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"The enemy of my enemy is my friend" - could bacterial viruses help us finally crack TB?

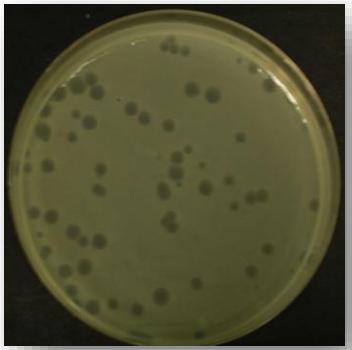
Prof Cath Rees

School of Biosciences

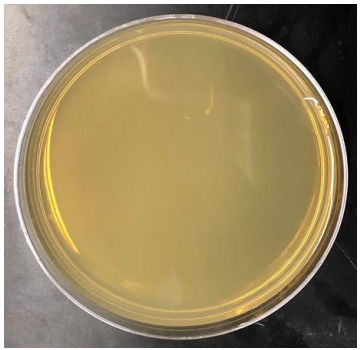
Microbiology, Brewing and Biotechnology

The discovery of bacteriophage

- Bacteriophage (phage) are viruses that specifically infect bacteria



Lawn of bacteria
with phage holes

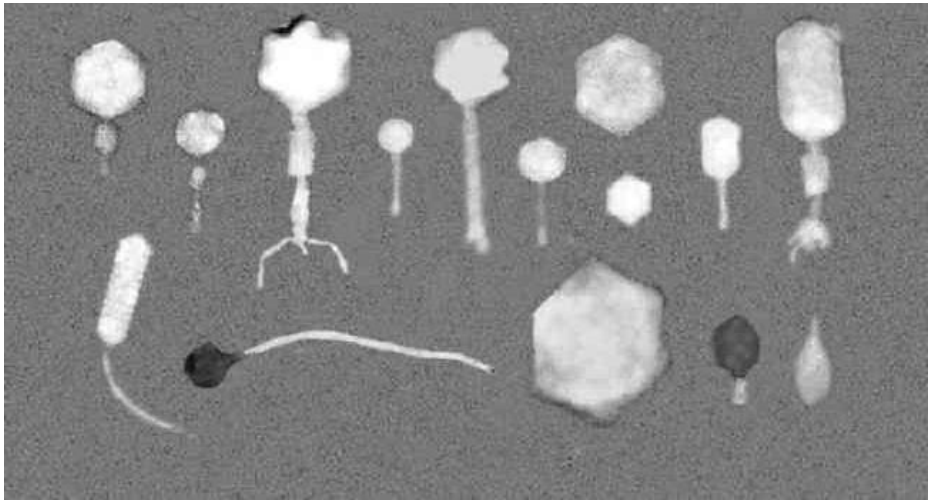


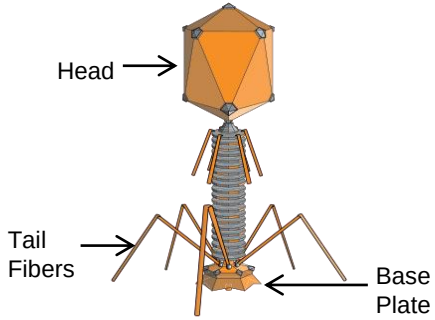
no bacteria

- First described by Felix d'Herelle (1917) & Frederick Twort (1915)
- Both noted that these unknown agents had the ability to "eat" bacterial cells
    - D'Herelle was investigating dysentery outbreak in a French cavalry squadron during World War I
    - mixed some of the material from a clear area on a plate with a culture of dysentery bacteria.
    - the bacteria were quickly and totally destroyed by an unknown agent in the filtrate
    - First called it "invisible microbe"; later renamed it a bacteriophage ("bacteria eater")

So what do we know now?

- All viruses consist of a shell that contains their genetic material





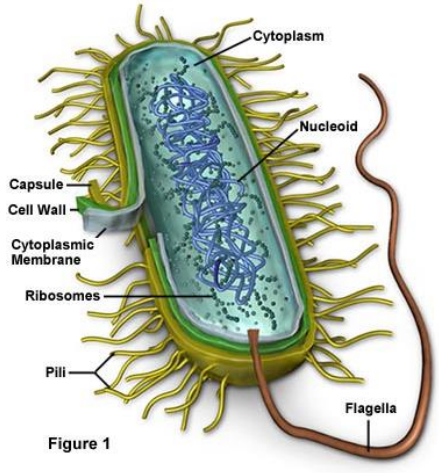
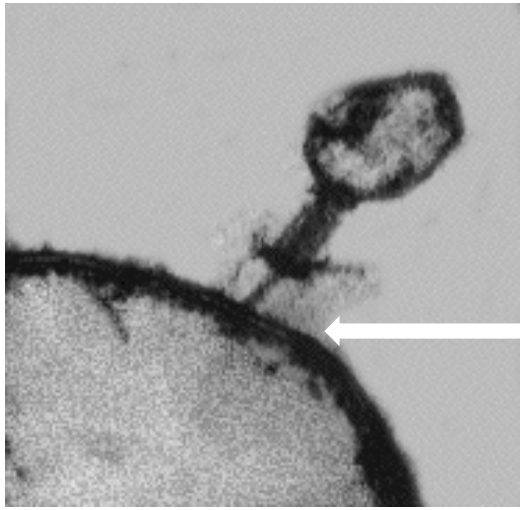


Figure 1

- Bacteriophage have additional tail structures
  - Helps the phage “inject” DNA into host

Bacteriophage only target specific cell types

- Like all viruses, phage have a limited Host Range
  - determines the type of cell infected
- They have evolved to bind to structures on the surface of just the correct host cell
  - so the infection process is <u>specific</u>



Surfaces if different types of bacterial cells are all different at this scale

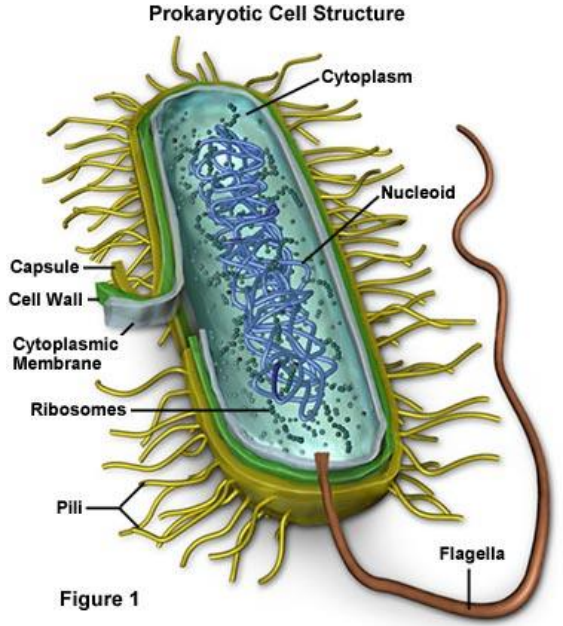
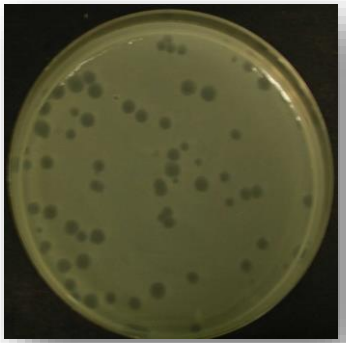
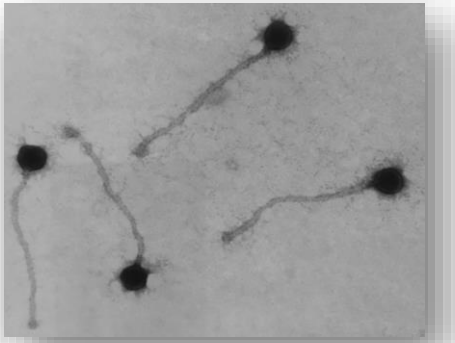


Figure 1

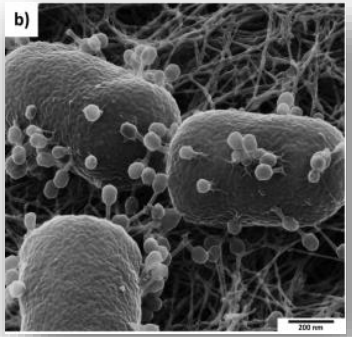
Phage life cycle



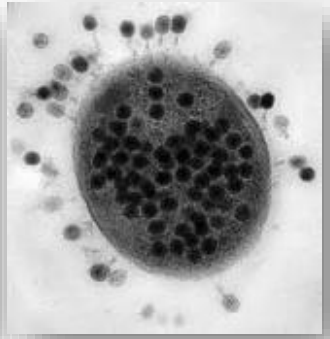
Bacteriophage = “bacteria eater”

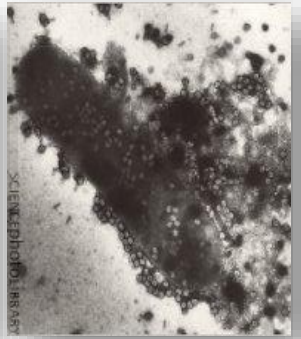


Electron Microscope



Helium-Ion Microscopy



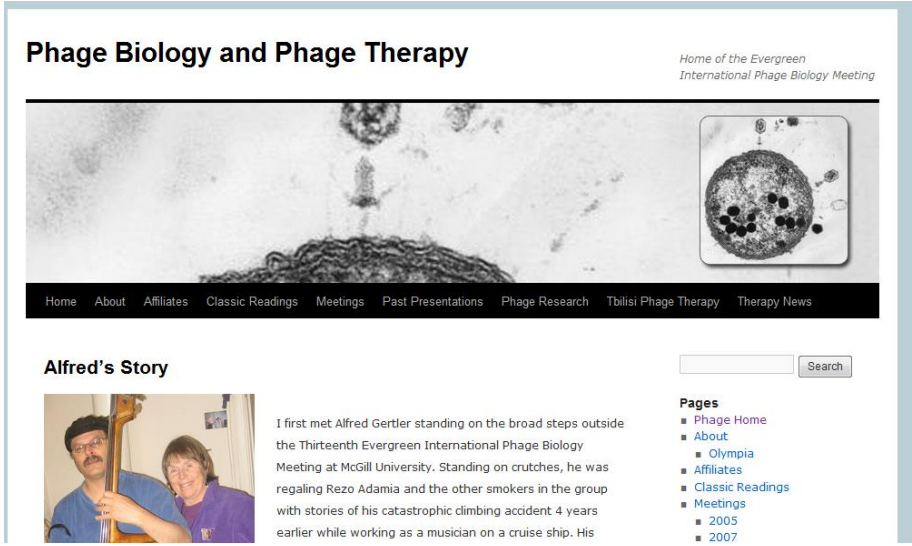


Electron Microscope

- Phage infect their host cell and take it over to make more copies of themselves
- Infected bacterial cells are broken open after phage infection
- Newly released phage in the environment can go and find a new host cell to infect

Phage therapy

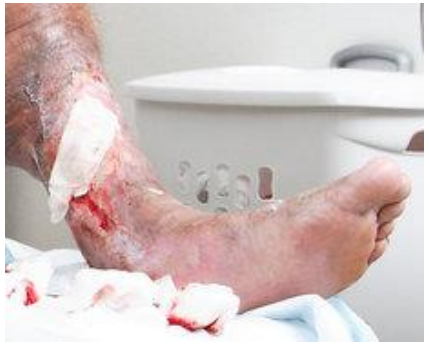
- Given the rise in antibiotic-resistant bacteria, there has been a renewed interest in using phage as antibiotic agents
  - Worries that no new antibiotics have been developed
  - Incidences of antibiotic-resistant bacteria increasing



[see Alfred's Story: http://blogs.evergreen.edu/phage/tbilisi-phage-therapy/alfreds-story/](http://blogs.evergreen.edu/phage/tbilisi-phage-therapy/alfreds-story/)

Phage therapy

- Much work has been carried out in Tibilisi, Georgia, but now being followed up by biotech companies in the West
- Shows potential in animal husbandry, agriculture as well as for human health



Chronic infections

Bacterial infections of crops

Personalised medicine for horses

intralytix
SAFETY BY NATURE

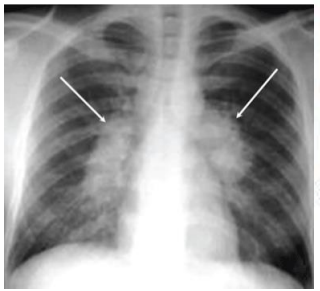
PHAXIAM

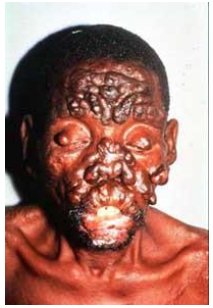
MICREOS

TECHNOPHAGE

Mycobacterial infections in Humans

- 2 major pathogens in humans
  - *Mycobacterium tuberculosis*
  - Described by Koch, 1882
    - Primary diagnosis by x-ray
    - **or** by detecting an immune response
    - **or** by detecting bacteria in sputum samples
      - "smear test"





  - *Mycobacterium leprae*
  - Described by Hansen, 1874
    - Primary diagnosis by skin biopsy and microscopy
    - **or** by looking for antibodies in blood

Despite being discovered early in the development of modern microbiology, these remain some of our most intractable disease

We need new ways to diagnose this disease….

THE END TB STRATEGY

Global strategy and targets for tuberculosis prevention, care and control after 2015

(The official text approved by the Sixty seventh World Health Assembly, May 2014)

We need new ways to diagnose this disease….

The Global Plan to End TB 2016 - 2020

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We need new ways to diagnose this disease….

The End TB Strategy

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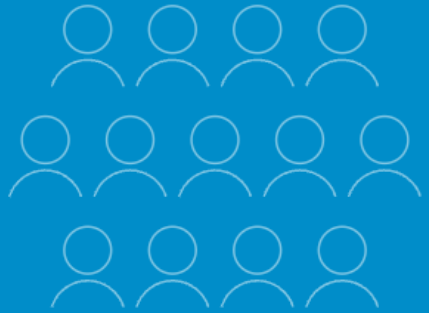
It serves as a blueprint for countries to reduce TB incidence by 80%, TB deaths by 90%, and to eliminate catastrophic costs for TB-affected households by 2030. The Strategy is not a "one size fits all" approach and its success depends on adaptation for diverse country settings.

80%
drop

in new cases by 2030.

The End TB Strategy

TB co-infection of HIV patients a huge problem in developing world



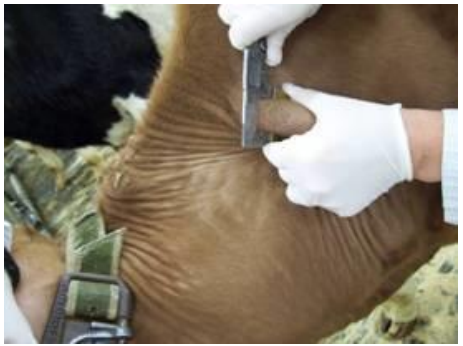
Mycobacterial infections in Animals

- Also a major animal pathogen
  - *Mycobacterium bovis*
  - Causes tuberculosis in cattle AND humans
    - Infection detected by immune response to tuberculin injection







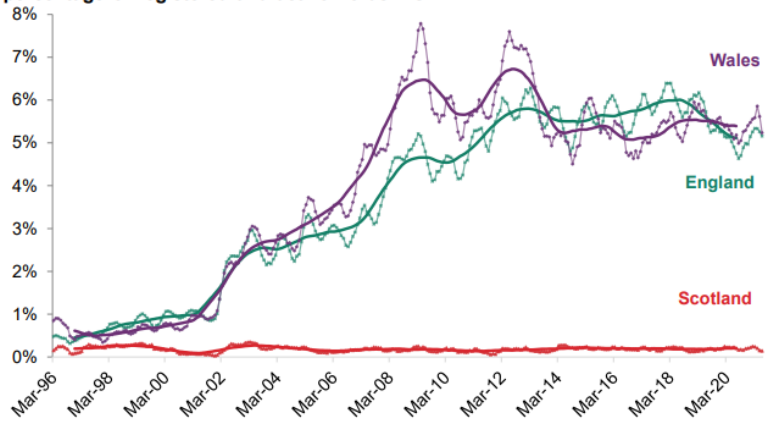



Vet carrying out a skin (SICCT) test

We need new ways to diagnose this disease….

- Annually approximately 40,000 cattle slaughtered every year in UK due to bovine TB
  - Huge economic impact on UK Agriculture sector
- Current control program for bovine TB in UK using skin test is not clearing the disease

Figure 5: Number of herds under disease restrictions at the end of the period as a percentage of registered and active herds – GB

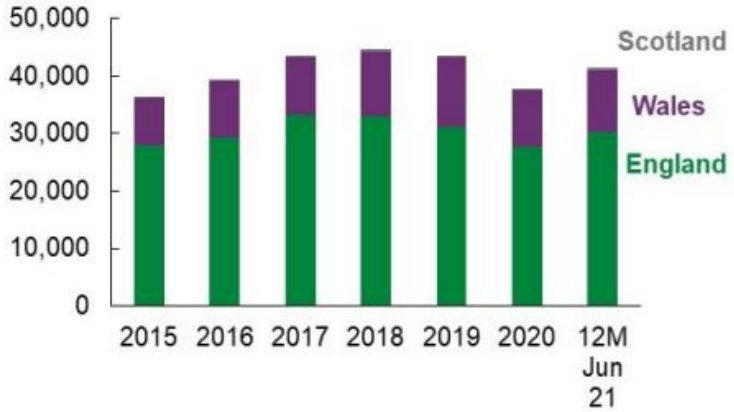


Bold lines represent 23 month centred rolling averages.

TB-free status

C – Total animals slaughtered

Figure C1 – Reactors, direct contacts, and inconclusive reactors (Wales only) slaughtered



So why are Mycobacterial disease so difficult to detect?

- Mycobacterial disease all develop slowly
  - Lesions in tissue only become visible in late stages of disease
- Most bacteria are found INSIDE host cells
  - Mycobacteria have developed ability to “hide” from the immune system
- Often detectable immune response only seen in late stages of infection

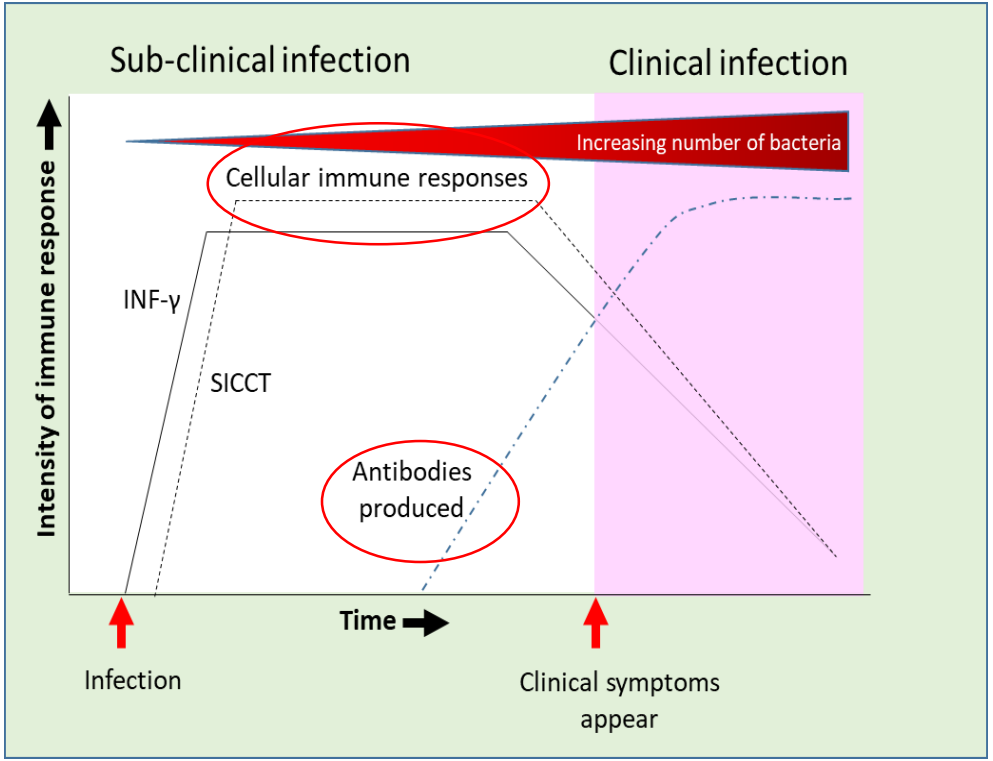
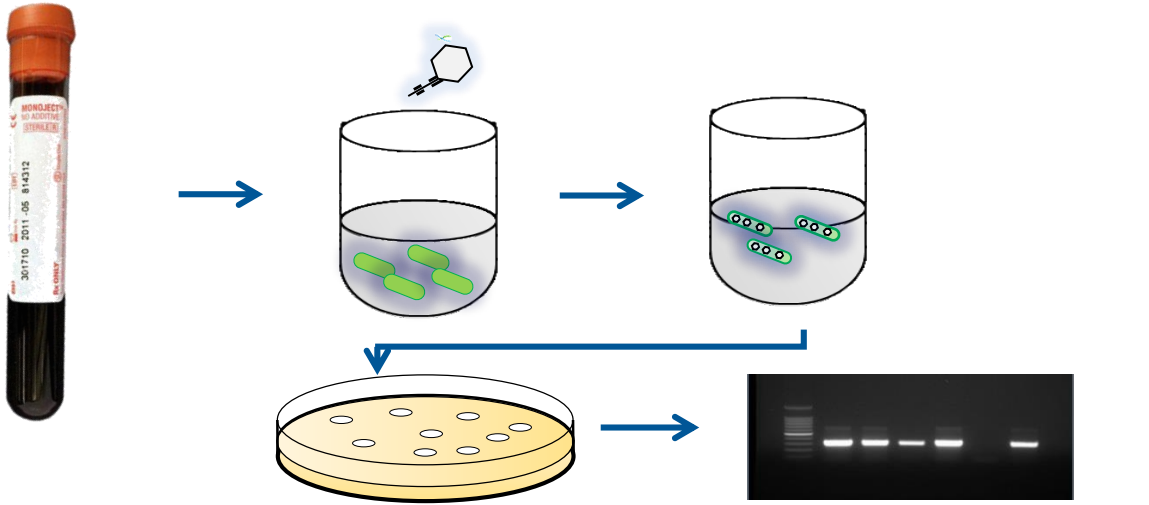


Figure 1: Summary of pattern of immune responses to mycobacterial infections. Based on diagrams produced by Vordermeier et al. (2004) and Rosseels & Huygen (2008)

Bacteriophage blood test

- Sample preparation method then developed to test blood samples
  - White blood cells purified and broken open to release mycobacteria
  - Phage used to break open mycyobacteria
  - Achieved rapid and sensitive detection within 48 h



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Contents lists available at ScienceDirect

Journal of Microbiological Methods

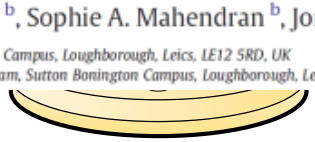
journal homepage: www.elsevier.com/locate/jmicmeth

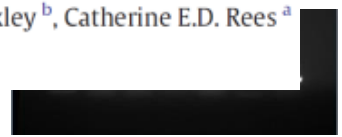
ELSEVIER

Development of a rapid phage-based method for the detection of viable *Mycobacterium avium* subsp. *paratuberculosis* in blood within 48 h ☆

Benjamin M.C. Swift [a,*], Emily J. Denton [b], Sophie A. Mahendran [b], Jonathan N. Huxley [b], Catherine E.D. Rees [a]

[a] School of Biosciences, University of Nottingham, Sutton Bonington Campus, Loughborough, Leics, LE12 5RD, UK
[b] School of Veterinary and Medicine Science, University of Nottingham, Sutton Bonington Campus, Loughborough, Leics, LE12 5RD, UK





Mycobacteria in blood

Detection of mycobacteria

DNA extraction & species Identification

Detection of Bovine TB in blood

- Method used to detect bovine TB in cattle blood

Virulence

RPA is an isothermal DNA amplification method

ISSN: 2150-5594 (Print) 2150-5608 (Online) Journal homepage: http://www.tandfonline.com/loi/kvir20

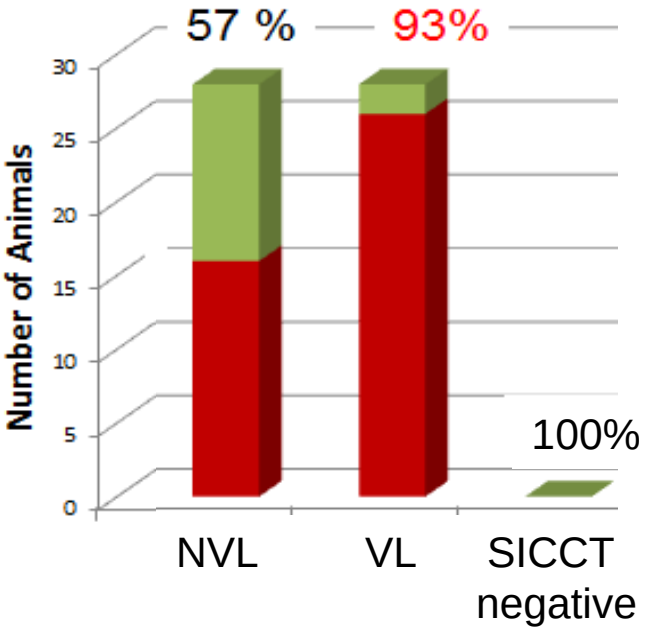
Evidence of Mycobacterium tuberculosis complex bacteraemia in intradermal skin test positive cattle detected using phage-RPA

Benjamin M. C. Swift, Thomas W. Convery & Catherine E. D. Rees

- Sensitivity of test allowed us to demonstrated that bacteraemia is established in bovine TB much earlier in infection than previously believed

Results with skin-test positive animals using Actiphage

- 66 % SICCT-test positive animals had detectable levels of *M. bovis in* blood



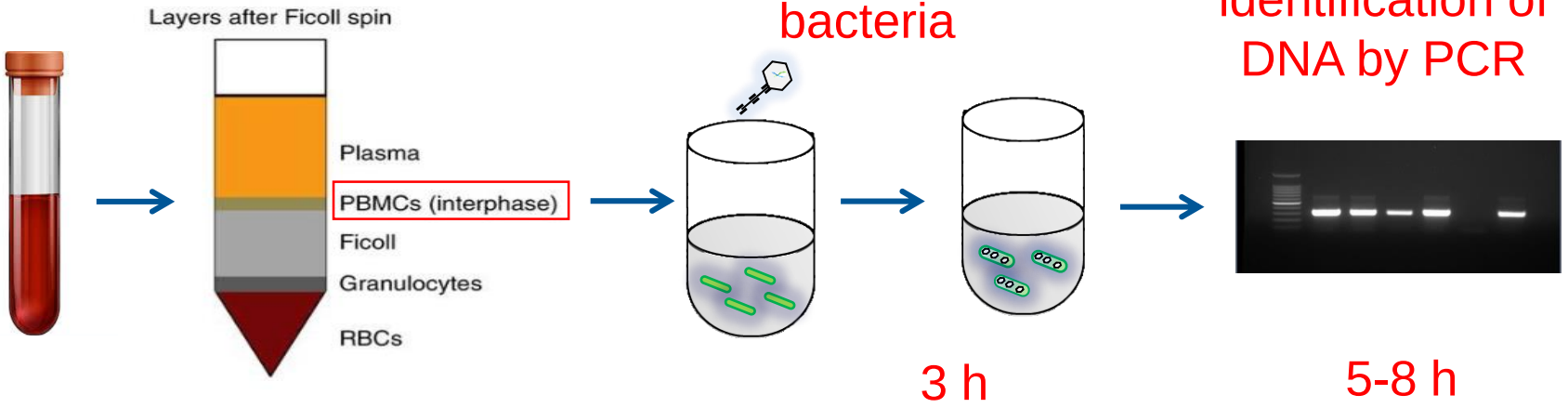
- Provided direct evidence for farmers that SICCT-positive, "No Visible Lesion" cattle ARE truly infected

VL= visible lesions
NVL = non-visible lesions

Swift et al., (2016) Virulence 7:779-88

New improved phage-based diagnostic test: Actiphage®

- Improved Actiphage method developed



- Detection and identification possible within 8 h
- Limit of Detection (LOD) = 5 cells

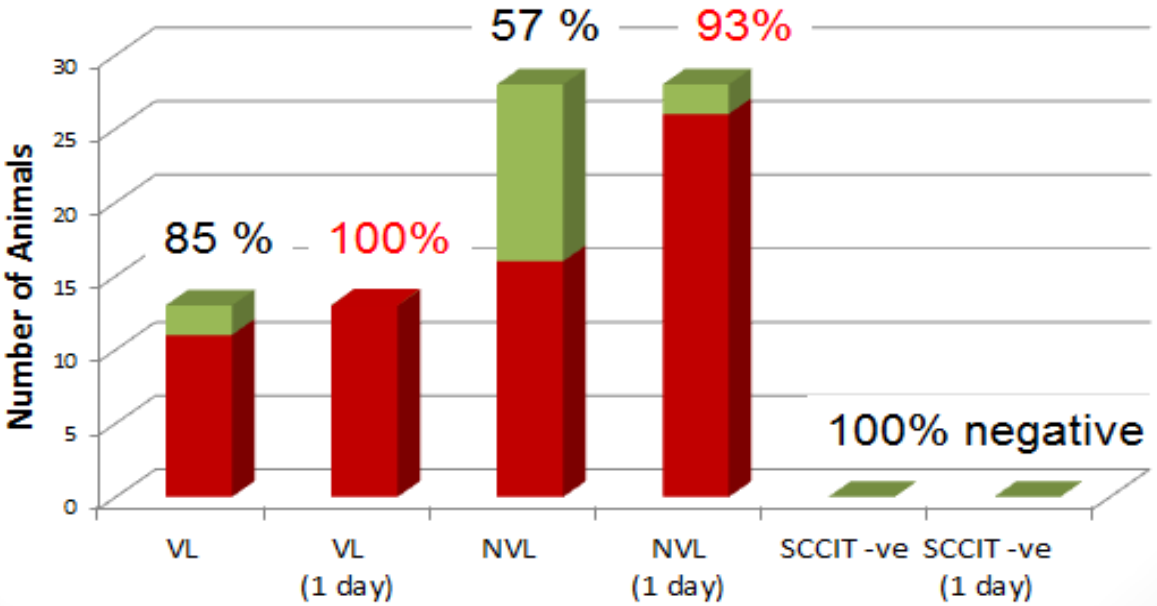
PBD BIOTECH

Improvements in Sensitivity

- Same samples retested using the 1 day test
- Overall 95 % SICCT-test positive animals now had detectable levels of *M. bovis* in blood

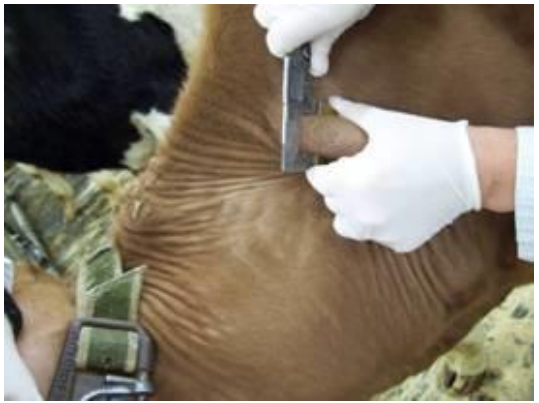
- Sensitivity = 95 % (95 % CI; 0.84–0.99)
- Specificity = 100 % (95% CI; 0.92–1)

Could this more sensitive method be used for earlier detection?



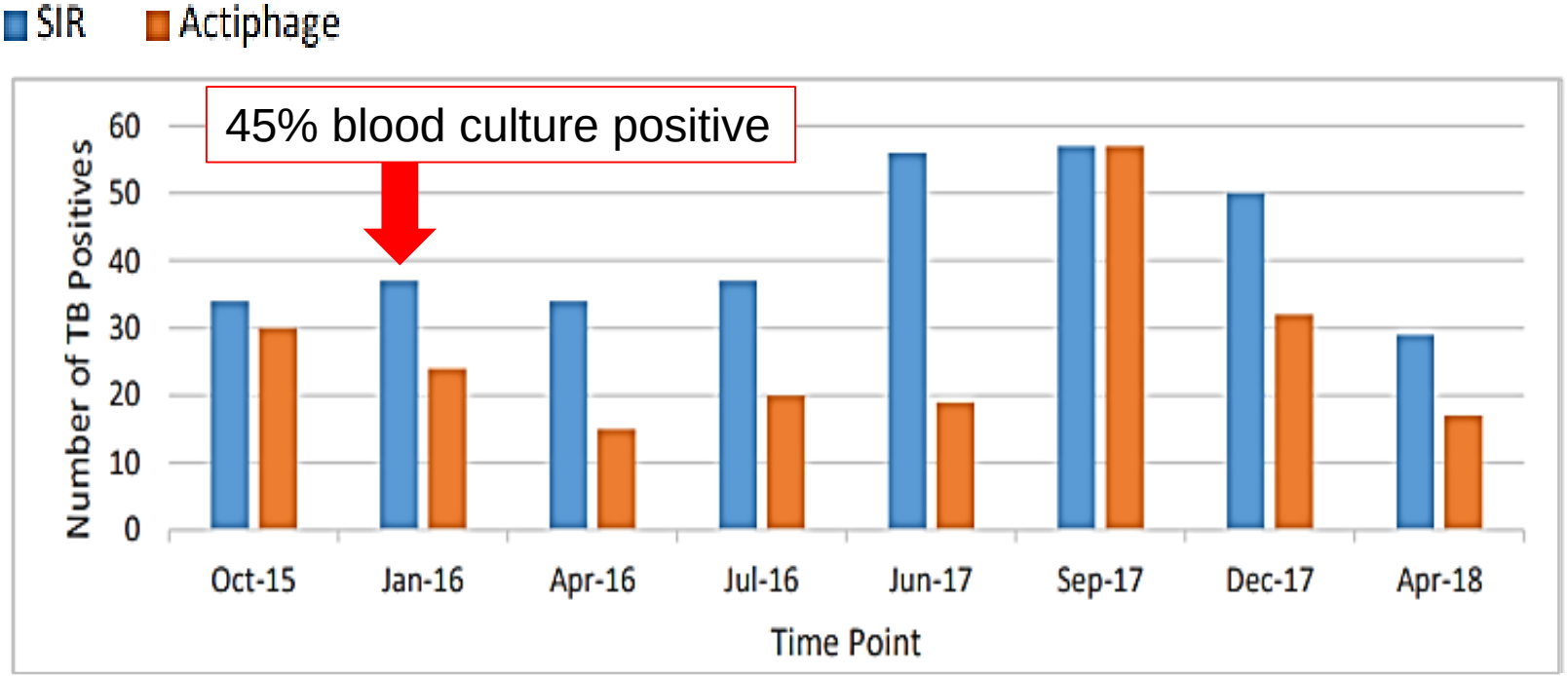
Earlier detection?

- Gatcombe project led by vet Dick Sibley
- SIR = Animals giving some indication of infection but not enough to be classed as a reactor
  - In total 154 animals tested
- Actiphage tests carried out after each round of SICCTs
  - 8 sampling points 2015-2018
  - Total of 321 tests performed

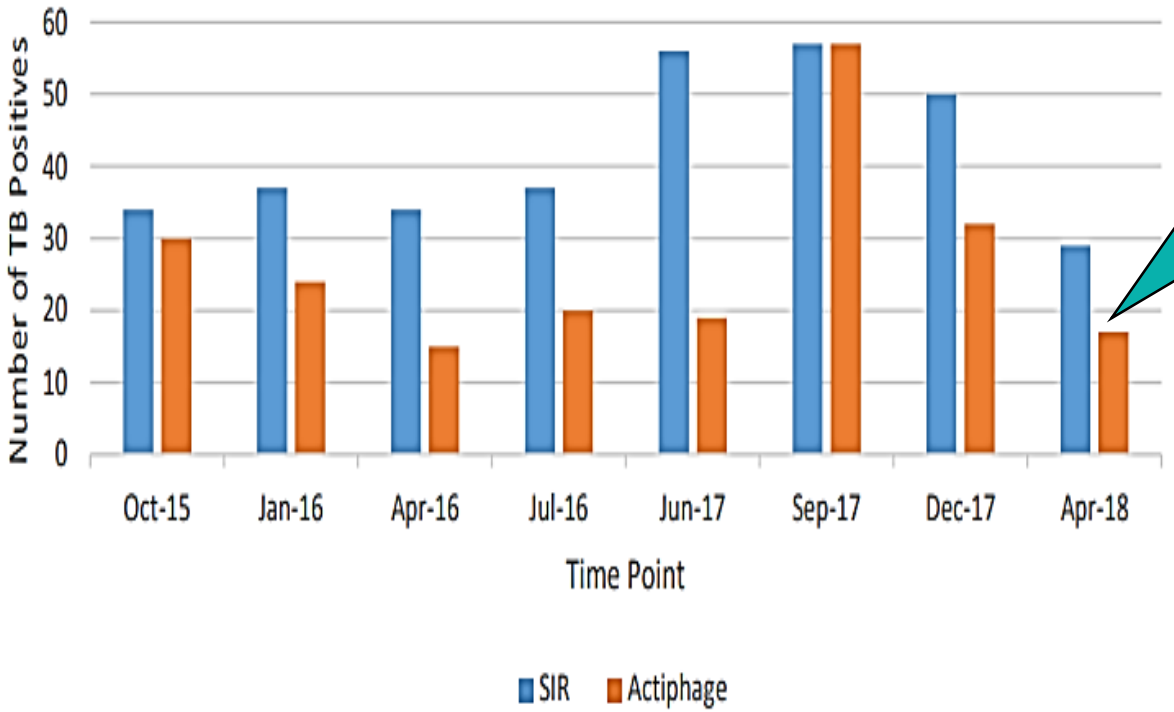


Summary of Gatcombe results

- Viable *M. bovis* detected in the blood of 64 % (av.) of each SIR cohort
- Culture confirmed *M. bovis* was present at detectable levels in blood



Evidence from Gatcombe that SICCT test is not clearing disease

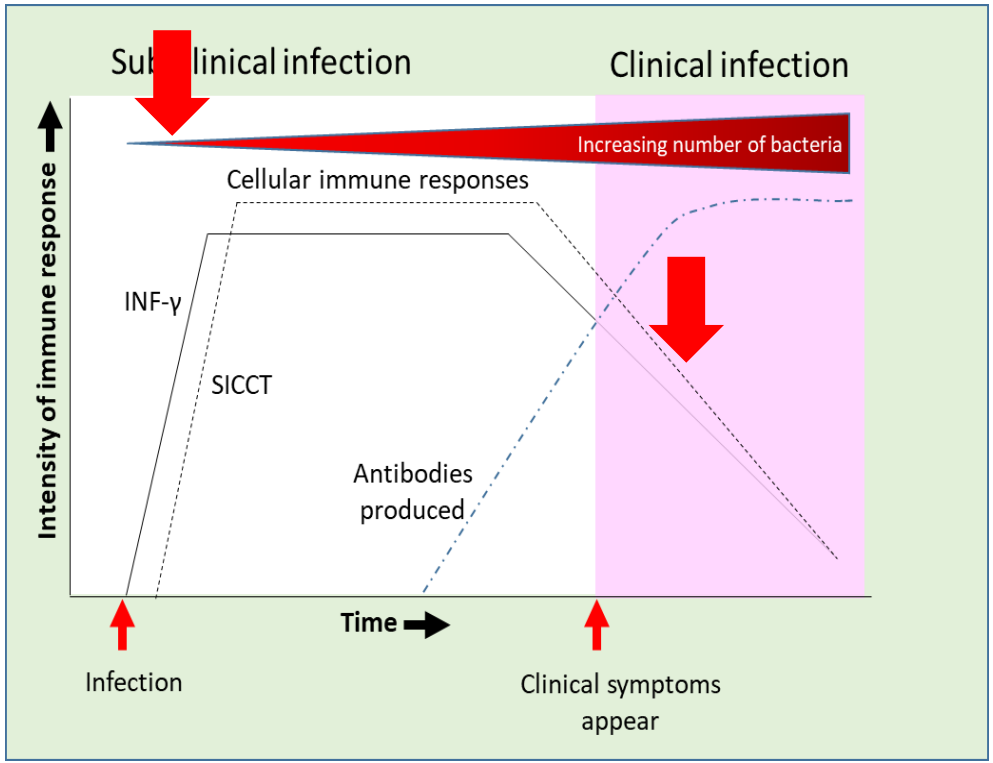


Clear TB test – program of enhanced testing stopped

TB breakdown again July 2020

- Later breakdowns are unlikely to be caused by new infections (*by badgers*)

APHA model of Immune response to TB infection

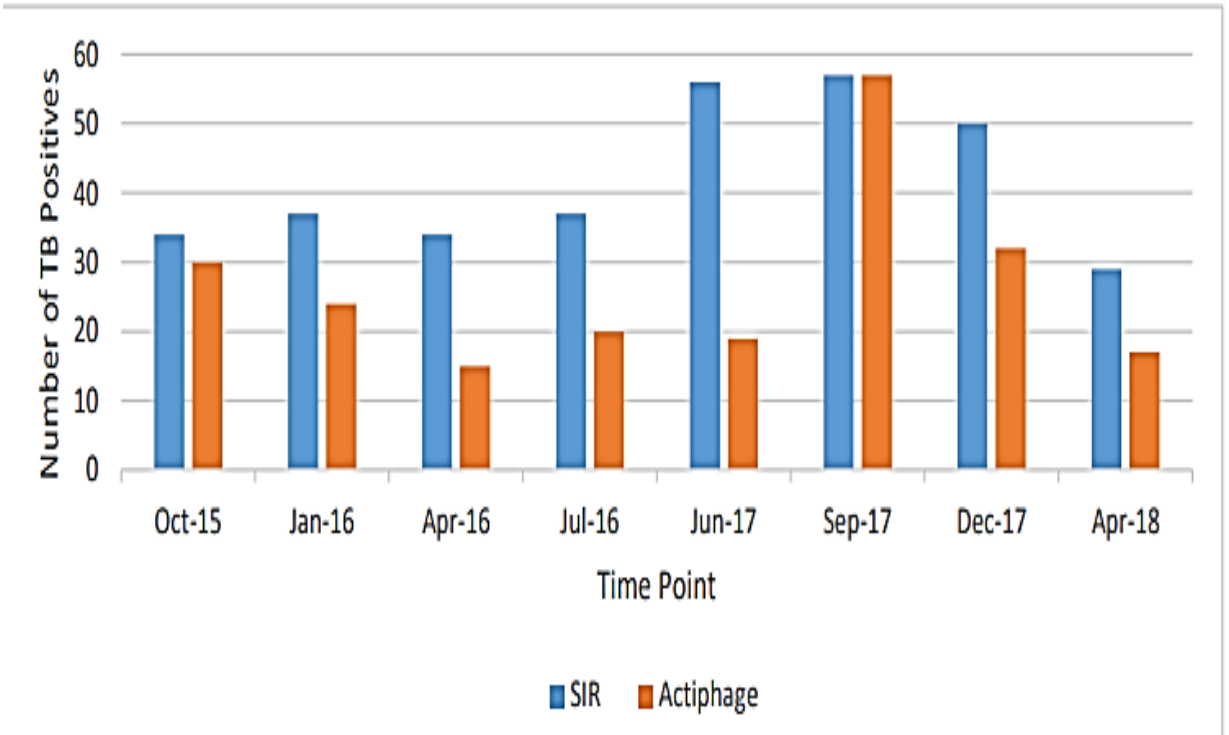


- SICCT positive result takes time to develop
- Actiphage can detect bacteraemia before SICCT response fully develops

- Both Gamma and SICCT responses decline over time
- Actiphage could be able to detect anergic animals remaining in herd

Detection of TB in blood indicates anergic population on farm

- Defra requested comparison of results with gamma interferon tests



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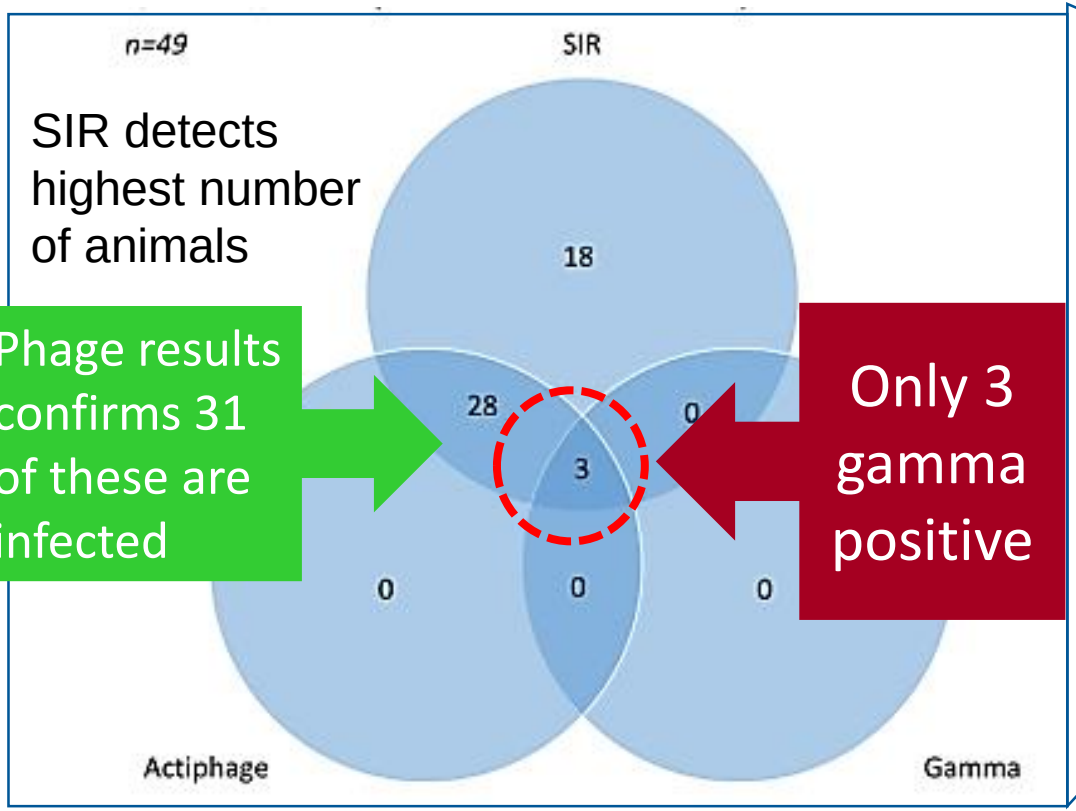
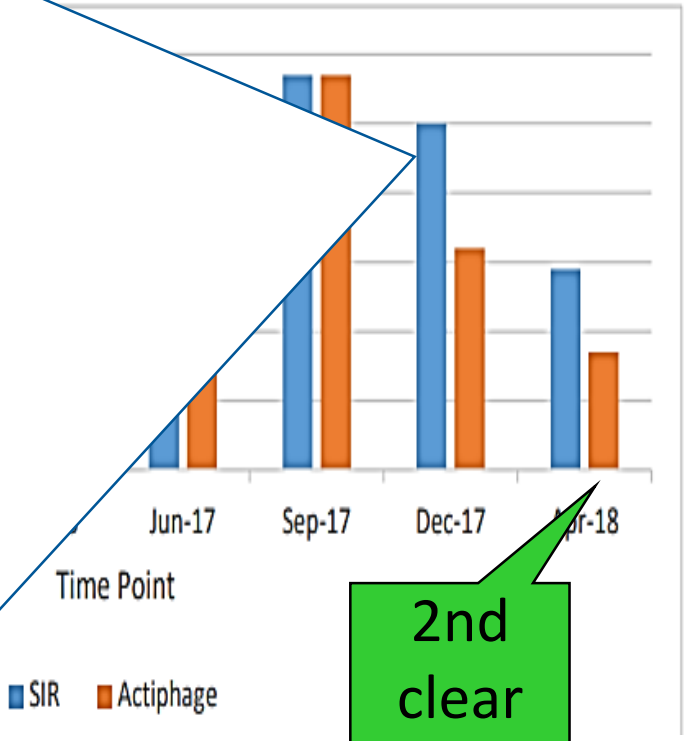


SIR detects highest number of animals

Phage results confirms 31 of these are infected

Only 3 gamma positive



2nd clear TB test

Figure 2. Relationship between positive Actiphage, SIR and Gamma tests in December 2017

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We need new ways to diagnose human TB too

The End TB Strategy

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drop

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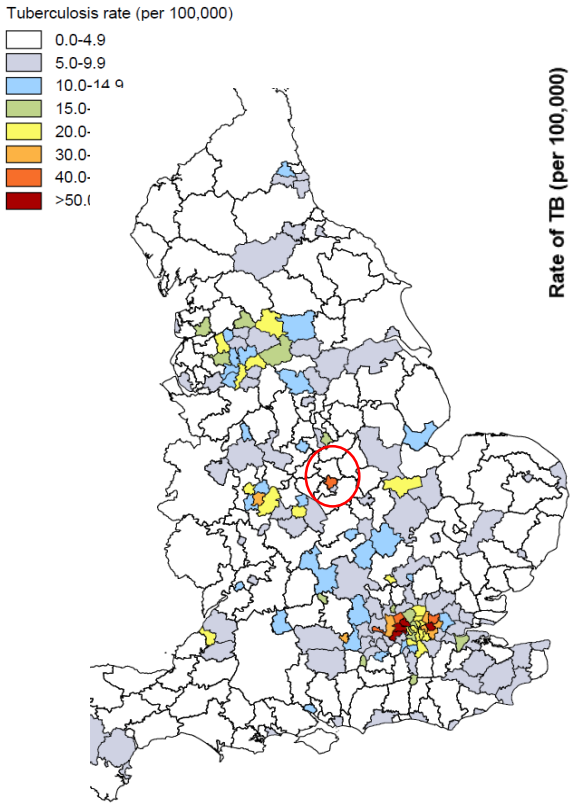
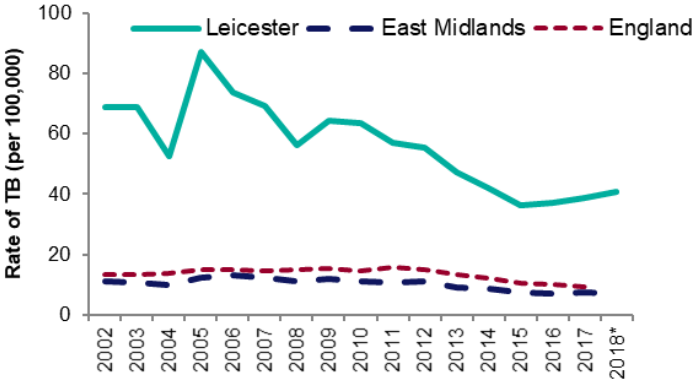
The End TB Strategy

Could Actiphage be used for detection of Human TB?

Human TB trials





UNIVERSITY OF LEICESTER

Actiphage for Mtb detection in active pulmonary TB

- Trial carried out with new TB patients

		Active Pulmonary TB (N=15)		Non-TB Acute Respiratory Illness (N=5)
Actiphage™ Result		Positive (n=11)	Negative (n=4)	All Negative
Male Gender (%)		5 (45.5)	2 (50)	2 (40)
Age (years; mean ±SD)		31.5 (±13.9)	38.8 (±13.5)	50 (±21.7)
UK Born (%)		3 (27.2)	1 (25)	2 (40)
BCG Vaccine	Yes (%)	4 (36.4)	2 (50)	2 (40)
	Unknown (%)	0	0	0
BMI (kg/m^2; mean ±SD)		19.9 (±3.6)	20.9 (±3.0)	25.7 (±5.3)
TB Disease Characteristic	Smear +ve	7	0	0
	Smear -ve	4	4	0
	GX-Ultra level	Medium - High	Very Low - Low	All Negative
	CRP (median, IQR)	63 (36-65)	41 (27-46)	84 (45-110)
	Days to Positive Culture (median, IQR)	15 (11-22)	21 (21-21)	N/A

- 11/15 Active Pulmonary TB patients were Actiphage +ve
  - Sensitivity 73%
  - Specificity 100%
- Mtb detectable in circulating blood of immunocompetent patients with clinically single compartment disease
- Actiphage-positive test result associated with markers of more severe or ***progressive*** infection:
  - sputum smear +ve
  - higher baseline C-reactive protein
  - shorter times to +ve culture

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Actiphage for Mtb detection in latent TB infections (LTBI)

- If someone in the UK is diagnosed with TB, family members and other close contacts are also tested using QFT (= human SICCT)

		Pulmonary TB Contacts With LTBI (N=18)		Healthy Controls: No LTBI (N=28)
Actiphage™ Result		**Positive (n=3)**	**Negative (n=15)**	**All Negative**
Male Gender (%)		1 (33.3)	10 (55.6)	**11 (39.3)**
Age (years; mean ±SD)		25.3 (±6.4)	54.7 (±12.3)	**38.9 (±14.6)**
UK Born (%)		1 (33.3)	5 (33.3)	**10 (35.7)**
BCG Vaccine	Yes (%)	2 (66.7)	7 (63.6)	**12 (50)**
	Unknown (%)	0	4 (26.7)	**4 (14.3)**
BMI (kg/m^2; mean ±SD)		21.9 (±2.0)	26.2 (±6.9)	**27.1 (±8.2)**
TB Disease Characteristic	Smear +ve	0	N/A	**N/A**
	Smear -ve	**2**	N/A	**N/A**
	GX-Ultra level	Medium	N/A	**N/A**

- 3/18 LTBIs were Actiphage +ve at BASELINE
- All had normal CXRs at baseline
- 2 developed symptoms after 6-7 months follow-up
  - Both had culture +ve PTB
  - Genome sequencing of Mtb isolates confirm their origin from their respective index cases

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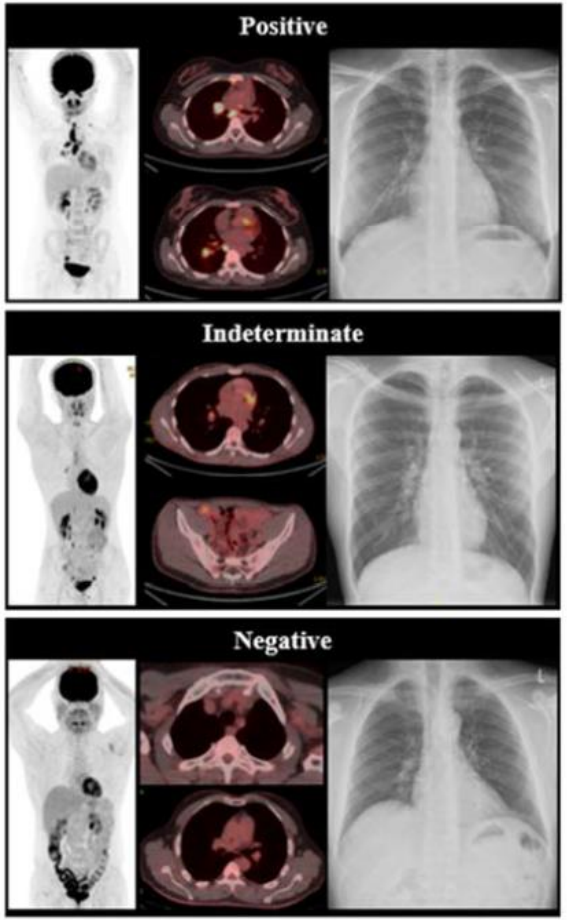
How do you prove something is there that you cannot find?

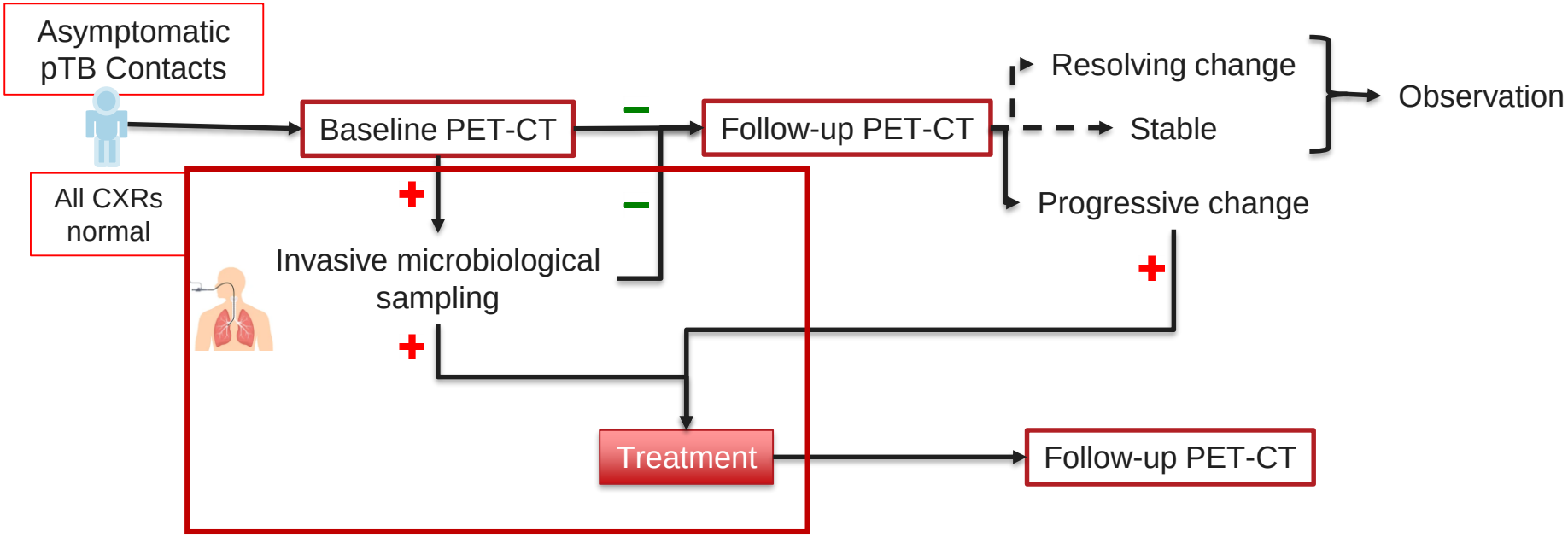
- Cannot carry out PM tissue analysis for humans!
- PET (Positron emission tomography)-CT scans
  - produce very detailed 3-dimensional images of the inside of the body including body structures
  - Highlight areas of inflammation/infection where radioactive glucose is taken up
- Could this be used to answer the question is Mtb detected in blood a new biomarker for progressive TB infection?

New PET-CT scoring method needed



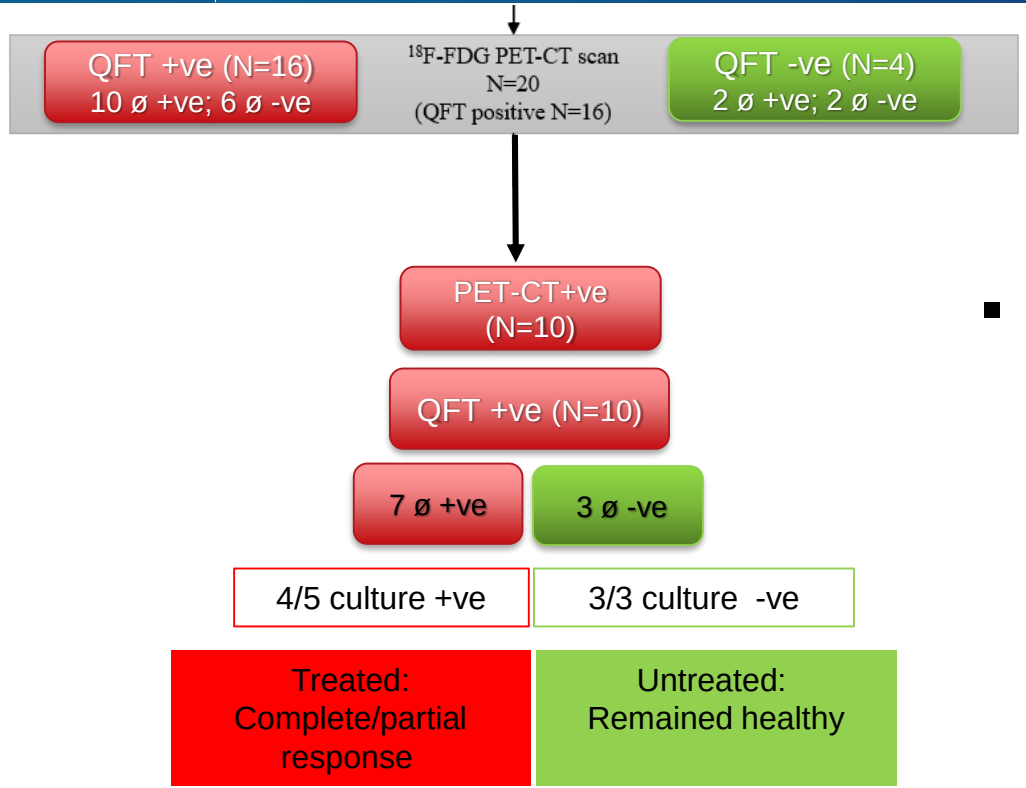
Classification of baseline PET-CT patterns	
Positive	^{18}F-FDG avid mediastinal / hilar lymph nodes (SUVmax >5) ± uptake in lung parenchyma
Indeterminate	Low absolute SUVmax uptake (SUVmax <5) Extrathoracic uptake at sites associated with Mtb infection
Negative	No ^{18}F-FDG uptake exceeding physiological uptake

Looking for TB in contacts



Kim *et al*., Lancet Microbe (2024)

Comparison of PET-CT with QFT and Phage results

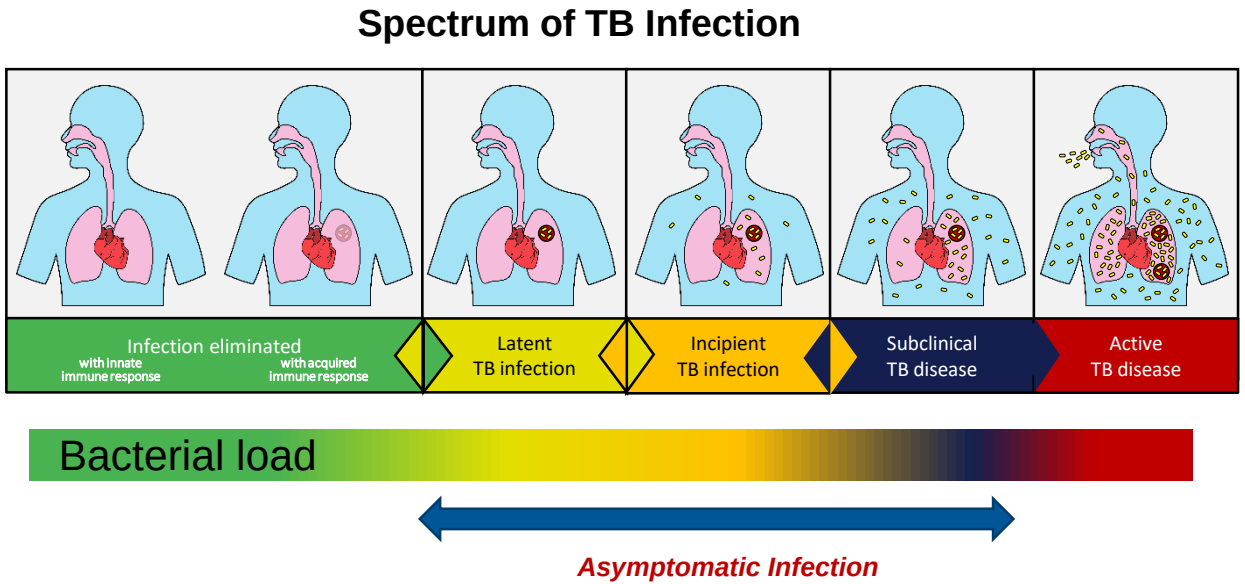


- Significant association between phage-positivity and active infection (p=0.018)

Kim *et al*., Lancet Microbe (2024)

So what have phage told us about TB?

- Being able to detect low levels of cells in clinical blood samples is changing our understanding of disease progression



"The real voyage of discovery consists not in seeking new landscapes, but in having new eyes"

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