MINGUIJON, E., VILLAR, M. & OTHERS (2009) First data on Eurasian wild boar response to oral immunization with BCG and challenge with a *Mycobacterium bovis* field strain. *Vaccine* **27**, 6662-6668

BALLESTEROS, C., SAGE, M., FISHER, P., MASSEI, G., MATEO, R., DE LA FUENTE, J., ROSSI, S. & GORTÁZAR, C. (2013) Iophenoxic acid as a bait marker for wild mammals: efficacy and safety considerations. *Mammal Review* **43**, 156-166

BARRETO, M. L., PEREIRA, S. M. & FERREIRA, A. A. (2006) BCG vaccine: efficacy and indications for vaccination and revaccination. *Jornal de Pediatria* **82**, S45-54

BELTRAN-BECK, B., ROMERO, B., SEVILLA, I. A., BARASONA, J. A., GARRIDO, J. M., GONZALEZ-BARRIO, D., DIEZ-DELGADO, I. & OTHERS (2014) Assessment of an oral *Mycobacterium bovis* BCG vaccine and an inactivated *M bovis* preparation for wild boar in terms of adverse reactions, vaccine strain survival, and uptake by nontarget species. *Clinical and Vaccine Immunology* **21**, 12-20

BENNETT, R. & BALCOMBE, K. (2012) Farmers' willingness to pay for a tuberculosis cattle vaccine. *Journal of Agricultural Economics* **63**, 408-424

BENNETT, R. & COOKE, R. (2005) Control of bovine TB: preferences of farmers who have suffered a TB breakdown. *Veterinary Record* **156**, 143-145

BHUJU, S., ARANDAY-CORTES, E., VILLARREAL-RAMOS, B., XING, Z., SINGH, M. & VORDERMEIER, H. M. (2012) Global gene transcriptome analysis in vaccinated cattle revealed a dominant role of IL-22 for protection against bovine tuberculosis. *PLOS Pathogens* **8**, e1003077

BLAKE, I. M. & DONNELLY, C. A. (2014) A simple incidence-based method to avoid misinterpretation of bovine tuberculosis incidence trends in Great Britain. *PLOS Currents* 6

BLANCOU, J., ARTOIS, M., GILOT-FROMONT, E., KADEN, V., ROSSI, S., SMITH, G. C., HUTCHINGS, M. R., CHAMBERS, M. A., HOUGHTON, S. & DELAHAY, R. J. (2009) Options for the control of disease 1: targeting the infectious or parasitic agent. In Management of Diseases in Wild Mammals. Eds R. J. Delahay, G. C. Smith, M. R. Hutchings. Springer. pp 97-120

BROCHIER, B., KIENY, M. P., COSTY, F., COPPENS, P., BAUDUIN, B., LECOCQ, J. P., LANGUET, B. & OTHERS (1991) Large-scale eradication of rabies using recombinant vaccinia-rabies vaccine. *Nature* **354**, 520-522

BROWN, E., COONEY, R. & ROGERS, F. (2013) Veterinary guidance on the practical use of the Badger BCG tuberculosis vaccine. *In Practice* **35**, 143-146

BUDDLE, B. M., ALDWELL, F. E., KEEN, D. L., PARLANE, N. A., HAMEL, K. L. & DE LISLE, G. W. (2006) Oral vaccination of brushtail possums with BCG: investigation into factors that may influence vaccine efficacy and determination of duration of protection. *New Zealand Veterinary Journal* **54**, 224-230

BUDDLE, B. M., ALDWELL, F. E., SKINNER, M. A., DE LISLE, G. W., DENIS, M., VORDERMEIER, H. M., HEWINSON, R. G. & WEDLOCK, D. N. (2005) Effect of oral vaccination of cattle with lipid-formulated BCG on immune responses and protection against bovine tuberculosis. *Vaccine* **23**, 3581-3589

BUDDLE, B. M., KEEN, D., THOMSON, A., JOWETT, G., MCCARTHY, A. R., HESLOP, J., DE LISLE, G. W., STANFORD, J. L. & ALDWELL, F. E. (1995) Protection of cattle from bovine tuberculosis by vaccination with BCG by the respiratory or subcutaneous route, but not by vaccination with killed *Mycobacterium vaccae*. *Research in Veterinary Science* **59**, 10-16

BUDDLE, B. M., WEDLOCK, D. N., DENIS, M., VORDERMEIER, H. M. & HEWINSON, R. G. (2011) Update on vaccination of cattle and wildlife populations against tuberculosis. *Veterinary Microbiology* **151**, 14-22

BUDDLE, B. M., WEDLOCK, D. N., PARLANE, N. A., CORNER, L. A., DE LISLE, G. W. & SKINNER, M. A. (2003) Revaccination of neonatal calves with *Mycobacterium bovis* BCG reduces the level of protection against bovine tuberculosis induced by a single vaccination. *Infection and Immunity* **71**, 6411-6419

CARTER, S. P., CHAMBERS, M. A., RUSHTON, S. P., SHIRLEY, M. D. F., SCHUCHERT, P., PIETRAVALLE, S., MURRAY, A. & OTHERS (2012) BCG vaccination reduces risk of tuberculosis infection in vaccinated badgers and unvaccinated badger cubs. *PLOS One* **7**, e49833-e49833

CHAMBERS, M., ROGERS, F., DELAHAY, R., LESELLIER, S., ASHFORD, R., DALLEY, D., GOWTAGE, S. & OTHERS (2011) Bacillus Calmette-Guerin vaccination reduces the severity and progression of tuberculosis in badgers. *Proceedings of the Royal Society B: Biological Sciences* **278**, 1913-1920

CORNER, L., COSTELLO, E., O'MEARA, D., LESELLIER, S., ALDWELL, F., SINGH, M., HEWINSON, R., CHAMBERS, M. & GORMLEY, E. (2010) Oral vaccination of badgers (*Meles meles*) with BCG and protective immunity against endobronchial challenge with *Mycobacterium bovis*. *Vaccine* **28**, 6265-6272

CROSS, M. L., HENDERSON, R. J., LAMBETH, M. R., BUDDLE, B. M. & ALDWELL, F. E. (2009) Lipid-formulated BCG as an oral-bait vaccine for tuberculosis: vaccine stability, efficacy, and palatability to brushtail possums (*Trichosurus vulpecula*) in New Zealand. *Journal of Wildlife Diseases* **45**, 754-765

DEAN, G., WHELAN, A., CLIFFORD, D., SALGUERO, F. J., XING, Z., GILBERT, S., MCSHANE, H., HEWINSON, R. G., VORDERMEIER, M. & VILLARREAL-RAMOS, B. (2014) Comparison of the immunogenicity and protection against bovine tuberculosis following immunization by BCG-priming and boosting with adenovirus or protein based vaccines. *Vaccine* **32**, 1304-1310

DEFRA (2014a) www.defra.gov.uk/animal-diseases/a-z/bovine-tb/. Accessed July 2, 2014

DEFRA (2014b) Badger vaccine deployment project. www.defra.gov.uk/ahvla-en/science/bovine-tb/bvdp. Accessed June 19, 2014.

DELAHAY, R. J., WILSON, G. J., SMITH, G. C. & CHEESEMAN, C. L. (2003) Vaccinating badgers (*Meles meles*) against *Mycobacterium bovis*: the ecological considerations. *Veterinary Journal* **166**, 43-51

DYE, C. (2013) Making wider use of the world's most widely used vaccine: Bacille Calmette-Guerin revaccination reconsidered. *Journal of the Royal Society Interface* **10**, 20130365

EFSA PANEL ON ANIMAL HEALTH AND WELFARE (2013) Scientific opinion on field trials for bovine tuberculosis vaccination. *EFSA Journal* **11**, 3475

ENTICOTT, G., MAYE, D., ILBERY, B., FISHER, R. & KIRWAN, J. (2012) Farmers' confidence in vaccinating badgers against bovine tuberculosis. *Veterinary Record* doi: 10.1136/vr.100079

EU (2014) Bovine TB eradication programme: letter from the European Commission to Owen Paterson. www.gov.uk/government/publications/bovine-tb-eradication-programme-letter-from-the-european-commission-to-owen-paterson. Accessed July 2, 2014.

GARRIDO, J. M., SEVILLA, I. A., BELTRAN-BECK, B., MINGUIJON, E., BALLESTEROS, C., GALINDO, R. C., BOADELLA, M. & OTHERS (2011) Protection against tuberculosis in Eurasian wild boar vaccinated with heat-inactivated *Mycobacterium bovis*. *PLOS One* **6**, e24905

GODFRAY, H. C., DONNELLY, C. A., KAO, R. R., MACDONALD, D. W., MCDONALD, R. A., PETROKOFSKY, G., WOOD, J. L., WOODROFFE, R., YOUNG, D. B. & MCLEAN, A. R. (2013) A restatement of the natural science evidence base relevant to the control of bovine tuberculosis in Great Britain. *Proceedings of the Royal Society B: Biological Sciences* **280**, 20131634

GOVERNMENT, W. (2013) Intensive Action Area Badger Vaccination Report – Year 1. http://wales.gov.uk/topics/environmentcountryside/ahw/disease/bovinetuberculosis/intensive-action-area/badger-vaccination-iaa/intensive-action-area-badger-vaccination-report-year-1/?lang=en. Accessed July 23, 2014.

GOVERNMENT, W. (2014) Intensive Action Area Badger Vaccination Report – Year 2. http://wales.gov.uk/topics/environmentcountryside/ahw/disease/bovinetuberculosis/intensive-action-area/badger-vaccination-iaa/intensive-action-area-badger-vaccination-report-year-2/?lang=en. Accessed July 23, 2014.

HOPE, J. C., THOM, M. L., VILLARREAL-RAMOS, B., VORDERMEIER, H. M.,

HEWINSON, R. G. & HOWARD, C. J. (2005) Vaccination of neonatal calves with *Mycobacterium bovis* BCG induces protection against intranasal challenge with virulent *M bovis*. *Clinical and Experimental Immunology* **139**, 48-56
JONES, G. J., WHELAN, A., CLIFFORD, D., COAD, M. & VORDERMEIER, H. M. (2012) Improved skin test for differential diagnosis of bovine tuberculosis by the addition of Rv3020c-derived peptides. *Clinical and Vaccine Immunology* **19**, 620-622
KIM, T. H., JOHNSTONE, J. & LOEB, M. (2011) Vaccine herd effect. *Scandinavian Journal of Infectious Diseases* **43**, 683-689
LESELLIER, S., PALMER, S., GOWTAGE-SEQUIERA, S., ASHFORD, R., DALLEY, D., DAVE, D., WEYER & CHAMBERS, M. A. (2011) Protection of Eurasian badgers (*Meles meles*) from tuberculosis after intra-muscular vaccination with different doses of BCG. *Vaccine* **29**, 3782-3790
LOPEZ-VALENCIA, G., RENTERIA-EVANGELISTA, T., WILLIAMS JDE, J., LICEA-NAVARRO, A., MORA-VALLE ADE, L. & MEDINA-BASULTO, G. (2010) Field evaluation of the protective efficacy of *Mycobacterium bovis* BCG vaccine against bovine tuberculosis. *Research in Veterinary Science* **88**, 44-49
MORTATTI, R. C., MAIA, L. C. & FONSECA, L. S. (1987) Absorption of *Mycobacterium bovis* BCG administered by the oral route. *Vaccine* **5**, 109-114
MURPHY, D., COSTELLO, E., ALDWELL, F. E., LESELLIER, S., CHAMBERS, M. A., FITZSIMONS, T., CORNER, L. A. & GORMLEY, E. (2014) Oral vaccination of badgers (*Meles meles*) against tuberculosis: comparison of the protection generated by BCG vaccine strains Pasteur and Danish. *Veterinary Journal* **200**, 362-367
NEAL, E. & CHEESEMAN, C. L. (1996) Badgers. T&AD Poyser
NOL, P., PALMER, M. V., WATERS, W. R., ALDWELL, F. E., BUDDLE, B. M., TRIANTIS, J. M., LINKE & OTHERS (2008) Efficacy of oral and parenteral routes of *Mycobacterium bovis* bacille Calmette-Guerin vaccination against experimental bovine tuberculosis in white-tailed deer (*Odocoileus virginianus*): a feasibility study. *Journal of Wildlife Diseases* **44**, 247-259
RAJA, A. (2004) Immunology of tuberculosis. *Indian Journal of Medical Research* **120**, 213-232
REYNOLDS, D. (2006) A review of tuberculosis science and policy in Great Britain. *Veterinary Microbiology* **112**, 119-126
ROPER, T. J. (2010) Badger. New Naturalist Library. Collins
SIDDERS, B., PIRSON, C., HOGARTH, P. J., HEWINSON, R. G., STOKER, N. G., VORDERMEIER, H. M. & EWER, K. (2008) Screening of highly expressed mycobacterial genes identifies Rv3615c as a useful differential diagnostic antigen for the *Mycobacterium tuberculosis* complex. *Infection and Immunity* **76**, 3932-3939
SLATE, D., RUPPRECHT, C. E., ROONEY, J. A., DONOVAN, D., LEIN, D. H. & CHIPMAN, R. B. (2005) Status of oral rabies vaccination in wild carnivores in the United States. *Virus Research* **111**, 68-76
SMITH, G. C. & CHEESEMAN, C. L. (2007) Efficacy of trapping during the initial proactive culls in the randomised badger culling trial. *Veterinary Record* **160**, 723-726
SMITH, G. C., MCDONALD, R. A. & WILKINSON, D. (2012) Comparing badger (*Meles meles*) management strategies for reducing tuberculosis incidence in cattle. *PLOS One* **7**, e39250
THOM, M. L., MCAULAY, M., VORDERMEIER, H. M., CLIFFORD, D., HEWINSON, R. G., VILLARREAL-RAMOS, B. & HOPE, J. C. (2012) Duration of immunity against *Mycobacterium bovis* following neonatal vaccination with bacillus Calmette-Guerin Danish: significant protection against infection at 12, but not 24, months. *Clinical and Vaccine Immunology* **19**, 1254-1260
TOMPKINS, D. M., RAMSEY, D. S., CROSS, M. L., ALDWELL, F. E., DE LISLE, G. W. & BUDDLE, B. M. (2009) Oral vaccination reduces the incidence of tuberculosis in free-living brushtail possums. *Proceedings of the Royal Society B: Biological Sciences* **276**, 2987-2995
VORDERMEIER, H. M., VILLARREAL-RAMOS, B., COCKLE, P. J., MCAULAY, M., RHODES, S. G., THACKER, T., GILBERT, S. C. & HEWINSON, R. G. (2009) Viral booster vaccines improve *Mycobacterium bovis* BCG-induced protection against bovine tuberculosis. *Infection and Immunity* **77**, 3364-3373
VORDERMEIER, M., GORDON, S. V. & HEWINSON, R. G. (2011a) *Mycobacterium bovis* antigens for the differential diagnosis of vaccinated and infected cattle. *Veterinary Microbiology* **151**, 8-13
VORDERMEIER, M., JONES, G. J. & WHELAN, A. O. (2011b) DIVA reagents for bovine tuberculosis vaccines in cattle. *Expert Review of Vaccines* **10**, 1083-1091
WATERS, W. R., PALMER, M. V., BUDDLE, B. M. & VORDERMEIER, H. M. (2012) Bovine tuberculosis vaccine research: historical perspectives and recent advances. *Vaccine* **30**, 2611-2622
WHELAN, A. O., CLIFFORD, D., UPADHYAY, B., BREADON, E. L., MCNAIR, J., HEWINSON, G. R. & VORDERMEIER, M. H. (2010) Development of a skin test for bovine tuberculosis for differentiating infected from vaccinated animals. *Journal of Clinical Microbiology* **48**, 3176-3181
WHELAN, A. O., COAD, M., UPADHYAY, B. L., CLIFFORD, D. J., HEWINSON, R. G. & VORDERMEIER, H. M. (2011) Lack of correlation between BCG-induced tuberculin skin test sensitisation and protective immunity in cattle. *Vaccine* **29**, 5453-5458
WILKINSON, D., SMITH, G. C., DELAHAY, R. J. & CHEESEMAN, C. L. (2004) A model of bovine tuberculosis in the badger *Meles meles*: an evaluation of different vaccination strategies. *Journal of Applied Ecology* **41**, 492-501
WILKINSON, D., SMITH, G. C., DELAHAY, R. J., ROGERS, L. M., CHEESEMAN, C. L. & CLIFTON-HADLEY, R. S. (2000) The effects of bovine tuberculosis (*Mycobacterium bovis*) on mortality in a badger (*Meles meles*) population in England. *Journal of Zoology* **250**, 389-395
WILSON, G. J., CARTER, S. P. & DELAHAY, R. J. (2011) Advances and prospects for management of TB transmission between badgers and cattle. *Veterinary Microbiology* **151**, 43-50
WILSON, G. J., DELAHAY, R. J., DE LEEUW, A. N. S., SPYVEE, P. D. & HANDOLL, D. (2003) Quantification of badger (*Meles meles*) sett activity as a method of predicting badger numbers. *Journal of Zoology* **259**, 49-56