

Controlled human infection with *Bordetella pertussis* induces asymptomatic, immunising colonisation

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Summary:

Asymptomatic *B. pertussis* nasopharyngeal colonisation can be induced in adults and results in seroconversion in most colonized volunteers, but not in non-colonized. Nasal wash sampling is more sensitive than pernasal swabbing. Azithromycin clears carriage in most people within 48 hours.

Abstract

Rationale: *Bordetella pertussis* is one of the leading causes of vaccine preventable death and morbidity globally. Human asymptomatic carriage as a reservoir for community transmission of infection might be a target of future vaccine strategies but has not been demonstrated to occur.

Objective: To demonstrate that asymptomatic nasopharyngeal carriage of *Bordetella pertussis* is inducible in humans and to define microbiological and immunological features of pre-symptomatic infection.

Methods: Healthy subjects aged 18-45 years with an anti-pertussis toxin IgG concentration of <20 IU/ml were inoculated intranasally with non-attenuated, wild type *Bordetella pertussis* strain B1917. Safety, colonisation and shedding were monitored over 17 days in an in-patient facility. Colonisation was assessed by culture and qPCR. Azithromycin was administered from day 14. The inoculum dose was escalated aiming to colonise at least 70% of participants. Immunological responses were measured.

Results: 34 participants were challenged in groups of four or five. The dose was gradually escalated from 10^3 colony forming units (0% colonised) to 10^5 colony forming units (80% colonised). Minor symptoms were reported in a minority of participants. Azithromycin eradicated colonisation in 48 hours in 88% of colonised individuals. Anti-pertussis toxin IgG seroconversion occurred in nine out of 19 colonised participants and in none of the participants who were not colonised. Nasal wash was a more sensitive method to detect colonisation than pernasal swabs. No shedding of *Bordetella pertussis* was detected in systematically collected environmental samples.

Interpretation: *Bordetella pertussis* colonisation can be deliberately induced and leads to a systemic immune response without causing pertussis symptoms.

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Keywords: *Bordetella pertussis*, human challenge, carriage, immune response

Introduction

Pertussis is the leading cause of vaccine-preventable death, resulting in approximately 24.1 million pertussis cases and 160,700 deaths from pertussis in children younger than 5 years worldwide in 2014 [1]. Pertussis vaccines have been included in national immunisation programmes since the 1940s-1950s, and many countries have switched from the original whole cell pertussis (wP) vaccine to acellular pertussis (aP) vaccines because aP vaccines have a favourable reactogenicity profile [2].

Despite high immunisation coverage, some developed countries have seen an increase in the incidence of pertussis over the past 20 years [3]. Transmission of *Bordetella pertussis* (*Bp*), the cause of pertussis, occurs by aerosolised respiratory droplets [4]. Studies using a baboon model of pertussis have shown that both aP and wP protect against severe disease, but not infection of the respiratory tract. Vaccination with wP induced a more rapid clearance compared with naïve and aP-vaccinated animals. By comparison, previously infected animals were not colonised upon secondary infection. This may be related to failure of aP to induce Th1 and Th17 memory responses required for sterilising mucosal immunity [5].

Whether *Bp* can exist in a human carrier state is an important question. Negligible carriage rates in epidemiological studies [6, 7] have not supported a carrier state of *Bp*. However, sero-epidemiological studies have revealed evidence for sero-conversion in the absence of clinical disease [8] suggesting that asymptomatic colonisation and transmission does occur sub-clinically in populations. The importance of this is that future vaccine strategies will need to reduce efficiently transmission between asymptomatic carriers. This has been demonstrated for medically-important nasopharyngeal pathobionts, such as *Streptococcus pneumoniae*, *Neisseria meningitidis* and *Haemophilus influenzae*; in each case herd protection conferred by potent glycoconjugate vaccines results from interruption of transmission by vaccine-induced protection against the carrier state [9, 10]. On the other hand, individuals harmlessly colonised benefit from immunity conferred by the carrier state which is a mechanism of natural protection against diseases such as pneumococcal or meningococcal disease [11].

As part of a European collaborative effort to accelerate pertussis vaccine development [12] we conducted controlled human infection with *Bp* to demonstrate that asymptomatic colonisation can occur, provide a safe human colonisation model for the development of

bioassays and testing of improved pertussis vaccines, and investigate the pathobiology of *Bp* infection.

Methods

This was a first in human study conducted in accordance with the provisions of the Declaration of Helsinki (1996) and the International Conference on Harmonization Guidelines for Good Clinical Practice. This study is registered with ClinicalTrials.gov: NCT03751514, ethical committee reference: 17/SC/0006. The protocol has been published ahead of this report [13], and can be found on www.periscope-project.eu.

Study population

Eligible participants were healthy males and females, aged 18–45 years, who were available for the admission period and all scheduled visits, with a history of being vaccinated against *Bp* no less than five years before enrolment, non-smokers, with no use of antibiotics within four weeks of enrolment, and no contact with people vulnerable to *Bp* disease. Participants with a serum anti-pertussis toxin immunoglobulin G (IgG) >20 international units/litre (IU/l), a positive *Bp* culture from a pernasal swab, pregnant women, nursing mothers, females of childbearing age who do not use acceptable birth control, people with impairment/alteration of the immune system (including immunosuppressive therapy) and people with a contra-indication to azithromycin were excluded.

Interventions

Participants received a nasal inoculum of 1 ml containing *Bp* strain B1917, which is a fully genotyped representative of current European isolates [14]. The strain, isolated from a Dutch patient with *Bp* disease, is characterised as *ptxP3-ptxA1-prn2-fim3-2, fim2-1* MLVA27,

PFGE BpSR11 and expresses pertactin (PRN), pertussis toxin (PT), fimbriae 3 (FIM 3), and filamentous hemagglutinin (FHA). The dose of the inoculum, starting at 1000 colony forming units (cfu), was adjusted after each fifth subject to achieve colonisation of 70% of the subjects. Colonisation was defined as any positive *Bp* culture from nasal or oral samples at any time point between day 3 and day 14.

Participants were admitted to the research facility for 17 days and monitored for any signs of early *Bp*-disease including cough, sore throat, nasal congestion, rhinorrhoea, sneezing and feeling generally unwell. Vital signs and adverse events were recorded four hourly during admission and at each follow up visit. Following discharge, subjects had four follow-up visits over 12 months. If early *Bp* disease was suspected on the basis of solicited adverse events, then additional bloods and a throat swab for viral PCR (influenza A, influenza B, parainfluenza type 1, 2 and 3, rhinovirus, RSV, adenovirus, and metapneumovirus) were taken to exclude an alternative aetiology for these symptoms.

All participants had pernasal swabs, nasal washes, throat swabs, and nasosorption fluid samples taken at pre-determined intervals (Table 1). Pernasal swabs and throat swabs were taken as per clinical protocol. Nasal wash samples were obtained by gently pushing 10 mL of normal saline in each nostril of the volunteer laying in in the supine position. After one minute the volunteer was asked to sit up and bend forward to allow the instilled fluid to be extruded from the nose by gravity into a petri dish. Nasosorption fluid samples were taken placing a strip of a hydrophilicpolyester absorptive matrix (Mucosal Diagnostics, Hunt Developments Ltd.) into the nostril for two min.

Bp colonisation was identified by culture of these samples and identification was confirmed by MALDI-TOF. The minimum detection rate of the culture was 6 cfu. qPCR was performed on pernasal swabs, nasal washes and throat swabs of the 15 volunteers who received the standard inoculum (see below). Detail of the microbiological methods used is provided in the supplementary material. Blood samples were taken at intervals and analysed for seroconversion against pertussis toxin (PT), pertactin, FHA, and Fim 2/3 at day 0 and day 28, and B cell responses using ELISPOT (day 0, day 7 and day 14). The IgG antibody concentrations were quantified using the fluorescent-bead-based multiplex immunoassay (MIA) as described by van Gageldonk *et al* [15]. ELISPOT methodology is provided in the supplementary material. Environmental samples were tested by culture and PCR to assess shedding from the volunteers. These included mask samples, fingertip samples, multiple surface samples, bedroom air samples, and air samples taken during standardised aerosol provoking procedures such as talking and coughing using the Coriolis air sampler (Bertin Technologies SAS, Montigny-le-Bretonneux, France).

Objectives

The primary objective was to determine the standard inoculum dose, defined as the inoculum dose that results in *Bp* carriage in at least 70% of the exposed subjects without causing *Bp* disease.

Predefined secondary objectives included: characterisation of the microbiological dynamics after challenge, the effectiveness of azithromycin eradication therapy, pre- and post-challenge *Bp*-specific immunity in healthy subjects, and the environmental shedding of *Bp* following nasal inoculation.

In this dose escalation study, safety and colonisation parameters were reviewed by an external committee after each fifth subject and the inoculum dose for the following five subjects was agreed. Enrolment ceased when at least 10 subjects had been colonised with the standard inoculum.

Statistical methods

The percentage of participants successfully colonised (colonisation fraction) with *Bp* at each dose and associated 95% confidence intervals (CI) were calculated using the modified Wald method. Conventional culture and qPCR data are presented as median, interquartile range (IQR), minimum and maximum. To compare conventional culture with qPCR, data were analysed using McNemar's test and sensitivity was calculated. These data are presented in contingency tables. Serological data comparing colonised and uncolonised participants were analysed using Wilcoxon test. Differences in ELISPOT assay readouts were compared using the Kruskal-Wallis test with Dunn's correction.

Results

A total of 54 subjects were screened between June 2017 and July 2018. No *Bp* was detected in any pernasal swab at screening and all participants had received wP vaccination in childhood. Thirty four healthy subjects were enrolled and inoculated intranasally with *Bp* in a dose-escalation study design (Figure 1). The demographic variables and baseline IgG concentration against common *Bp* antigens; PT, PRN, FHA, and FIM 2/3 are shown in Table 2. All subjects were followed up for at least three months.

***B. pertussis* colonisation**

The dose of intranasal inoculum was gradually increased following an algorithm, starting at a dose of 1000 cfu, which did not result in colonisation. As the inoculum dose increased, so did the colonisation fraction; 55% (95% CI 27-82%) at a dose of 10,000 cfu, 40% (95% CI 12-77%) at a dose of 50,000 cfu, reaching 80% (95% CI 54-94%) at a dose of 100,000 cfu. On completion of the protocol, 19 participants had become colonised. There was no significant difference between the baseline demographic characteristics and pertussis antibody levels of the colonised and uncolonised group (Table 2). *Bp* was cultivable from nasal wash samples by day 4 in most colonised subjects and the quantitative count then rose gradually and peaked on day 11 (Figure 2A), and a substantial decrease on day 15 and 16 following commencement of azithromycin eradication therapy (Supplementary figure S3 for colonisation density plotted individually). This is mirrored in the qPCR data (Figure 2B); *Bp* DNA was still detectable on day 15 and 16 in culture-negative samples, as would be expected.

Comparison of microbiological sampling method

Nasal wash was the most sensitive technique for microbiological detection of *Bp* colonisation; conventional culture was equally sensitive at detecting *Bp* colonisation as qPCR of nasal wash samples (40 out of 48 samples, 83%; Table 3). Regarding pernasal swabbing, the conventional sampling procedure for laboratory diagnosis of *Bp* infection, qPCR was more sensitive at detecting *Bp* colonisation than conventional culture, 77% versus 36%, respectively. However, PCR of pernasal swabs was not as sensitive as PCR of nasal washes, 52% versus 87%, respectively. Comparing cultures of nasal wash samples and pernasal swabs taken at the same sampling times, *Bp* was significantly more frequently detected in

nasal wash samples ($p < 0.01$, McNemar's test) (Table 4). PCR of throat swabs detected 36% of all PCR positive samples taken at the same sampling times ($n = 70$), for pernasal swabs this was 54% and for nasal washes 94% (Table 5). Only one throat swab was culture positive and nasosorption fluid culture was never positive.

Clearance and eradication of *Bp* colonisation

Of the 19 participants who were successfully colonised with *Bp*, three cleared colonisation prior to receiving azithromycin. Eradication therapy rendered all samples culture negative by 48 hours in 14 out of 16 subjects (88%). The remaining two volunteers who were still colonised at day 16 were brought back for an additional follow up visit at day 21 by which time neither was carrying any detectable *Bp*.

Experimental infection with *Bp* is safe

There were no serious adverse events during the course of the study, no participants received rescue-eradication therapy, and no subjects withdrew due to study-related adverse events. Solicited adverse events occurred equally frequently in the colonised group and the uncolonised group. Mild symptoms of cough, rhinorrhoea and nasal congestion were reported more frequently in the groups receiving higher inoculum doses (Supplementary figure S1 and S2). Viral PCR was negative in all tested subjects. Overall, controlled human pertussis infection was safe with no significant safety concerns in any subject.

Immune response to colonisation

Serological response assessed by multiplex immune-assay

Serum antibody concentrations were measured against the following *B. pertussis* antigens; PT, PRN, FHA, and FIM 2/3 on day 0 and day 28. A significant rise in serum IgG concentration was found against PT, PRN, and FHA at the highest inoculum dose (Figure 3). Comparing colonised with uncolonised participants, five out of 12 of those colonised after inoculation with 10^5 cfu (n=15) exhibited a four-fold or more increase in serum anti-PT IgG concentration. Conversely, none of the uncolonised subjects exhibited a rise in serum anti-PT IgG concentration (Figure 4). All participants with a rise in anti-PT concentration also had a rise in anti-IgG concentration against other antigens (Table 6 and Supplementary table S4).

Detection of antibody-secreting plasma cells specific to *B. pertussis* antigens by ELISPOT

No antigen-specific IgG or IgA secreting cells was detected by ELISPOT in any of the subjects at day 0 or 7, above background concentrations detected in the PBS blank control or the tetanus toxin negative control antigen. (Figure 5). At day 14, there was a trend for increased numbers of antigen-specific IgG and IgA secreting cells in colonised participants, compared to day 0 and day 7, and compared to uncolonised participants. This increase was significant ($=<0.05$) for FHA-specific Ig secreting cells (Figure 5 C-D).

Environmental sampling

In a controlled aerosolisation experiment, median 17% (IQR 1-40%) *Bp* which had been aerosolised into an environmental chamber at various concentrations was recovered using

the air sampler with a limit of detection of 15 cfu/ml(Supplementary table S5). Following extensive sampling and culture, no environmental shedding of *Bp* from colonised participants was detected. Mask sample cultures (n=442), air samples taken during aerosol-provoking procedures (n=1088), bedroom air samples (n=272), contact cultures (n=1904), or fingertip cultures (n=442) all tested negative for *Bp* culture.

Discussion

This first-in-human study has demonstrated that asymptomatic colonisation can be induced safely by intranasal inoculation with wild type *Bp*, and is associated with seroconversion, suggesting true biological colonisation of the host. The dose needed to induce colonisation of approximately 80% of the exposed subjects is 10^5 cfu. Nasal washing was the most sensitive technique to detect colonisation; pernasal swabbing, the conventional diagnostic technique, was even negative in one individual who seroconverted. Induced colonisation causes a systemic immune response in the form of a rise in antigen specific serum IgG concentration and detectable specific B-cells in some, but not all colonised individuals. Azithromycin clears carriage in most people by 48 hours.

This study adds *prima facie* evidence to support epidemiological and serological observations which suggest that asymptomatic *Bp* colonisation is part of the natural life cycle of the organism. The significance of this is that transmission from this reservoir to susceptible people is probably responsible for sporadic pertussis cases and outbreaks [16], and will need to be targeted in future successful vaccination strategies to achieve herd protection. Screening studies have failed to detect asymptomatic colonisation [6, 7], which

has been demonstrated in only a few cases during contact studies [17, 18], but this study shows that the microbiological sampling technique used is likely to be critical.

This is not the first time that human subjects have been infected deliberately with wild type *Bp*. In a very small paediatric study carried out in 1933, *Bp* disease was induced by exposing two presumably immunologically naïve children to 140 cfu *Bp* bacteria. No asymptomatic colonisation was detected as the participants developed symptoms at the same time as positive cultures were obtained from cough samples one week after exposure [19]. A Phase 1 trial of a live, genetically attenuated *Bp* intranasal vaccine has been reported.

Asymptomatic colonisation was demonstrated after inoculation with BPZE1, a *Bp* strain in which dermonecrotic toxin and tracheal cytotoxin is genetically deleted, and PT is genetically detoxified by two independent mutations, removing the toxic activity of PT without affecting its immunogenic properties [20]. In the BPZE1 study, colonisation was detected in one out of 12 (8%) subjects inoculated with 10^3 cfu, one out of 12 (8%) inoculated with 10^5 cfu and five out of 12 (42%) inoculated with 10^7 cfu. These colonisation fractions are significantly lower than those at the equivalent, and lower doses of *Bp* in our study using a wild type strain, suggesting that the toxins removed from *Bp* are important for colonisation. In addition our study demonstrates that asymptomatic colonisation can still occur in the presence of these potent toxins. Other nasal challenge studies such as the *S. pneumoniae* challenge studies [21] and the *N. lactamica* [22, 23] studies show a similar increase in colonisation fraction with increasing dose.

In the current study, subjects who were inoculated but not colonised exhibited no rise in anti *Bp* antigen specific IgG concentration. Specific antibody was not produced in some colonised participants, unlike other nasal challenge studies in which all colonised volunteers

exhibit seroconversion [21, 22]. This may reflect our protocol requirement to terminate colonisation using azithromycin at 14 days, in order to avoid progression to a lengthy syndrome of cough [24]. All participants will have received a whole cell pertussis vaccine in infancy as acellular pertussis vaccine was introduced into the UK in 2005. The increase in anti *Bp* serum IgG concentrations seen in colonised subjects in our study is modest compared to that which is observed after *Bp* disease [25], or *Bp* vaccination [26].

The gold standard for diagnosis of pertussis is qPCR or culture of pernasal swabs [27]. Epidemiological studies looking for asymptomatic colonisation have also used PCR of pernasal swabs [6, 7]. Our study has revealed that culture or PCR of nasal wash samples is much more sensitive than PCR of pernasal swab samples, likely due to the surface area sampled, which may explain negative findings in previous epidemiological studies.

In patients being treated for whooping cough, azithromycin eradicates *Bp* from the nasopharynx in 97% of individuals with disease within 3 days [28]. In this study, azithromycin eradicated colonisation in 88% of colonised subjects within two days, supporting current public health outbreak guidelines [27]. Although the sample used in our study is small, there was a trend suggesting that adults with higher anti-PRN and anti-FIM 2/3 antibody concentrations are protected against colonisation, consistent with epidemiological studies [20, 29, 30].

The absence of environmental shedding in these asymptomatic participants is striking. Our participants received wP in infancy. It is possible that this impacts on the likelihood of shedding during asymptomatic colonisation, and might be different amongst individuals who receive aP. Alternatively, it is possible that the methods used are not sensitive enough to detect subtle degrees of shedding. In summary, asymptomatic colonisation of the human

upper respiratory tract by *Bp* can be induced by experimental inoculation and is associated with a modest serological response in the majority of colonised volunteers. This has important implications for future vaccine strategies, and may explain the high seroprevalence of anti-PT IgG in populations and the epidemiological peaks that have been observed in *Bp* disease which suggest continued circulation of *Bp* in populations vaccinated with wP or aP.

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Author contribution:

HdG, RCR, AG, SNF, KEK, DD, AP and AB contributed to the study design. HdG, RCR, AG, DD, MI, ARH, ATV, GAMB and DG contributed to data collection, analyses and interpretation. HdG and RCR drafted and then finalised the manuscript. All authors reviewed and approved the final draft. All authors had full access to study data and hold final responsibility for publication submission.

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following article publication. To gain access, data requestors will need to sign a data access agreement.

Conflicts of interest

HdG, RCR, AG, SNF, DD, AP, AB, MI, ARH, ATV, GAMB and DG reports grants from IMI, during the conduct of the study. KEK is an employee of Sanofi Pasteur. SNF reports fees were paid to his institution (with no personal payment of any kind) from AstraZeneca/Medimmune, Sanofi, Pfizer, Sequerius, Sandoz, Merck, GSK, Alios, J&J, and Merck, outside the submitted work. DD reports personal fees from Sanofi Pasteur, outside the submitted work.

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Figure legends

Figure 1. Subject flowchart

Figure 2. Colonisation density in nasal wash samples of colonised subjects (n=19) over time

A) Culture results in total colony forming units (cfu) measured by dilutional plating.
B) Quantitative PCR results expressed as cycle threshold (Ct) value. Day 0 is day of inoculation. Results are presented as boxplots with median and 25% and 75% interquartiles, and whiskers representing minimum and maximum values.

Figure 3. Antigen specific serum IgG concentration after *Bp* exposure comparing dose groups

A) Anti-PT, B) Anti-PRN, C) Anti-FHA, D) Anti-Fim 2/3. Infected with: ○ inoculum dose 10^3 cfu (n=5), ◐ inoculum dose 10^4 cfu (n=9), ● inoculum dose 5×10^4 cfu (n=5), ● inoculum dose 10^5 cfu (n=15). Day 0 is day of inoculation. PT, pertussis toxin; PRN, pertactin; FHA, filamentous hemagglutinin; FIM 2/3, fimbriae 2/3; CfU, colony forming units. IU, international units. AU, Arbitrary units. Results are presented as scatter plots with median value.

*Significance between time points ($P < 0.05$) (Wilcoxon test)

Figure 4. Serum IgG concentration against *Bp* specific antigens after challenge with 10^5 cfu *Bp*.

IgG concentration of n=15 subjects exposed to 10^5 cfu *Bp*. A) Anti-PT, B) Anti-PRN, C) Anti-FHA, D) Anti-Fim 2/3. Black lines, colonised; dashed lines, not colonised. CfU, colony forming units. IU, international units. AU, Arbitrary units. Day 0 is day of inoculation. PT, pertussis toxin; PRN, pertactin; FHA, filamentous hemagglutinin; FIM 2/3, fimbriae 2/3.

Figure 5. IgG and IgA-secreting plasma B cell responses to *Bp* challenge

Numbers of plasma B cells secreting IgG and IgA specific for PT (A-B), FHA (C-D), PRN (E-F) and FIM 2/3 (G-H) by ELISPOT. PT, pertussis toxin; PRN, pertactin; FHA, filamentous hemagglutinin; FIM 2/3, fimbriae 2/3; TT, tetanus toxoid; PBS, phosphate buffered saline. PBS (I-J) and TT (K-L) were used as a background control and negative control antigen respectively. Results for volunteers who were uncolonised (□; n=9) and colonised (■; n=16) are shown as box plots representing the median with 25% and 75% interquartiles (IQRs), and whiskers representing minimum and maximum values. *Significance between time points ($P<0.05$) (Kruskal-Wallis test with Dunn's correction). #Significance between not-colonised and colonised response ($P<0.05$) (Kruskal-Wallis test with Dunn's correction)

Table 1. Overview of visits, admission and procedures during the study

	Day																Week							
	-30	-7	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	4	8	26	52	
Visit	x	x																			x	x	x	x
Admission				x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x				
Challenge				x																				
Blood sample	x		x	x	x	x	x		x	x		x					x				x	x	x	x
Nasal wash sample		x					x		x		x						x	x	x	x	x	x	x	x
Nasal fluid sample		x				x	x	x		x		x					x	x	x	x	x	x	x	x
Pernasal swab	x	x				x		x		x		x					x	x	x	x				
Throat swab		x				x		x		x		x					x	x	x	x				
Azithromycin																		x	x	x				
Shedding samples				x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x					

Table 2. Demographic characteristics base line anti *Bp* specific IgG concentration

	Excluded	Enrolled	Colonised	Uncolonised
	N=20	N=34	N=19	N=15
Age, median years (IQR)	24(20-35)	26 (21-35)	26 (21-37)	26 (22-34)
Males, n (%)	16 (80)	20 (59)	11 (58)	6 (40)
Weight, median kg (IQR)	81 (73-95)	74 (65-83)	73.7 (65-83)	74.2 (65-83)
Height, median cm (IQR)	177 (171-180)	176 (170-183)	175 (163-181)	177 (172-186)
Anti PT IgG, median IU/ml (IQR)	29.2 (9.7-46.5)	4.6 (2.1-9.3)	6.8 (1.1-9.5)	5.5 (2.2-8.6)
Anti PRN IgG, median IU/ml (IQR)	NA	13.2 (3.3-21.2)	9.8 (3.3-14.6)	14.7 (3.5-60.1)
Anti FIM 2/3 IgG, median AU/ml (IQR)	NA	5.2 (2.2-21.4)	2.7 (0.9-5.7)	12.3 (6.5-31)
Anti FHA IgG, median IU/ml (IQR)	NA	16.6 (8.4-32)	15.7 (6.1-26)	26.0 (8.4-50.8)

IQR, interquartile range; PT, Pertussis Toxin; PRN, Pertactin; FIM 2/3, Fimbriae 2/3; FHA, Filamentous hemagglutinin; IU, international units. AU, Arbitrary units. NA, not available

Table 3. Nasal wash and pernasal swab comparing detection of *Bp* by qPCR and culture at day 0-14

			Culture		
			Nasal wash		
			Positive	Negative	Total
qPCR	Pernasal swab	Positive	22	5	27
		Negative	19	14	33
		Total	41	19	60
qPCR	Nasal wash	Positive	40	8	48
		Negative	8	19	27
		Total	48	27	75

Number of samples presented of subjects exposed to 10^5 cfu *Bp* (n=15). Pernasal swab taken day 3, 5, 7, 9, 11, 14, nasal wash sample taken day 4, 7, 9, 11, 14

Table 4. Culture results of *Bp* in nasal wash and pernasal swab samples at day -7 till day 16

		Pernasal swab		
		Positive	Negative	Total
Nasal wash	Positive	24	46	70
	Negative	0	134	134
	Total	24	180	204

Number of samples presented off all subjects (n=34). Pernasal swabs and nasal wash both taken day 7, 9, 11, 14, 15, 16.

Table 5. *Bp* PCR results of nasal wash, pernasal swab and throat swab samples at day -7 till

day 16

	Pernasal swab			Throat swab			
		Positive	Negative	Total	Positive	Negative	Total
Nasal wash	Positive	34	32	66	23	43	66
	Negative	5	41	46	2	37	39
	Total	39	73	112	25	80	105
Throat swab	Positive	23	3	26			
	Negative	16	93	109			
	Total	39	96	135			

Number of samples presented of subjects exposed to 10^5 cfu *Bp* (n=15)

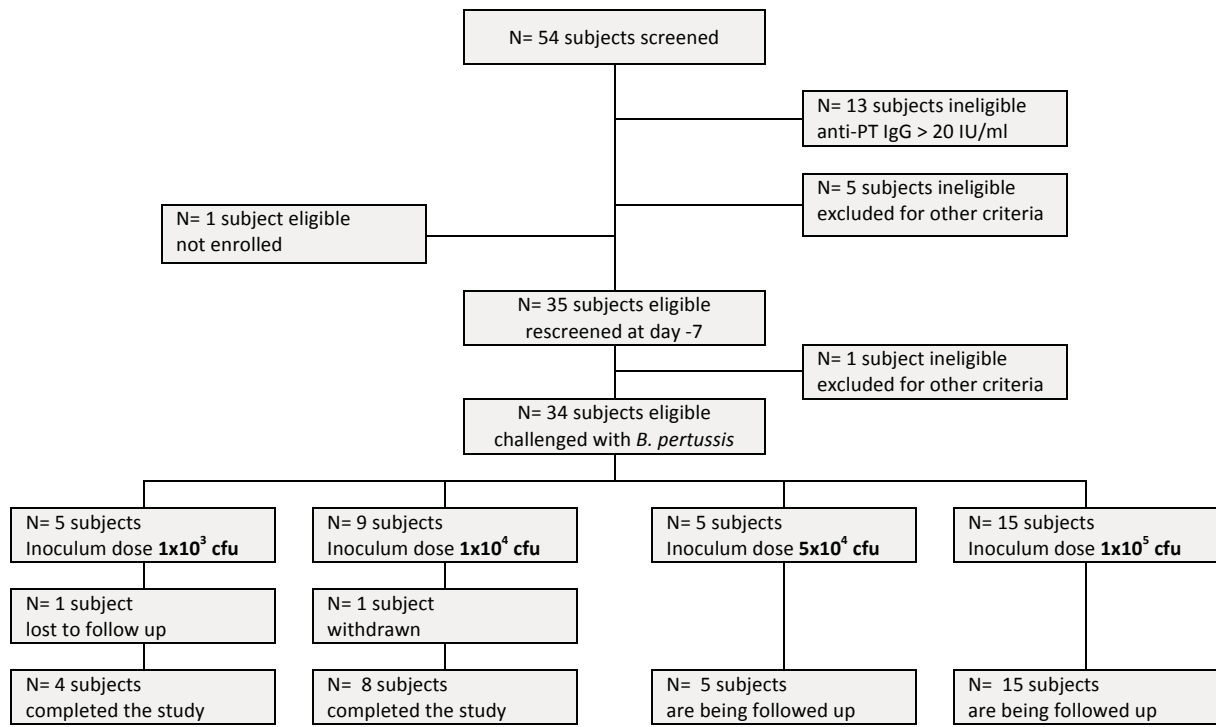
Table 6. Baseline and fold change in serum IgG concentration against *Bp* antigens

comparing day 0 and day 28

Subject no.	Inoculum dose (cfu)	PT		FHA		PRN		FIM2/3	
		Baseline IU/ml	Fold change	Baseline IU/ml	Fold change	Baseline IU/ml	Fold change	Baseline AU/ml	Fold change
1	10 ³	8.8	4.7	30.9	1.0	9.8	1.0	1.5	4.1
2	10 ⁴	2.1	22.5	9.7	9.1	4.3	9.9	4.1	9.4
3	10 ⁴	0.2	55.3	5.1	4.2	1.3	9.1	1.0	5.8
4	5x 10 ⁴	7.6	5.1	21.1	4.4	18.1	1.3	2.9	1.2
5	5x 10 ⁴	10.5	2.6	44.4	1.4	13.8	2.3	0.5	5.6
6	10 ⁵	7.5	55.6	10.4	6.7	1.3	2.8	5.7	8.2
7	10 ⁵	1.9	21.8	5.2	16.1	5.9	16.9	1.5	5.1
8	10 ⁵	22.4	4.0	15.9	3.9	2.0	1.9	0.6	2.6
9	10 ⁵	0.7	45.1	26.0	3.9	2.6	1.9	2.7	13.7
10	10 ⁵	1.1	6.6	24.1	2.0	15.1	1.4	0.1	55.4

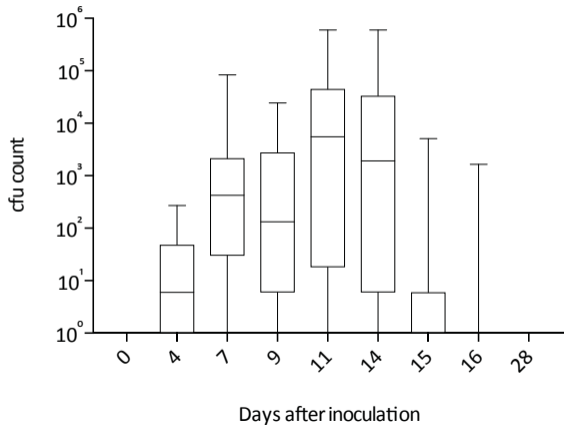
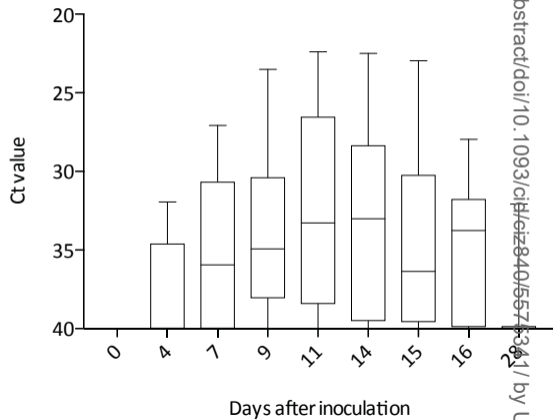
Subjects presented who showed at least four-fold IgG concentration change against at least one antigen. PT, pertussis toxin; PRN, pertactin; FHA, filamentous hemagglutinin; FIM 2/3, fimbriae 2/3. CfU, colony forming units; IU, international units. AU, Arbitrary units; Green, at least four-fold increase in IgG concentration

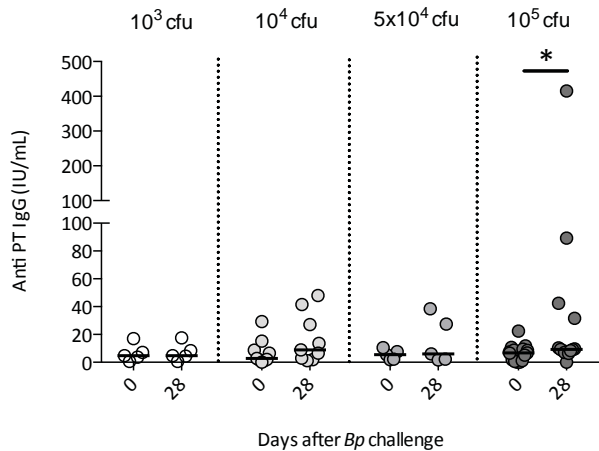
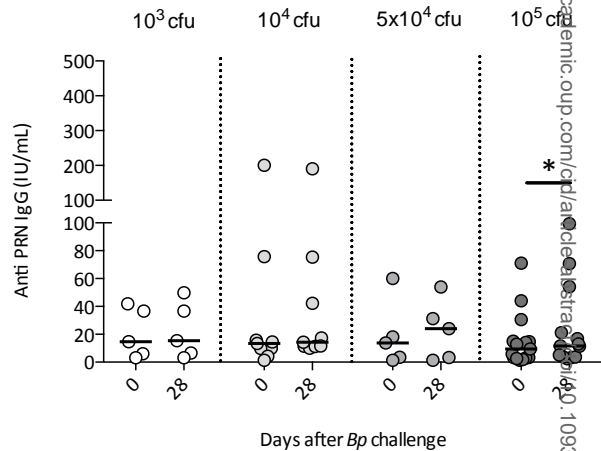
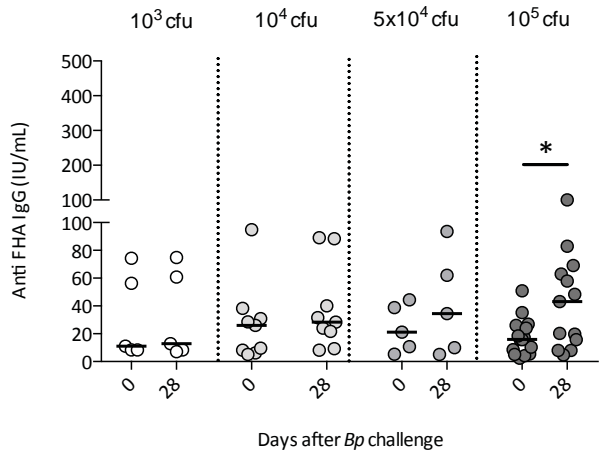
1 **Figure 1. Subject flowchart**



2

3

A**B**

A**B****C****D**