Progress in the development of tuberculosis vaccines for cattle, goats and wildlife

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Welsh welcome
Topic: recent TB vaccine studies

• Cattle
  – BCG
  – Attenuated live vaccines
  – Subunit vaccines (DNA, protein & virus-vector)

• Goats

• Wildlife
  – Possums, badgers, wild boar and deer
Goals of vaccination against bovine TB

Reduce infection and spread of TB in cattle or wildlife species

Developing countries – prevent spread of TB in cattle

Developed countries (wildlife reservoirs of TB)
• vaccinate cattle in at risk areas (or buffer zones)
• vaccinate wildlife (maintenance hosts), if culling not possible
BCG vaccination of cattle

Advantages
- Inexpensive (low dose can be used)
- Safe
- DIVA tests to differentiate from *M. bovis* infection

Disadvantages
- Proportion of vaccinated animals react in skin test
- Protection may be incomplete
- No therapeutic effect
Summary: BCG vaccination of cattle

• Dose (10^3 to 10^6 CFU)\textsuperscript{1,2,5} similar protection

• Strain of BCG (Pasteur and Danish)\textsuperscript{8,9} similar protection

• Lyophilised v fresh culture\textsuperscript{9} similar protection

• Age of animal\textsuperscript{4,6} very young

• Pre-exposure to environmental mycobacteria\textsuperscript{3,7} + or -

\textsuperscript{Ref. Buddle et al., 1995a\textsuperscript{1};b\textsuperscript{2}; 2002\textsuperscript{3}; 2003\textsuperscript{4}; 2013\textsuperscript{5}; Hope et al 2005a\textsuperscript{6};b\textsuperscript{7}; 2011\textsuperscript{8}; Wedlock et al., 2008\textsuperscript{9}}
Vaccination with oral BCG

• Animals exposed to mycobacteria via mucosal surfaces

• Long history of oral immunisation with BCG in humans

• May reduce tuberculin skin test reactivity
Titration of oral dose of BCG (Skin test; 15 weeks post-vaccination)

<table>
<thead>
<tr>
<th>Vaccine group</th>
<th>Skin test responses (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NV</td>
<td>0.5</td>
</tr>
<tr>
<td>SC BCG</td>
<td>3.5</td>
</tr>
<tr>
<td>Oral BCG (10^8 CFU)</td>
<td>5.0</td>
</tr>
<tr>
<td>Oral BCG (10^7 CFU)</td>
<td>3.0</td>
</tr>
<tr>
<td>Oral BCG (10^6 CFU)</td>
<td>1.0</td>
</tr>
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</table>

* Significantly protection against challenge with *M. bovis* (Wedlock et al., 2011)
# Duration of protection

## Median gross pathology scores (range)

<table>
<thead>
<tr>
<th>Vaccine groups</th>
<th>Total LN score</th>
<th>Total lung score</th>
<th>Total path score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n=9)</td>
<td>11 (0.20)</td>
<td>6 (0.19)</td>
<td>16 (0.38)</td>
</tr>
<tr>
<td>BCG 10⁶ CFU (s/c; n=9)</td>
<td>7 (0.12)</td>
<td>0 (0.7)*</td>
<td>8 (0.16)*</td>
</tr>
</tbody>
</table>

### 24 month challenge

<table>
<thead>
<tr>
<th>Vaccine groups</th>
<th>Total LN score</th>
<th>Total lung score</th>
<th>Total path score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n=9)</td>
<td>5 (4.10)</td>
<td>5 (4.10)</td>
<td>10 (8.17)</td>
</tr>
<tr>
<td>BCG 10⁶ CFU (s/c; n=9)</td>
<td>4 (0.9)</td>
<td>4 (3.9)</td>
<td>8 (5.17)</td>
</tr>
</tbody>
</table>

- Significantly reduced scores compared to that for controls, $P<0.05$
- Ref. Thom et al., 2012
Effect of BCG revaccination in young calves

Calf vaccine groups (n=10)
- Not vaccinated
- BCG within 8 hours of birth
- BCG at 6 weeks old
- BCG 8 hours + 6 weeks

Challenge with *M. bovis* at 14-17 weeks, necropsy 4 months later

<table>
<thead>
<tr>
<th>Proportion with TB lesions</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Not vaccinated</td>
<td>10/10</td>
</tr>
<tr>
<td>BCG within 8 hours of birth</td>
<td>0/10</td>
</tr>
<tr>
<td>BCG at 6 weeks old</td>
<td>1/9</td>
</tr>
<tr>
<td>BCG 8 hours + 6 weeks</td>
<td>4/10</td>
</tr>
</tbody>
</table>

Ref. Buddle et al., 2003
Animals which subsequently developed lesions had the highest post-vaccination IFN-γ responses
Long term effects of BCG vaccination and can immunity be boosted

**Vaccine groups (total 79 calves)**

1. Non-vaccinated (n=17)

2. S/c BCG (n=16)

3. S/c BCG, at 2 years revaccinate BCG (n=15)

4. S/c BCG, at 2 years revaccinate with *M. bovis* culture filtrate protein (CFP)/adjuvant (n=15)

5. S/c BCG, at 2 years revaccinate with biobeads displaying mycobacterial proteins, ESAT-6 and Ag85A on the surface (n=16)

Vaccinate calves at 2-4 weeks of age

Revaccinate some groups at 2 years of age

Challenged with TB at 2½ years of age and slaughter 3 months later
Whole blood IFN-γ responses to bovine PPD

A

Significant different to the non-vaccinated group, * P <0.05, ** P <0.01, *** P<0.001
Total lung and pulmonary lymph node lesion scores following challenge with *M. bovis*

BCG-revaccinated group had significantly lower lesion scores than no vaccine group (*P* < 0.001)
Serum antibody responses to *M. bovis* CFP and ESAT-6

Significant different to the non-vaccinated group, * P<0.05, **P<0.01, ***P<0.001

![Graph showing antibody responses to M. bovis CFP and ESAT-6](image-url)
Field BCG trial in cattle - Mexico

- Vaccinated 70 one to two week old calves, BCG s/c
- Equivalent number non-vaccinated

- Followed until 12 months of age

- Positive case of TB defined by the following tests
  - Tuberculin skin test
  - IFN-\( \gamma \) PPD-B
  - IFN-\( \gamma \) ESAT-6/CFP10

BCG-vaccinated group: 6 of 65 classified as TB-infected
Non-vaccinated group: 15 of 66 classified as TB-infected

Ref. Lopez-Valencia et al., 2010
## Field BCG trial in cattle - Ethiopia

- Calves < 3 wks old vaccinated $10^6$ CFU BCG s/c
- In contact with reactor cattle from 3 mths post-vaccination
- In-contact period 10-22 mths, then killed and necropsied

<table>
<thead>
<tr>
<th></th>
<th>Gross pathology (VL) % (n)</th>
<th><em>M. bovis</em> culture +ve % (n)</th>
<th>Spread outside head and lung regions % (n)</th>
<th>Condemned at meat inspection % (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naïve (n=14)</td>
<td>86 % (12)</td>
<td>79 % (11)</td>
<td>21 % (3)</td>
<td>71 % (10)</td>
</tr>
<tr>
<td>BCG (n=13)</td>
<td>38 % (5)</td>
<td>31 % (4)</td>
<td>0 % (0)</td>
<td>23 % (3)</td>
</tr>
<tr>
<td><strong>P-values</strong></td>
<td><strong>0.018</strong></td>
<td><strong>0.021</strong></td>
<td>NS</td>
<td><strong>0.021</strong></td>
</tr>
<tr>
<td><em>(Fisher’s exact test)</em></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

Ref. Ameni et al., 2010
Cattle vaccination trial:  
Muzzle Station  (LandcareResearch, NZ)

- Isolated farm
- TB incidence 5 -10% of cattle/yr

**BCG vaccination trial**
- Five cohorts of ‘free ranging’ cattle, skin-tested, +ves excluded.
- Approx. half vaccinated with BCG orally (mostly $10^8$ CFU)
- Cattle inspected for TB at slaughter 1-3 yr later.
## Progress results

Provisional diagnoses, some cultures pending

<table>
<thead>
<tr>
<th>Cohort birth year</th>
<th>Oral BCG Dose</th>
<th>Vaccinates</th>
<th>Non Vaccinates</th>
<th>$P$ 2 x 2 contingency table</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.5 yr cattle 2006</td>
<td>$10^8$</td>
<td>0/30 (0.00%)</td>
<td>5/130 (3.85%)</td>
<td>0.58</td>
</tr>
<tr>
<td>1.5 yr cattle 2007</td>
<td>$10^8$</td>
<td>5/172 (2.91%)</td>
<td>8/118 (6.78%)</td>
<td>0.15</td>
</tr>
<tr>
<td>Weaners 2008</td>
<td>$10^8$</td>
<td>11/177 (6.21%)</td>
<td>12/85 (14.12%)</td>
<td>0.039*</td>
</tr>
<tr>
<td>Weaners 2009</td>
<td>$10^8$</td>
<td>10/167 (5.88%)</td>
<td>21/106 (19.81%)</td>
<td>&lt;0.001***</td>
</tr>
<tr>
<td>Weaners 2010</td>
<td>$2 \times 10^7$</td>
<td>2/98 (2.04%)</td>
<td>7/84 (8.33%)</td>
<td>0.083</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>28/644 (4.35%)</td>
<td>53/523 (10.13%)</td>
<td>&lt;0.001***</td>
</tr>
</tbody>
</table>
Differentiate BCG-vaccinated from *M. bovis*-infected cattle (DIVA tests)

- Whole blood IFN-\(\gamma\) test (ESAT-6, CFP10, Rv3615c)\(^1\+)

- Differential skin test (ESAT-6, CFP10, Rv3615c, Rv3020c)
  - Recombinant proteins or peptides\(^2,3\)
  - Proteins displayed on biobeads\(^4\)

Ref. Vordermeier et al., 2011\(^1\); many other references\(^+\); Whelan et al., 2010\(^2\): Jones et al., 2012\(^3\); Chen et al., 2014\(^4\)
Attenuated mycobacterial TB vaccines
Attenuated *M. bovis* vaccines

- **Attenuated *M. bovis* ∆RD1 mutant**
  - Vaccination reduced TB pathology and bacterial counts (thoracic LNs)
  - Protection similar to that from vaccination with BCG
  - Neither vaccine induced IFN-γ ESAT-6/CFP10 prior to challenge

- **Attenuated *M. bovis* ∆mce2 double deletion mutant**
  - Lower histopathological lesion score for lungs and LNs v BCG group (P<0.05)

- **BCG strain overexpressing Ag85B**
  - Lower histopathological lesion score for lungs v BCG group (P<0.05)

- **BCG ∆zmp1 mutant (Zmp1 inhibit phagosome-lyosome fusion)**
  - Superior T cell memory responses v BCG

Ref. Waters et al., 2009; Blanco et al., 2013; Rizzi et al., 2012; Khatri et al., 2014
TB DNA vaccines
DNA vaccines for cattle

- DNA alone – little protection

DNA vaccines + immunopotentiators → some protection
- DNA + co-stimulatory molecules (CD80 and CD86) (Maue et al., 2004)
- DNA + adjuvant (DDA) (Cai et al., 2005)

DNA prime/ BCG boost better than BCG alone
(Skinner et al., 2003; Cai et al., 2006)

Both DNA/BCG or BCG/DNA effective
(Skinner et al., 2005)
TB protein vaccines
TB protein vaccines

- Proved difficult to induce protection against TB in cattle with protein vaccines

- Co-administration of BCG and *M. bovis* culture filtrate vaccine at adjacent sites s/c induced improved protection v BCG alone\(^1,2\)

- Display of antigens on particles (Biobeads)

- Use of Toll-like receptors to induce a CMI response
  - TLR2 – Pam3Cys, Pam3CSK4, mycobacterial PIMs\(^2\)
  - TLR4 – Lipid A, glucopyranosyl lipid A\(^3\)
  - TLR7/8 – Resiquimod\(^3\)
  - TLR9 – CpG oligonucleotides\(^1\)

Ref. Wedlock et al., 2005\(^1\); 2008\(^2\); Jones et al., 2014\(^3\)
Virus vectored TB vaccines
Vaccination/challenge doses/routes:

- MVA85A: $1 \times 10^9$ pfu, i.d.
- Ad85A: $2 \times 10^9$ pfu, i.d.
- BCG (SSI): $10^6$ CFU, s.c.
- *M. bovis* (AF2122): 2000 cfu, intratracheal route
Viral vector vaccines reduced pathology with no visible (or histological) signs of infection in a proportion of animals.
Further studies with virus-vector vaccines

- Boosting with adenovirus 5 (Ad5) expressing Ag85A provided better protection than Ad5 expressing 4 mycobacterial proteins (Ad5-TBF)\(^1\)

- Dose and route of immunisation with Ad5-TBF\(^2\)
  - Strongest IFN-\(\gamma\) responses
    - 2 \(\times\) 10\(^9\) infectious units
    - Delivered intradermally

Ref. Dean et al., 2014\(^a\); b\(^2\)
Summary of TB vaccines for cattle

- New TB vaccines for cattle are promising, but no single vaccine is better than BCG

- Combinations of BCG + other TB vaccines (virus vector, protein or DNA) induced better protection than BCG alone

- BCG vaccine shown to reduce disease in experimental and field trials

- Revaccination with BCG – effective when immunity has waned

- Effective non-sensitising TB vaccine for cattle?
Caprine tuberculosis

- Goats are susceptible to *M. caprae* and *M. bovis*
  - no difference in the pathology

- *M. caprae* infection of goats
  - *M. caprae* may cause infections in cattle and humans, and problem for bovine TB diagnosis
  - Cavities in granulomas (similar to pathology in humans)

- Development of a vaccine against caprine TB
  - Assist in control of this disease in goats
  - Valuable model for development of TB vaccines in cattle and humans
Vaccination of goats against TB

- Experimental challenge - low dose of *M. caprae* endobronchially\(^1\)

- BCG vaccination (s/c) significantly reduced pathology and bacterial loads\(^2,4\)

- BCG prime/ adenovirus (Ad) - Ag85A or Ad - Ag85A/TB10.4/TB9.8/Acr2 boost improved protection \(^2,4\)

- DIVA reagents not compromised by vaccination of goats with BCG, BCG/Ad or Johne’s disease (JD) vaccine

- Vaccination of goats with JD vaccine → partial protection against *M. caprae*\(^3\)

- Aerosol challenge of goats with *M. bovis* → lung and LN pathology\(^5\)

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Ref. Perez de Val et al., 2011\(^1\); 2012a\(^2\); 2012b\(^3\); 2014\(^4\); Gonzales-Juarrero et al., 2013\(^5\)
TB vaccines for wildlife

- Brushtail possums - New Zealand
- White-tailed deer - USA
- Badgers - UK and Ireland
- Wild boar - Spain
Oral BCG vaccine for possums

- Encapsulation in a lipid matrix,
  - Small numbers of BCG shed in faeces\(^1\)
  - Protect against experimental aerosol challenge with *M. bovis*
    decrease severity of disease\(^2\)
  - Protection wanes between 6 to 12 months\(^3\)

- Baits stable for 3-5 wks field conditions\(^4\)

- 85 and 100\% of possums accessed baits at bait densities 40-80 sachets/ha\(^4\)

Ref. Wedlock et al., 2005\(^1\); Aldwell et al., 2003\(^2\); Buddle et al., 2006\(^3\); Cross et al., 2009\(^4\)
Protection against natural exposure to *M. bovis* in possums

- Approx. 50% of possums orally vaccinated with BCG
- Trap every 2 mths and check for TB lesions
- Kill out all possums after 2 yrs and necropsy
- Proportion with TB lesions
  12/71 control v. 1/51 vaccinated (P<0.05)

Ref. Tompkins et al., 2009
Duration of protection

- Duration of protection following a single oral BCG

- Possums from a TB-free area were orally vaccinated with BCG released back into the field

- 28 months later, vaccinated and non-vaccinated possums captured, relocated to a PC3 unit and challenged s/c in the paw

- BCG-vaccinated group, significant reduction in LN bacterial counts

Ref. Tompkins et al., 2013
Vaccination of badgers with BCG

- BCG vaccine shown to be safe in badgers\textsuperscript{1}

- Vaccination with BCG via s/c or mucous membranes\textsuperscript{3}, I/mus\textsuperscript{2} or oral BCG in lipid matrix\textsuperscript{4} protected against \textit{M. bovis} challenge

- Comparison of oral vaccination with Pasteur and Danish BCG strains induced similar levels of protection against \textit{M. bovis} challenge\textsuperscript{5}

- Ready uptake of buried baits, 51\% of captured badgers after 7 days\textsuperscript{6}

- Modelling – revealed that combination of vaccination and culling may be more effective than either strategy alone\textsuperscript{7}

- Ref. Lesellier et al., 2006\textsuperscript{1}; 2011\textsuperscript{2}; Corner et al., 2008\textsuperscript{3}; 2010\textsuperscript{4}; Murphy et al., 2014\textsuperscript{5}; Palphramand et al., 2012\textsuperscript{6}; Smith et al., 2012\textsuperscript{7}
Field BCG badger trials

Field trial, 3 years duration (2006-09)$^1$
- 179 vaccinated with $10^6$ CFU BCG i/mus, 83 non-vaccinated
- Vaccination reduced incidence of seroconversion by 74%

Follow up from trial, risk of unvaccinated cubs testing positive to a series of TB tests reduced significantly as proportion of vaccinated individuals in the social group increased$^2$

Irish 3-year field trial –
- 3 zones: 100%, 50% & 0% vaccination with oral BCG
- Annual vaccination: Vaccine/placebo blind coded
- Capture/release, monitor serology, final necropsy all

On-going trials in Wales and England (licenced i/mus BCG vaccine)
- Ref. Chambers et al., 2011$^1$; Carter et al., 2012$^2$
Summary: badger vaccination

- Convincing evidence implicating badgers in spread of TB to cattle

- Culling and segregation in long term may be unsustainable for ethical, economic and practical reasons

- Vaccination is a practical method provided it is efficacious and cost-effective

- I/mus vaccination – useful to determine efficacy, practicality, costs and building confidence in the principle of vaccination
  - Issues – high cost, selection of animals trapped, welfare issues

- Oral bait – cost effective method for vaccine delivery, wide coverage
  - Issues – identify delivery system, proportion vaccinated not known, non-target species
BCG vaccination of white-tailed deer

Protection of white-tailed deer against intratonsillar challenge with *M. bovis*
- Orally with $10^9$ CFU of BCG in lipid matrix or PBS\(^1\)
- Orally with $10^8$ CFU of BCG in PBS\(^2\)
- Subcutaneously with $10^6$ CFU Pasteur or Danish strains of BCG\(^3\)

Subcutaneous revaccination with BCG after 6 weeks did not enhance protection\(^4\), in contrast to studies in red deer\(^5\)

Ref. Nol et al., 2008\(^1\); Palmer et al., 2007\(^2\); 2009\(^3\), 2014\(^4\); Griffin et al. 1999\(^5\)
Persistence and transmission of BCG in deer

Persistence of BCG in vaccinated deer
- BCG recovered from lymphatic tissue of deer at 1, 3, 6, 9, 12 months after oral vaccination with $10^9$ CFU of BCG, but not from muscle nor from any tissues after a $10^8$ CFU oral dose
- BCG recovered from lymphatic tissue of deer up to 9 months after s/c vaccination with $10^6$ CFU, but not from muscle

Transmission of BCG
- Immunological evidence of transmission of BCG to non-vaccinated deer co-housed with deer orally vaccinated with $10^9$ CFU of BCG, but not to cattle which alternatively shared pen space with deer

Ref. Palmer et al., 2010; 2012; Nol et al., 2013
TB in Eurasian wild boar

- Wild boar – wildlife reservoirs for *M. bovis*, high densities in hunting estates and national parks (often >40 % TB prevalence in dry Mediterranean sites)

- TB in wild boar identified in at least 10 European countries

- Lesions predominantly found in head LNs (mandibular), may become generalised with lung lesions (in 50% of cases)

- Role of wild pigs as a reservoir for *M. bovis*?
Vaccination of wild boar

Selective feeders for wild boar piglets (2-4 months of age) and apply baits in early and late summer in south-central Spain

Vaccine baits- cereal-based matrix containing a capsule to deliver vaccine

Survival of BCG in baits
- No loss of viability up to 36 h in the field (temperature range 11 to 41°C)
- Loss of 2 logs by 24 h with storage at 37°C in laboratory

After oral BCG vaccination of $10^5$ CFU, no isolation from tissues of piglets or from faeces from 1-71 days after vaccination

Ref. Ballesteros et al., 2011; Beltrán-Beck et al., 2012; 2014
Evaluation of TB vaccines in wild boar

- Challenge model established – $10^4$ to $10^6$ CFU via oropharyngeal route\(^1\)
- Vaccination - oral live BCG ($10^5$ CFU), oral or parenteral heat-inactivated *M. bovis* vaccine IV ($10^6$ bacilli)\(^2\)
  - Lower pathology and bacterial loads, but not significantly different v controls
- Revaccination – oral live BCG ($10^6$ CFU, 52 day interval)\(^3\)
  - Significantly lower pathology and bacterial loads v controls
- Revaccination – oral IV ($10^6$ CFU, 52 day interval)\(^4\)
  - Significantly lower pathology and bacterial loads v controls
- Ongoing field trial of oral BCG and IV
  - Started in 2012. 1\(^{st}\) two years yield significant reduction of piglet lesion scores in IV treatment sites v controls

Ref. Ballesteros et al., 2009, Garrido et al., 2011; Gortazar et al., 2014; Beltrán-Beck et al. 2014b
Future directions for wildlife TB vaccines

- Improvements in oral bait formulation
- Systems to avoid uptake by non-target species (attractants and delivery systems)
- New improved TB vaccines (killed or attenuated *M. bovis*)
- Therapeutic TB vaccine for wildlife?
- Field trials to demonstrate prevention or reduction of spread of *M. bovis* infection to domestic animals
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