Bovine tuberculosis - exploiting host and pathogen genetics

Robin Skuce
Veterinary Research Officer
Bacteriology
Veterinary Sciences

www.afbini.gov.uk
Bovine TB host genetics:

A genetic basis for resistance?
TB host genetics

- “Infectious diseases are hereditary”
  - Louis Pasteur (1830s)
- When individuals are exposed to infection, some contract the disease and others do not
  - A spectrum of susceptibility
  - Exploitable genetic variation
- Primary immuno-deficiencies (PID)
  - Susceptible to certain infections and not others
- Could host genes determine whether infection occurs in the first place?
• The genetic basis of complex diseases
• Most are the sum of many minor susceptibility alleles and a very small number of alleles of larger effect
• TB resistance - likely a highly polygenic trait
• ‘Simple’ Mendelian Susceptibility to Mycobacterial Disease (MSMD)

• Genetic factors have long been suspected to contribute to onset and outcome
  - Different inter-individual clinical outcomes
  - Different progression from infection
  - Familial clustering of cases
  - Higher concordance of TB in identical v non-identical twins (exposure?)

• Complex inheritance - confounded by environment
  - Includes pathogen phenotype
- Inbred mouse strains with different TB susceptibilities
  - KOs, KDs
- Genes involved - positional cloning
  - Nramp1, IFNgR etc
- Candidates for human genetic epidemiology studies
  - Some associations in some populations
- *Detected a major locus controlling binary skin test response (T-cell independent)
- *Detected a second major locus controlling skin test QTL or intensity of DTH (T-cell dependent)
Context: bovine TB incidence

- $3 billion worldwide (2003)
- Initial control for public health
- Now includes trade-based considerations
- “One of the most complex and difficult multi-species endemic diseases currently facing government, the veterinary profession and the farming industry in the United Kingdom and Ireland”
Context: bovine TB incidence

• GB: largely unexplained increase year-on-year?  
  – £1,000M by 2011
• NI: *down* ~50% 2002-2008  
  – Levelled off – stable endemic  
    • 5.57% herd incidence  
    • 0.57% animal incidence  
  – NIAO/PAC Report £200M in 10 years  
    • Compensation and testing
Bovine TB - control

• Directive 64/432 EEC
• Computer database
• Test-and-slaughter protocol
  – Field diagnosis
    • Annual TB skin test (SICTT)
    • Abattoir
      – Reactors - post-mortem examination
      – Non-reactors - targeted surveillance
  – Lab confirmation
• Abattoir and lab confirmation - low sensitivity
• Remove reactors
• Consequences
  – Movement restrictions
  – Short interval retests
Phenotype data – bTB skin test

• Interpretation
  – Standard
  – Severe

• Performance*
  – Sensitivity = 80.0% (52-100%)
    • 93.5% severe
  – Specificity = 99.5% (78.8-100%)
  – GB Sensitivity = 75.0-95.5%
  – GB Specificity = 99.99%

Phenotype data – bTB skin test

• Disease geneticists are looking for measurable variation in outcome (population level) as evidence for genetic component
• Variation is found at population scale
Phenotype data – bTB confirmation

• TB reactors
  – Visible lesions
  – No Visible lesions

• Passive surveillance
  – Lesions at routine slaughter
    • 20% breakdowns

• Lab confirmation
  – Histology
    • H&E, ZN
  – Bacteriology
    • Presumptive
    • Molecular confirmed
    • Molecular type

confirmed

| (VL) | 40% | 99% |
| (NVL) | 60% | 02% |
| (LRS) | 75% |
Review

- Much current effort
  - Deployment of control programmes
  - Improving diagnosis
  - Vaccines and adjuvants - cattle and wildlife
  - Badger culling trials

- *Failure of Ireland, the UK and other EU member states to reach TB-free bovine herd status plus the high cost of existing eradication programmes…

- Other measures still required

Review

• The contribution that both host and pathogen genetic variation makes to disease outcome - largely overlooked

• Both the host and pathogen are sources of genetic variation which may influence the outcome of exposure to bTB

• Genetic selection for improved resistance to bTB in cattle?
bTB resistance in livestock

- Evidence* that *Bos indicus* is more resistant than *Bos taurus*
- Anecdotal evidence of resistance/susceptibility differences within some families

*Ameni and others (2007). Clin Vaccine Immunol 14: 1356-1361*
bTB resistance in livestock

- bTB in farmed deer (NZ)
- Differential susceptibility in outbreaks and transmission rates
- Continuous normal distribution response to disease
- From experimental infection studies identified highly R and highly S sires*
- Strong genetic basis to R phenotype
- $h^2 = 0.48!$

Genetic epidemiology - resources

- Large epi-field phenotype datasets
  - Abattoir and lab confirmation
- Large pedigree and performance datasets
- Mathematical models
- Experimental models
- Genomics
  - The bovine genome
  - The Bovine HapMap (~2.4M SNPs)
Genetic epidemiology - concepts

- **Heritability**: the relative contributions of differences in genetic and non-genetic factors to the total phenotypic variance in a population (0-1)
  - Human height $h^2 = 0.80$
  - Milk yield $h^2 = 0.50$
  - Mastitis $h^2 = 0.09$
- Population stratification
- Phenotype ($P$) = Genotype ($G$) + Environment ($E$)
- Definition of *phenotype*?
  - Exposure
  - Infection
  - Disease
- **Infectious disease genetics - concerns**?
  - Incomplete exposure
  - Imperfect diagnosis ($Se/Sp$)
On the Genetic Interpretation of Disease Data

Stephen C. Bishop*, John A. Wooilliams
The Roslin Institute and Royal (Dick) School of Veterinary Studies, University of Edinburgh, Roslin, Midlothian, United Kingdom


- Genetic variation in host resistance to infectious diseases ubiquitous
- Derived models to predict impact on heritability of
  - Incomplete exposure
  - Imperfect diagnosis (Se/Sp)
- All tend to underestimate true heritability
- Not fatal in demonstrating host genetic differences in resistance - just reduces power
- Finding detectable genetic variation in field data implies that the true heritability is likely to be much higher
- Helps explain low apparent disease heritability under field conditions
Genetics of tuberculosis in Irish Holstein-Friesian dairy herds

M. L. Bermingham,*† S. J. More,‡ M. Good,‡ A. R. Cromie,§ I. M. Higgins,‡ S. Brotherstone,# and D. P. Berry*

*Movipark Production Research Centre, Fermoy, Co. Cork, Ireland
†Centre for Veterinary Epidemiology and Risk Analysis, UCD School of Agriculture, Food Science and Veterinary Medicine, University College Dublin, Belfield, Dublin 4, Ireland
‡Department of Agriculture, Fisheries and Food, Kildare St., Dublin 2, Ireland
§The Irish Cattle Breeding Federation, Bandon, Co. Cork, Ireland
#Institute of Evolutionary Biology, University of Edinburgh, West Mains Road, Edinburgh EH9 3JT, United Kingdom

- ICBF pedigree and performance data
- DAFF AHC bTB test and movement data
- Data extracts and edits - episodes
- Phenotype = skin test and *M. bovis* infection
- Significant genetic variation for PPD response
- Significant genetic variation for confirmed infection - genetic improvement possible
- **Heritability = 0.18** (underestimated)
- Strong correlation between susceptibility to confirmed bTB and bTB skin test responsiveness
- Skin test can be used as indirect bTB susceptibility measure
- Select for increased resistance to PPD response will reduce susceptibility to infection in the national herd - complement and benefit existing measures
Evidence of genetic resistance of cattle to infection with *Mycobacterium bovis*


*School of Biological Sciences, University of Edinburgh, West Mains Road, Edinburgh EH9 3JT, United Kingdom
†Scottish Agricultural College, Bush Estate, Penicuik, Midlothian EH26 0PH, United Kingdom
‡Centre for Epidemiology and Risk Analysis, Veterinary Laboratories Agency-Weybridge, New Haw, Addlestone, Surrey KT15 3NB, United Kingdom
¶Centre for Veterinary Epidemiology and Risk Analysis, School of Agriculture, Food Science and Veterinary Medicine, University College Dublin, Belfield, Dublin 4, Ireland
‖Department of Agriculture, Fisheries and Food, Agriculture Hse., Kildare Street, Dublin 2, Ireland
§The Roslin Institute, Roslin Biocentre, Roslin, Midlothian EH25 9PS, United Kingdom

- Different farming practices ROI and GB
- Defra VetNet data
- NMR-CDI-HUK pedigree and performance data
- Data extracts and edits - episodes
- Heritable variation in bTB risk for culled and confirmed cattle
  - $h^2 = 0.18$ (underestimated), $P<0.001$
- Selection for milk yield unlikely to have contributed to current epidemic and *vice versa*
- Select breeding stock to produce offspring with enhanced resistance
- Genetics could play an important role in controlling bTB
- Reduce incidence and severity of herd breakdowns
Are high producing cows more or less likely to be detected as bTB reactors?

ICBF and DAFF data extracts and edits - for all lactations and years - milk yield lower for bTB reactor cows

This study:
- Low milk yield is a risk factor for being a bTB reactor in Irish dairy herds
- Low milk yield tends to precede infection

Consistent with ROI and GB data - genetic basis to bTB susceptibility
- Genetic selection for bTB resistance should not have negative impact on herd productivity - maybe the opposite
- No evidence that bTB reactors are on average the most productive cows in a herd
- National bTB programme should not be leading to loss of highest producing cows
- Other work ongoing - association with other traits (MB)
The ‘on/off’ relationship

• Quantitative geneticists
  - Exploitable variation in risk
  - Estimated breeding values (EBVs)
  - Artificial selection
  - Genomic selection
  - Less concerned with identifying ‘genes’!
The ‘on/off’ relationship

• **Molecular geneticists**
  - Genes (variants), pathways, networks - the molecular basis of the phenotype - how?
  - Host mechanisms of protection
  - Dissecting the host/pathogen interaction
    - Gene expression profiling
      - HD microarrays, RNAseq
  - Rational targets
  - Vaccines
  - Diagnostics
  - Marker-assisted selection

• Dialogue important!
Genetic epidemiology analysis

- Look for genetic variants associated with phenotype
- Study design
  - Genotype cases and controls
  - Genotype extremes of sire risk ranking
- Definition of phenotype
- Based on Holstein-Friesian dairy cattle
  - Population stratification
- Logistics - sampling cases and controls
- Molecular genetics analysis
  - High-density SNP arrays
    - Illumina SNP800K
  - Power to detect associations
Phenotype: case-control study

Figure 3: A spectrum of responses to tuberculosis infection. Infection with M. tuberculosis is usually viewed in terms of a binary outcome: as active disease or latent infection. We propose that a model that includes a spectrum of responses provides a better representation of the biology of infection and might assist in formulation of appropriate research questions.

DB Young TRENDS in Microbiology 2009
Phenotypes at extremes of spectrum may provide most robust Case & Control definitions.

Also - occupying extremes of distribution is likely to maximise detection of any underlying genetic difference.

**Phenotype 1** - Cases: skin test positive, macroscopic lesions & bacterial culture.

**Phenotype 5** - Controls: exposed but skin test negative on multiple occasions
Case-control study

700 DISEASED ANIMALS
SAMPLED > 3,000

700 CONTROL ANIMALS
SAMPLE >1,000
Whole genome SNP scan

Next steps:
• Select ‘case’ and ‘control’ samples for **whole-genome scan** (WGS) genotyping
• New genetic tools from the bovine genome sequence (2009)
  – Illumina SNP800 chip
  – Denser arrays, re-sequencing, CNVs
• **Analyse for genetic variation statistically-associated with cases or controls**
  – SNPs will direct towards causative variation
  – Map and clone genes (pathways) which predispose to disease
    • Diagnosis
    • Vaccinology
    • Genetic tests
Beneficiaries

• Advance our understanding of bovine TB
  – Host and pathogen genetics
  – Pathogenesis
  – Epidemiology

• The prospect of breeding cattle for increased TB resistance
  – Sire selection
  – Marker-assisted genetic selection

• Potential engagement with cattle industry
  – Rapid genetic turnover in UK dairy herd
  – May obtain benefits quickly
Policy relevance

• Reduced susceptibility would support other control measures
• May inadvertently improve resistance to other pathogens
• Powerful demonstration of exploiting Government and industry databases
• No negative impact on wildlife populations
• Genetics can play an important complementary role in control strategies
  – Not the sole strategy (assuming R not absolute)
• A more sustainable approach to disease control
Bovine TB pathogen genetics:

Getting to know the enemy
Bovine TB

- Infectious respiratory disease caused by infection with *Mycobacterium bovis*
- Complex epidemiology
  - An epidemic involving multiple species
  - Transmission within and between populations of cattle and wildlife
  - Wildlife reservoir in badgers
  - Relatively low transmissibility
- Disease *source* and *spread* not clearly understood
- Difficulties in identifying epidemiologically-linked cases
Genomes

• Circular chromosome (5 million bp) coding ~4,000 genes
• Human-adapted (*M. tuberculosis*) and bovine-adapted (*M. bovis*) >99.95% identical!!
  • *M. tuberculosis* H37Rv (1998)
  • *M. tuberculosis* CDC1551 and others
  • *M. bovis* AF2122/97 (2003)
  • *M. bovis* BCG

• Exploiting the genome sequence
  – Comparative genomics, physiology and pathogenesis
  – Understanding attenuation
  – Diagnostics and vaccine candidates

• Mutations: powerful markers of genome evolution
Molecular sub-typing

• Molecular sub-typing and epidemiology:
  – Research tool
    • Epidemiology questions
    • Population structure
    • Genotype:phenotype
  – Decision-support tool
    • Source of infection
    • Route of transmission

• Require a fundamental understanding of selective and neutral forces that shape bacterial populations
  • Influence on current TB epidemic?
Biogeography of human TB lineages

- Important phenotypic consequences
  - Differences in virulence
  - Differences in immunogenicity

- Might impact new diagnostics, drugs and vaccines

- Biogeography has important consequences for geographical distribution of relevant lineage-specific phenotypic traits
  - A large proportion of variation in BCG efficacy linked to geography
TB bacteria - molecular epidemiology

• Until recently assumed that all bTB isolates were the same
• Post genomics - different tools (markers) inform on different evolutionary scales

• Evolution and phylogenetics
  - lineages = LSPs, RDs, SNPs
• Population structure
  - clones = spoligotype
• Outbreak investigation
  - strains = VNTRs
Mycobacterium tuberculosis complex

- Species?
- A nested set of host-adapted 'clones' ('ecotypes')*
- Each with its own host preference (niche)
- Consequences?
- Separated by mutations

### M. bovis spoligotypes: geographical localisation by country (%)

<table>
<thead>
<tr>
<th>Spoligotype</th>
<th>GB</th>
<th>NI</th>
<th>ROI</th>
<th>FR</th>
</tr>
</thead>
<tbody>
<tr>
<td>SB0140</td>
<td>36</td>
<td>66</td>
<td>52</td>
<td>1</td>
</tr>
<tr>
<td>SB0142</td>
<td>1</td>
<td>19</td>
<td>13</td>
<td>0</td>
</tr>
<tr>
<td>SB0263</td>
<td>24</td>
<td>7</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>SB0130</td>
<td>1</td>
<td>1</td>
<td>15</td>
<td>1</td>
</tr>
<tr>
<td>SB0274</td>
<td>13</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>SB0120 (BCG-like)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>26</td>
</tr>
<tr>
<td>SB0121</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>12</td>
</tr>
</tbody>
</table>

British Isles TB epidemic

• Now relatively limited diversity*
  – Population ‘bottleneck’?
    • ‘Test and slaughter’ policy?
• Clonal expansion…series of local epidemics

*NH Smith (2003) PNAS 100(25): 15271-15275
British Isles TB epidemic

• UK and Ireland dominated by *M. bovis* of a particular lineage
  – Spoligotype SB0140 ‘clonal complex’
    • From which most other UK spoligotypes readily derived
  – Genetic distance?

*NH Smith (2003) PNAS 100(25): 15271-15275*
High-resolution pathogen genotyping - microbial forensics

• Genetic tracking of pathogens
  – Analysis of their *mutagenic drift*
  – Important surveillance data
• Potential to help clarify:
  – sources of infection
  – chains of transmission
  – new tools to investigate spread
**M. bovis** VNTR typing  
(Northern Ireland)

- High-performance PCR-based
- Genotype = Haplotype - a string of ‘copy number variants’
- Listed in increasing discrimination*
  - 2163b - 4052 – 2461 – 1955 – **1895** – 2165 – 2163a - 3232
  - 4 4 5 3 4 8 10 8
- Can be simplified to surveyed ‘prevalence’
  - Database…link animal-level data (APHIS)
  - Map to herd of slaughter (MapInfo)
  - Combination of spoligotyping* and VNTR

* Skuce and others (2005) Vet Record 157: 501-504
Herd-level surveillance 2003-present

- Population sample - first (disclosing) isolate per confirmed TB breakdown
- Non-random geographical distribution
  - Significant association with region (p<0.0001)
  - Significant inter-annual differences and trend for some genotypes (p<0.0001)
- Animal-level surveillance 2008
- Animal test and movement history (APHIS)
NI surveillance 2003 - present

- Striking geographical localisation of molecular type
- Each molecular type...
  - Has its own **home range** (unless imported)
  - Responsible for its own micro-epidemic
  - Is a separate experiment!
    - Target eradication of specific genotype(s)?
Geographical localisation

• Severe geographical localisation of *M. bovis* molecular types in UK
  - *Absence of recombination between bovine TB strains*
  - *Natural and imposed movement limitation on domestic cattle*
  - Natural social structure and movement of wildlife?
  - Populations genuinely separate (compartmentalised)?
  - Environmental contamination?
  - *Local and national efficiency of control and eradication*
NI surveillance 2003 - present

- Why do bTB strains cluster?
- Translocation - what’s moving them about?
- Frequency of most genotypes relatively stable
- Contraction/expansion of some genotypes
- Emergence/transmission/extinction of new variants
Cattle-badger association

• That TB in badgers and cattle is linked is **NOT** debated
• Strain typing indicates *epidemiological association*
• Data cannot be used to infer *direction* or to *quantify* the badger or cattle component
NI surveillance 2003 - present

- Role (unquantified) for cattle movement in ‘translocation’
  - Movement and social networks?
- Data implies:
  - Stable and local sources
  - A series of local epidemics
- Significant diversity within regions
  - Despite appearance of domination by strains which may have reached fixation
Applications

- Surveillance
  - Is current situation due to rapid expansion of particular TB types?
  - Need surveillance data over years to help interpret current outbreaks

- Outbreak investigation
  - Source, contacts and route
  - Patterns, prevalence and trends of strains Informed picture of epidemiological features of transmission
  - Inform deployment of additional tests
Pathogen genotype-phenotype

• Co-variable in genetic epidemiology study
• Genetic association studies
  – Only now sufficiently powered to detect significant interactions
  – Does *M. bovis* molecular type influence, for example:
    • Skin test responses?
    • Lesion status?
    • Outbreak size?
    • Persistence?
    • Latency?
    • Transmissibility?
    • Host susceptibility?
Conclusions - pathogen

• Isolates have a genetic signature characteristic of their geographic origin
• *M. bovis* population structure and performance characteristics of genotyping support its use to monitor current control and future interventions
• Potential to refine modelling and analyses of policy questions
• *M. bovis* genotyping - with cattle movement databases and wildlife surveillance
  – A powerful tool for investigating bovine TB source, maintenance and spread
• Unique and valuable insight into current TB epidemic
Conclusions - host

• Significant and exploitable genetic variation exists in cattle in response to *M. bovis* infection
• Both the *host* and *pathogen* are sources of genetic variation which may influence the outcome of exposure to bTB
• Several interesting bTB phenotypes
• Advances in genetics and genomics should allow dissection of the host/pathogen interaction
  – Identification of genes (variants) associated with resistance
  – Impact of pathogen type on outcome
• Breeding for enhanced resistance – benefits may accrue quickly
• Should complement existing and future control measures
• Contribute to bTB control