A human challenge model of *Bordetella pertussis* infection

Robert C Read, Hans de Graaf and Alison Hill on behalf of 12 co-investigators

• University of Southampton
Disclosures

• GSK Novartis Non-personal, Non-specific
• DSMB Chair, *S.pneumoniae/S.pyogenes* EHCPs
• Member, UK Joint Committee for Vaccination and Immunisation
• Editor in Chief, Journal of Infection
• Co-editor in Chief, Current Opinion in Infectious Disease
An update of the global burden of pertussis in children younger than 5 years: a modelling study

Karene Hoi Ting Yeung, Philippe Duclos, E Anthony S Nelson, Raymond Christiaan W Hutubessy

- 24.1 million cases in 2014 (sensitivity analyses: up to 40 million)
- 160,700 deaths in 2014 (sensitivity analyses: up to 670,000)
Pertussis Vaccines

- **Whole cell vaccines (wP)**
  - Developed in 1930s, combined as DTP in 1940s
  - 70-90% efficacy after 3 doses
  - Adverse reactions fairly common

- **Acellular pertussis vaccines (aP)**
  - Purified subunit vaccines
  - Developed in the 1980s
  - 70-80% efficacy after 3 doses
  - Reduced adverse reactions

- **aP vaccines contain**
  - Pertussis Toxin
  - Pertactin
  - Filamentous haemagglutinin
  - +/- Fimbriae
Pertussis Vaccines

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[Diagram showing vaccination age and countries with different pertussis vaccines]
Worldwide resurgence of pertussis
Althouse and Scarpino 2015
Hypotheses to explain resurgence:
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• Waning of protective immunity from vaccination or natural infection over time,
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• Asymptomatic transmission from individuals vaccinated with the currently used acellular *B. pertussis* vaccines.
Hypotheses to explain resurgence:

• Waning of protective immunity from vaccination or natural infection over time,
• Evolution of *B. pertussis* to escape protective immunity
• Low vaccine coverage
• Asymptomatic transmission from individuals vaccinated with the currently used acellular *B. pertussis* vaccines.
  • Failure of cocooning to protect newborns in outbreaks (*Healy CM et al 2015*)
  • wP vaccine obliterates 4 year cycles, aP does not (*Gay N and Miller E, 2000*)
  • Phylodynamic (genetic epi) data supports asymptomatic not case transmission (*Bart MJ et al 2014*)
Baboons vaccinated with current acellular B.pertussis vaccines (aP) - become asymptotically infected, - can then transmit infection to susceptible individuals

wP vaccinated baboons do not

(Warfel JM et al PNAS 2014)
Althouse and Scarpino 2015
PERISCOPE consortium

1. PRE-CLINICAL MODELS
2. IMMUNITY TO INFECTION
3. VACCINES & IMMUNITY
4. VACCINE-INDUCED IMMUNITY
5. BIOMARKER DISCOVERY PLATFORM
6. COORDINATION & MANAGEMENT
7. COMMUNICATION, DISSEMINATION & TRAINING

BUILDING CAPACITY IN EU FOR PERTUSSIS RESEARCH

CLINICAL DATA & BIOLOGICAL SAMPLES

MOUSE MODEL

HUMAN CHALLENGE MODEL

PERTUSSIS VACCINES

pertussis patients

MATERNAL IMMUNIZATION

aP, wP, wP

Bill & Melinda Gates Foundation
Human Challenge model - pertussis

• Considerations:
  • Natural history of wild disease
  • Reproduction number
  • Natural occurrence of asymptomatic colonisation
  • Pre-existing immunity in adults (In UK, aP introduced from 2004)
  • Historical example of human challenge (very rapid onset of severe disease)
  • Macrolide clearance of carriage
Clinical Pertussis

Days

0 10 20 30 40 50

Catarrhal stage: Conjunctival suffusion Nasal congestion and rhinorrhea Cough (mild)

Paroxysmal stage: Paroxysmal **Cough** Nocturnal **Cough** Post-tussive vomiting

Convalescent stage: Protracted cough
Human Challenge model - pertussis

• Considerations:
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The Microbe-scope

CONTAGIOUSNESS
average basic reproduction number (R0)
no. of people one person will likely infect

DEADLIENESS
Case fatality rate

PRIMARY TRANSMISSION METHOD
airborne  bits  body fluids  fecal-oral  food  sexual contact  surfaces

not very  quite contagious  very  highly  vaccine now!  extremely deadly  death likely  deadly  high chance  quite deadly  unilkely or unhealthy  not too deadly  high-risk groups (infants, the aged)

R0 = 1 disease not likely to spread

sources: Centers for Disease Control, World Health Org., CIDRAP, studies

David McCandless v1.02 / Oct 2014
InformationIsBeautiful.net

Rotavirus

data: bit.ly/KIB_MicrobeScope

part of KnowledgesBeautiful
Human Challenge model - pertussis

• Considerations:
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Days

Catarrhal stage:
- Conjunctival suffusion
- Nasal congestion and rhinorrhea
- Cough (mild)

Paroxysmal stage:
- Paroxysmal Cough
- Nocturnal Cough
- Post-tussive vomiting

Convalescent stage:
- Protracted cough
Clinical Pertussis

**Complications in Adults/Adolescents**

<table>
<thead>
<tr>
<th>Complication</th>
<th>Percent</th>
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<tbody>
<tr>
<td>Insomnia</td>
<td>77</td>
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<tr>
<td>Weight loss</td>
<td>3-33</td>
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<tr>
<td>Urinary incontinence</td>
<td>3-28</td>
</tr>
<tr>
<td>Death</td>
<td>0.01</td>
</tr>
</tbody>
</table>

**Kilgore PE, et al, CMR 2016**

- **Catarrhal stage:**
  - Conjunctival suffusion
  - Nasal congestion and rhinorrhea
  - Cough (mild)

- **Paroxysmal stage:**
  - Paroxysmal **Cough**
  - Nocturnal **Cough**
  - Post-tussive vomiting

- **Convalescent stage:**
  - Protracted cough

**Days**
Controlled Human Infection

Catarrhal stage:
- Conjunctival suffusion
- Nasal congestion and rhinorrhea
- Cough (mild)

Paroxysmal stage:
- Paroxysmal \textbf{Cough}
- Nocturnal \textbf{Cough}
- Post-tussive vomiting

Convalescent stage:
- Protracted cough

Days

`Colonisation model`

`Disease model`
Disease model

<table>
<thead>
<tr>
<th>ADVANTAGES</th>
<th>DISADVANTAGES</th>
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<tbody>
<tr>
<td>Clear Physiological Relevance</td>
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<tr>
<td>Easily measured</td>
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<tr>
<td>Correlate micro/immunology with clinical endpoints</td>
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<tr>
<td>Study the full/mature disease process</td>
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<tr>
<td>Interventions measured against undeniable endpoints</td>
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Controlled Human Infection

Days

- Colonization model
- Disease model
- Catabolic stage: Congestive puffiness
- Nasal congestion and rhinorrhea
- Cough (mild)
- Paroxysmal stage: Paroxysmal Cough
- Nocturnal Cough
- Post-tussive vomiting
- Convalescent stage: Protracted cough
# Disease model

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<th>ADVANTAGES</th>
<th>DISADVANTAGES</th>
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</thead>
<tbody>
<tr>
<td>Clear Physiological Relevance</td>
<td>Unable to quantify risk</td>
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<tr>
<td>Easily measured</td>
<td>Unable to reverse disease after cough onset</td>
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<tr>
<td>Correlate micro/immunology with clinical endpoints</td>
<td>Secondary cases</td>
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<tr>
<td>Study the full/mature disease process</td>
<td>Risk to nursing staff</td>
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<tr>
<td>Interventions measured against undeniable endpoints</td>
<td>Reputational risk</td>
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</tbody>
</table>

## Controlled Human Infection

![Disease model diagram](image-url)

- **Colonization model**: Confluent culture
- **Pathogenic stage**: Pharyngeal Cough, Nasal Cough
- **Convalescent stage**: Protracted cough

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<thead>
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<th>Days</th>
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<td>10</td>
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<td>30</td>
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<tr>
<td>40</td>
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<tr>
<td>50</td>
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## Colonisation model

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<td>Probably less infection control issues</td>
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<td>Vaccine/Challenge studies would inform herd protection estimates</td>
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### Colonisation model

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</thead>
<tbody>
<tr>
<td>Safer</td>
<td><em>Bordetella pertussis</em> is difficult to detect in asymptomatic people</td>
</tr>
<tr>
<td>Shorter finite period of infection</td>
<td>The site of colonisation is unknown</td>
</tr>
<tr>
<td>Probably less infection control issues</td>
<td>What duration of carriage is physiologically relevant?</td>
</tr>
<tr>
<td>May be the commonest manifestation of wild infection</td>
<td>Detection of <em>B.pertussis</em> in an asymptomatic person may not imply active biological interaction</td>
</tr>
<tr>
<td>Vaccine/Challenge studies would inform herd protection estimates</td>
<td>Different carriage states – density??</td>
</tr>
</tbody>
</table>

**Controlled Human Infection**

- **Colonisation model**
  - Catarrhal stage: Constrictive (pharynx, nasal congestion and sneezing, cough (mild))
  - Convalescent stage: Protracted cough

- **Disease model**
  - Convalescent stage: Protracted cough
Overview

*B. pertussis* human challenge study

**Phase A**
Finding the inoculum dose causing safe colonization of 70% of exposed volunteers

**Phase B**
Confirmation of inoculum dose and searching for correlates of protection against colonization
Selected organism

• GMP manufactured by Q Biologicals, Belgium
• B1917, characterised by \textit{ptxP3-pxA1-prn2-fim3-2, fim2-1} MLVA27, PFGE BpSR11
• Expresses
  – Pertactin
  – Pertussis Toxin
  – Filamentous haemagglutinin
• Representative of current isolates in Europe
Participants

• Healthy volunteers aged 18 – 45 year
• Intranasal inoculation 1ml maximum volume
• Endpoint colonization without disease
• No recent pertussis infection
  – Anti-pertussis toxin IgG <20 IU/mL (In UK this should be 70%)
• Vaccinated > 5 years ago
Logistic challenges

• Safety
• Prolonged hospital admission (17 days)
• Multiple blood and URT samples
• Strict infection control
• Will we be able to recruit??
Logistic challenges

• Safety
• Prolonged hospital admission
• Multiple blood and URT samples
• Strict infection control
Study overview

- **Screening**
  - Day -30
- **Visit**
  - Day -7
  - Day 0
- **Inoculation**
  - Day 14
- **Eradication**
  - Day 16
  - Day 28
  - Day 56
  - Day 183
  - Day 365
- **Visit**
  - Admission
To determine the dose of the standard inoculum: Dose escalation

**Inoculum doses to be used in cfu**

- $5 \times 10^2$
- $10^3$
- $5 \times 10^3$
- $10^4$
- $5 \times 10^4$
- $10^5$
- $5 \times 10^5$

After 5 volunteers

- 5/5 colonized → One dose lower
- 3-4/5 colonized → Repeat the same dose
- 1-2/5 colonized → One dose higher
- 0/5 colonized → Dose 10x higher

After 10 volunteers with one dose

- 9-10/10 colonized → One dose lower
- 7-8/10 colonized → Repeat the same dose
- <7/10 colonized → One dose higher
Enrolment
June 2017-July 2018

N= 54 volunteers screened

N= 37 volunteers meet inclusion criteria

N= 13 volunteers anti-PT IgG > 20 IU/mL

N= 34 volunteers rescreened at day -7

N= 2 volunteers meet an exclusion criterion

N= 1 volunteer eligible, not enrolled

N= 36 volunteers challenged
Time line dose escalation

Cohort 1
June 2017
N=5
1000 cfu

None colonized

Cohort 2
August 2017
N=4
10,000 cfu

3 colonized

Cohort 3
November 2017
N=5
10,000 cfu

2 colonized

Cohort 4
January 2018
N=5
50,000 cfu

55%

Cohort 5
March 2018
N=5
100,000 cfu

4 colonized

Cohort 6
June 2018
N=5
100,000 cfu

4 colonized

Cohort 7
July 2018
N=5
100,000 cfu

4 colonized

Standard inoculum dose: $10^5$ cfu

80%
Colonization fraction

% colonized of challenged volunteers

Inoculum dose in cfu
Evaluation of accuracy of the inoculum dilution

Intended inoculum dose in cfu

- $10^3$
- $10^4$
- $5 \times 10^4$
- $1 \times 10^5$

Mean residuum count in cfu

- 964
- 8,952
- 41,800
- 143,555
Symptoms persisting >4 hour

<table>
<thead>
<tr>
<th>Symptom</th>
<th>$10^3$ cfu (n=5)</th>
<th>$10^4$ cfu (n=9)</th>
<th>$5x10^4$ cfu (n=5)</th>
<th>$10^5$ cfu (n=15)</th>
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<tr>
<td>Headache</td>
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<td>Feeling generally unwell</td>
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% of volunteers during admission

Legend:
- Severe
- Moderate
- Mild
- None
## Maximum severity reported (yellow: mild, orange: moderate)

| Day | Dose | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | Symptoms |
|-----|------|---|---|---|---|---|---|---|---|---|---|----|----|----|----|----|----|--------|
|     |      |   |   |   |   |   |   |   |   |   |   |    |    |    |    |    |    |        |

### Six volunteers inoculated with $10^4$ cfu had no solicited symptoms lasting >4 hours
- **AA10**: $10^4$
- **AA13**: $10^4$
- **AA16**: $10^4$

### Three volunteers inoculated with $5 \times 10^4$ cfu had no solicited symptoms lasting >4 hours
- **AA21**: $5 \times 10^4$
- **AA29**: $5 \times 10^4$

### Nine volunteers inoculated with $10^5$ cfu had no solicited symptoms lasting >4 hours
- **AA26**: $10^5$
- **AA27**: $10^5$
- **AA33**: $10^5$
- **AA39**: $10^5$
- **AA46**: $10^5$
- **AA50**: $10^5$

Dose: $10^4$ cfu

<table>
<thead>
<tr>
<th></th>
<th>Day 4</th>
<th>Day 7</th>
<th>Day 9</th>
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</table>
Colonization dynamics

**Culture nasal wash over time**

- Inoculum $10^4$ cfu
- Inoculum $5 \times 10^4$ cfu
- Inoculum $10^5$ cfu

**qPCR nasal wash over time**

- Ct value
Nasal wash culture vs qPCR

![Graphs showing nasal wash culture vs qPCR results]
Culture positive

Nasal wash day

Ct value

AA26 nasal wash PCR/cult

CfU

AA27 nasal wash PCR/cult

Ct value

AA31 nasal wash PCR/cult

CfU

AA33 nasal wash PCR/cult

Ct value

AA34 nasal wash PCR/cult

CfU

AA45 nasal wash PCR/cult

Ct value

AA49 nasal wash PCR/cult

CfU

AA53 nasal wash PCR/cult

Ct value

AA37 nasal wash PCR/cult

CfU

AA39 nasal wash PCR/cult

Ct value

AA46 nasal wash PCR/cult

CfU

AA48 nasal wash PCR/cult

Ct value

AA50 nasal wash PCR/cult

CfU

AA55 nasal wash PCR/cult

Ct value

AA56 nasal wash PCR/cult

CfU

Culture negative
## Eradication efficacy

**Azithromycin: 88% in 48 hours**

<table>
<thead>
<tr>
<th>Day 4</th>
<th>Day 7</th>
<th>Day 9</th>
<th>Day 11</th>
<th>Day 14</th>
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Culture of *Bp* comparing nasal wash and pernasal swab at day -7 till day 16 (n=34)

<table>
<thead>
<tr>
<th></th>
<th>Pernasal swab</th>
<th></th>
<th>Nasal wash</th>
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<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
<td>Total</td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>Nasal wash</td>
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<td>70</td>
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<td></td>
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<td>134</td>
<td>0</td>
<td>134</td>
</tr>
<tr>
<td>Total</td>
<td>24</td>
<td>180</td>
<td>204</td>
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<td>180</td>
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Nasal wash is more sensitive than per-nasal swab
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<th></th>
<th>Culture Nasal wash</th>
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<tbody>
<tr>
<td>qPCR Pernasal swab</td>
<td>Positive 10 Negative 18 Total 28</td>
<td>Positive 22 Negative 5 Total 27</td>
<td>Positive 10 Negative 33 Total 45</td>
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<tr>
<td>qPCR Nasal wash</td>
<td>Negative 3 Positive 59 Negative 62 Total 62</td>
<td>Positive 19 Negative 14 Total 33</td>
<td>Negative 2 Positive 15 Negative 17 Total 17</td>
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<tr>
<td>qPCR Nasal wash</td>
<td>Total 13 Negative 77 Total 90</td>
<td>Total 41 Negative 19 Total 60</td>
<td>Total 12 Negative 48 Total 60</td>
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Nasal wash culture and PCR are equivalent
qPCR of pernasal swab misses 46% of positives
**Bp PCR comparing nasal wash, pernasal swab and throat swab at day -7 till day 16 (n=15)**

<table>
<thead>
<tr>
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<td>Negative</td>
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<td><strong>Throat swab</strong></td>
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<td>Positive</td>
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<td>93</td>
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<tr>
<td>Negative</td>
<td>39</td>
<td>96</td>
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qPCR of throat swab is relatively insensitive
Diagnostics summary

- Nasal wash most sensitive sampling technique
- Nasal wash culture and PCR equivalent
- Pernasal swab- culture 36% sensitive versus PCR 77%
- PCR of throat swabs detected 36% of all PCR positive samples (for pernasal swab this was 54%, and nasal wash 94%)
IgG levels absolute

- Anti pertussis toxin IgG
- Anti pertactin IgG
- Anti filamentous haemagglutinin IgG
- Anti fimбриae IgG
IgG levels absolute – fold change

Anti pertussis toxin IgG

Anti pertactin IgG

Anti filamentous haemagglutinin IgG

Anti fimbriae IgG

Fold change in Anti PT IgG level

Fold change in Anti PRN IgG level (IU/mL)

Fold change in anti FHA IgG level (IU/mL)

Fold change in Anti PT IgG level (IU/mL)

Fold change in Anti FIM IgG level (EU/mL)

Days

Days

Days

Days

- Not colonized
- Colonized - low density (<1000 cfu/mL)
- Colonized - high density (>1000 cfu/mL)
Serum IgG levels after *Bp* exposure comparing dose groups

A

Dose $10^3$ cfu
Dose $10^4$ cfu
Dose $5 \times 10^4$ cfu
Dose $10^5$ cfu

Days after *Bp* challenge

Anti PT IgG level (IU/mL)

B

Anti PRN IgG level (IU/mL)

C

Anti FHA IgG level (IU/mL)

D

Anti FIM 2/3 IgG level (EU/mL)
Correlate of protection? T=0

**Anti PT colonised vs non colonised all**

![Graph showing Anti PT IgG level (IU/mL) for colonised vs non colonised](image)

**Anti PRN colonised vs non colonised all**

![Graph showing Anti PRN IgG level (IU/mL) for colonised vs non colonised](image)

**Anti FHA colonised vs non colonised all**

![Graph showing Anti FHA IgG level (IU/mL) for colonised vs non colonised](image)

**Anti FIM colonised vs non colonised all**

![Graph showing Anti FIM IgG level (IU/mL) for colonised vs non colonised](image)
Plasma B cell ELISPOT

Day 0
Pre-challenge
(Representative of 25 volunteers)

Day 7
(Representative of 25 volunteers)

Day 14
Uncolonised
(Representative of 9 volunteers)
Colonised
(Representative of 16 volunteers)

Bp-specific IgG secreting cells are detected 14 days after B. pertussis challenge, in colonised individuals
ELISPOT for Bp-specific IgG-secreting plasma cells

**Negative controls**

**PBS**

- Uncolonised
- Colonised

**Bp antigens**

**PTx**

- Uncolonised
- Colonised

**FHA**

- Uncolonised
- Colonised

**TT**

- Uncolonised
- Colonised

**PRN**

- Uncolonised
- Colonised

**FIM**

- Uncolonised
- Colonised

- From last 5 cohorts in Phase A, receiving either 10,000, 50,000 or 100,000 CFU.

*P≤0.05 for comparison between time points; # P≤0.05 for comparison between uncolonised and colonised
ELISPOT for Bp-specific IgA-secreting plasma cells

**Negative controls**

- **PBS**
  - Uncolonised
  - Colonised

- **PTx**

- **FHA**
  - * indicates a significant difference

**Bp antigens**

- **TT**

- **PRN**

- **FIM**

Uncolonised n=9
Colonised n =16

From last 5 cohorts in Phase A receiving either 10,000, 50,000 or 100,000 CFU.

*P \leq 0.05 for comparison between time points*
Plasma cell ELISPOT data correlates with serology data

PTX

$r_s = 0.6575$

$P = 0.0007$

FHA

$r_s = 0.6738$

$P = 0.0004$

PRN

$r_s = 0.3222$

$P = 0.1338$

FIM

$r_s = 0.3249$

$P = 0.1249$

Uncolonised $n=9$

Colonised $n=16$

From last 5 cohorts in Phase A, receiving either 10,000, 50,000 or 100,000 CFU.

$r_s =$ Spearman correlation coefficient. $P≤0.05$ considered significant.
Memory B cell ELISPOT

**Negative control**

**Positive control memory responses**

**Control for polyclonal activation**

**Bp antigens**

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<th>HA</th>
<th>Total IgG</th>
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<th>FHA</th>
<th>PRN</th>
<th>FIM</th>
<th>Bp lysate</th>
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</table>

*Bp-specific IgG memory cells are detectable 7-14 days after B. pertussis challenge, in one of the colonised individuals*
ELISPOT for Bp-specific memory IgG cells

**Negative control**

**Positive control memory responses**

**Control for polyclonal activation**

**Bp-specific memory responses**

Uncolonised n= 7  
Colonised n = 14  
From last 5 cohorts in Phase A receiving either 10,000, 50,000 or 100,000 CFU.
T cell assays

Stimulation of diluted whole blood with *B. pertussis* antigens

- Heat inactivated *B. pertussis* lysate (BpLys)
- Heat inactivated Pertussis toxin (PTx)
- Filamentous hemagglutinin (FHA)
- SEB (positive control)

37°C 24h

19h

Sample processing

Cytokine secretion blockade
+ Brefeldin A
+ Goligistop (monesin)

37°C 72h

Supernatant collection

Supernatants sent to Sanofi for detection of cytokine release by Luminex (N=5)

Cytokine release assay

Flow cytometric assay

Staining for T cell markers (Th1, Th2, Th17, activation) and flow cytometric analysis

No significant differences in T cell marker expression over time post-challenge, or between colonised and uncolonised volunteers (N=10)

Stored in LN₂ and shipped to PERISCOPE collaborators for analysis with final optimised antibody panel (N=10)

Cytokine secretion blockade

Supernatant collection

Supernatants sent to Sanofi for detection of cytokine release by Luminex (N=5)

CyTOF assay

+ Proteomic stabiliser

Stored at -80°C for future analysis by CyTOF

Euroflow Assay

Standardised multidimensional flow cytometric immunophenotyping
Peter Piper picked a peck of pickled peppers.

A peck of pickled peppers Peter Piper picked.

If Peter Piper picked a peck of pickled peppers,

Where’s the peck of pickled peppers Peter Piper picked?

John Harris (1756–1846)

Environmental shedding
Conclusion phase A

1. Asymptomatic colonisation can be induced after intranasal inoculation with *B. pertussis* without causing disease.
2. The inoculum required for >70% colonisation is \( \geq 10^5 \) cfu
3. By 11 days 100% of colonised volunteers are culture positive
4. Azithromycin clears carriage in 88% of the colonised volunteers within 48 hours
5. Culture and qPCR of nasal wash is more sensitive than nasopharyngeal swab
6. Seroconversion occurs in some but not all colonised individuals
7. No environmental shedding has been detected in any of the volunteers
Controlled Human Infection With *Bordetella pertussis* Induces Asymptomatic, Immunizing Colonization

H. de Graaf,1 M. Ibrahim,2 A. R. Hill,2 D. Ghesemete,3 A. T. Vaughan,2 A. Gorringe,3 A. Preston,4 A. M. Buisman,5 S. N. Faust,1 K. E. Kester,5 G. A. M. Berbers,5 D. A. Diavatopoulos,7 and R. C. Read1

1 Faculty of Medicine and Institute for Life Sciences, University of Southampton, Academic Unit of Clinical Experimental Sciences, NIHR Clinical Research Facility and NIHR Southampton Biomedical Research Centre, University Hospital Southampton NHS Foundation Trust, 2 Faculty of Medicine and Institute for Life Sciences, University of Southampton, Southampton General Hospital, 3 Public Health England, Salisbury, and 4 The Milner Centre for Evolution and Department of Biology and Biochemistry, University of Bath, United Kingdom, 5 Centre for Infectious Disease and Control, National Institute for Public Health and the Environment, Bilthoven, the Netherlands, 6 Translational Science and Biomarkers, Sanofi Pasteur, Swiftwater; and 7 Section Paediatric Infectious Diseases, Laboratory of Medical Immunology, Radboud Institute for Molecular Life Sciences, Radboud UMC, Radboud Center for Infectious Diseases, Nijmegen, The Netherlands
B. pertussis human challenge study
University of Southampton, UK

Phase B

Hans de Graaf, Chief investigator: Robert C. Read
Southampton
02/10/2019
Overview of the study

Phase A
Inoculum dose finding phase to cause safe colonisation of 70% of volunteers

Phase B
Confirmation of inoculum dose and Searching for correlates of protection against colonisation
• **Intervention:** Nasal inoculation with *B. pertussis* strain B1917 dose $10^5$ cfu

• **Endpoint:** Colonisation
  – Culture positive – pernasal swab/throat swab/nasal wash/nasolacrimal fluid
  – At any point over 2 weeks
Phase B - Objectives

• To confirm the dose of the standard inoculum *B. pertussis* (*Bp*)

• To measure kinetics and density of *Bp* colonisation

• To assess *Bp*-specific immunity in healthy volunteers before and after nasal inoculation with *Bp*

• To assess transmission of *Bp* to bedroom contacts of inoculated volunteers

• To identify biomarkers that correlate with protection against *Bp* colonisation

• To conduct a re-challenge
Challenge volunteers

• Healthy volunteers aged 18 – 55 years
• Vaccinated > 5 years ago
• **Not** exclude an anti pertussis toxin antibody level >20 IU/mL).
• No children aged < 12 years in the household

Contact volunteers (max 25)

• Bedroom sharers of challenged volunteers
Overview

• Approximately 66 individuals, then review the power of the study and adjust sample
• Out patient setting
• Optional re-challenge with the standard inoculum 14 weeks after the initial inoculation.
• Eradication therapy 14 days after each challenge to both the challenge volunteer and the contact volunteer.
# Challenge volunteers

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<th>D-30</th>
<th>D-7</th>
<th>D0</th>
<th>D3</th>
<th>D7</th>
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</table>
## Challenge volunteers

<table>
<thead>
<tr>
<th>Time-point</th>
<th>W13</th>
<th>W14</th>
<th>W15</th>
<th>W16</th>
<th>W18 (TC)</th>
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</thead>
<tbody>
<tr>
<td>Visit</td>
<td>x</td>
<td>X</td>
<td>x</td>
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<tr>
<td>Challenge with SI</td>
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<td>Clinical review</td>
<td>x</td>
<td>X</td>
<td>x</td>
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<tr>
<td>Bloods (mL)</td>
<td>90</td>
<td>39</td>
<td>39</td>
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<tr>
<td>Nasal and oral samples</td>
<td>x</td>
<td>X</td>
<td>x</td>
<td>x</td>
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<tr>
<td>Azithromycin start</td>
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</table>
### Contact volunteers

<table>
<thead>
<tr>
<th>Time-point</th>
<th>D-30 Screening</th>
<th>D7</th>
<th>D14</th>
<th>W16</th>
<th>W18 (TC)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Contact volunteers</td>
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<tr>
<td>Visit window (days)</td>
<td>-30/+23</td>
<td>-2/2</td>
<td>-2/2</td>
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<tr>
<td>Visit</td>
<td>x</td>
<td>x</td>
<td>x</td>
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<tr>
<td>Informed consent</td>
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<tr>
<td>Screening examinations</td>
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<tr>
<td>Clinical review</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
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<tr>
<td>Nasal wash</td>
<td>x</td>
<td>x</td>
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<td>x</td>
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<tr>
<td>Azithromycin start</td>
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</tbody>
</table>
Visit overview phase B

- Challenge volunteers
  - Screening
  - Visit
  - Inoculation
  - Visit
  - Eradication

- Contact volunteers
  - Screening
  - Visit
  - Eradication

Day: -30 -7 0 3 7 14 28
Overview phase B – re-challenge

Challenge volunteers

Contact volunteers

Visit  Inoculation  Visit  Eradication  Telephone contact

-7 0 7 14 28
• Ethical approval initial amendment phase B - 21/3/2019
• Ethical approval amendment re-challenge – 19/8/2019
• 1st volunteer screened - 16/8/2019
• 1st volunteer challenged - 23/9/2019
Bacterial count of stock vials

![Graph showing bacterial count in CFU across different cohorts](https://www.periscope-project.eu)

- Pre-study check
- Cohort 7/2017
- Cohort 8/2017
- Cohort 11/2017
- Cohort 1/2018
- Cohort 3/2018
- Cohort 6/2018
- Cohort 7/2018

**Y-axis:** Vial count in CFU

**X-axis:** Cohorts
Thanks to the volunteers, the team in Southampton, Public Health England, RIVM and our partners within PERISCOPE
Questions?