Clinical Infectious Diseases

MAJOR ARTICLE

Controlled Human Infection With *Bordetella pertussis* Induces Asymptomatic, Immunizing Colonization

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(See the Editorial Commentary by Merkel on pages 412–4.)

Background. *Bordetella pertussis* is among the leading causes of vaccine-preventable deaths and morbidity globally. Human asymptomatic carriage as a reservoir for community transmission of infections might be a target of future vaccine strategies, but has not been demonstrated. Our objective was to demonstrate that asymptomatic nasopharyngeal carriage of *Bordetella pertussis* is inducible in humans and to define the microbiological and immunological features of presymptomatic infection.

Methods. Healthy subjects aged 18–45 years with an antipertussis toxin immunoglobin G (IgG) concentration of <20 international units/ml were inoculated intranasally with nonattenuated, wild-type *Bordetella pertussis* strain B1917. Safety, colonization, and shedding were monitored over 17 days in an inpatient facility. Colonization was assessed by culture and quantitative polymerase chain reaction. Azithromycin was administered from Day 14. The inoculum dose was escalated, aiming to colonize at least 70% of participants. Immunological responses were measured.

Results. There were 34 participants challenged, in groups of 4 or 5. The dose was gradually escalated from 10^3 colony-forming units (0% colonized) to 10^5 colony-forming units (80% colonized). Minor symptoms were reported in a minority of participants. Azithromycin eradicated colonization in 48 hours in 88% of colonized individuals. Antipertussis toxin IgG seroconversion occurred in 9 out of 19 colonized participants and in none of the participants who were not colonized. Nasal wash was a more sensitive method to detect colonization than pernasal swabs. No shedding of *Bordetella pertussis* was detected in systematically collected environmental samples.

Conclusions. *Bordetella pertussis* colonization can be deliberately induced and leads to a systemic immune response without causing pertussis symptoms.

Clinical Trials Registration. NCT03751514.

Keywords. *Bordetella pertussis*; human challenge; carriage; immune response.

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

Despite high immunization coverage, some developed countries have seen increases in the incidences of pertussis over the past 20 years [3]. The transmission of *Bordetella pertussis (Bp)*, the cause of pertussis, occurs by aerosolized 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. More rapid clearance was induced in wP-vaccinated animals, compared with naive and aP-vaccinated animals. By comparison, previously infected animals were not colonized upon secondary infection. This may be related to a failure of aP to induce the Th1 and Th17 memory responses required for sterilizing 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 seroconversion

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in the absence of clinical disease [8], suggesting that asymptomatic colonization and transmission does occur sub-clinically in populations. This is important, as future vaccine strategies will need to efficiently reduce 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, the herd protection conferred by potent glycoconjugate vaccines results from an interruption of transmission by vaccine-induced protection against the carrier state [9, 10]. However, individuals harmlessly colonized 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 infections with *Bp* to demonstrate that asymptomatic colonization can occur, provide a safe human colonization 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 was 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, had a history of being vaccinated against *Bp* no less than 5 years before enrollment, were nonsmokers, had no use of antibiotics within 4 weeks of enrollment, and had not had contact with people vulnerable to *Bp* disease. Participants with a serum antipertussis toxin immunoglobulin G (IgG) level >20 international units/liter or a positive *Bp* culture from a pernasal swab, pregnant women, nursing mothers, females of childbearing age who did not use acceptable birth control, people with impairments/alterations of the immune system (including immunosuppressive therapy), and people with a contraindication 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 characterized 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 colonization of 70% of the subjects. Colonization 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 every 4 hours during admission and at each follow-up visit. Following discharge, subjects had 4 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 polymerase chain reaction (PCR; influenza A, influenza B, parainfluenza [types 1, 2 and 3], rhinovirus, RSV, adenovirus, and metapneumovirus) were taken to exclude an alternative etiology for these symptoms.

All participants had pernasal swabs, nasal washes, throat swabs, and nasosorption fluid samples taken at predetermined 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, who was lying in the supine position. After 1 minute, the

Table 1. Overview of Visits, Admissions, 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	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	x	…	…	…
Throat swab	…	x	…	…	…	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	…	…	…	…

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 by placing a strip of a hydrophilicpolyester absorptive matrix (Mucosal Diagnostics, Hunt Developments Ltd.) into the nostril for 2 minutes.

Bordetella pertussis colonization was identified by culture of these samples and identification was confirmed by matrix-assisted laser desorption/ionisation time of flight mass spectrometry (MALDI-TOF). The minimum detection rate of the culture was 6 cfu. Quantitative PCR (qPCR) was performed on pernasal swabs, nasal washes, and throat swabs of the 15 volunteers who received the standard inoculum (see below). Details of the microbiological methods used are provided in the Supplementary Material. Blood samples were taken at intervals and analyzed for seroconversion against PT, pertactin, FHA, and FIM 2/3 on Days 0 and 28 and analyzed for B-cell responses using enzyme-linked immune absorbent spot (ELISPOT) on Days 0, 7, and 14. The IgG antibody concentrations were quantified using the fluorescent bead–based multiplex immunoassay, as described by van Gageldonk et al [15]. The 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 standardized 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 resulted in *Bp* carriage in at least 70% of the exposed subjects without causing *Bp* disease.

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

In this dose escalation study, safety and colonization parameters were reviewed by an external committee after each fifth subject and the inoculum dose for the following 5 subjects was agreed. Enrollment ceased when at least 10 subjects had been colonized with the standard inoculum.

Statistical Methods

The percentage of participants successfully colonized (colonization 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 medians, interquartile ranges, minimums, and maximums. To compare conventional culture with qPCR, data were analyzed using McNemar's test and sensitivity was calculated. These data are presented in contingency tables. Serological data comparing colonized and uncolonized participants were analyzed using the 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 vaccinations in childhood. There were 34 healthy subjects 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 3 months.

Bordetella pertussis Colonization

The dose of intranasal inoculum was gradually increased following an algorithm, starting at a dose of 1000 cfu, which did not result in colonization. As the inoculum dose increased, so did the colonization fraction, for 55% (95% CI 27–82%) at a dose of 10 000 cfu, 40% (95% CI 12–77%) at a dose of 50 000 cfu, and 80% (95% CI 54–94%) at a dose of 100 000 cfu. On completion of the protocol, 19 participants had become colonized. There was no significant difference between the baseline demographic characteristics and pertussis antibody levels of the colonized and uncolonized group (Table 2). *Bordetella pertussis* was cultivable from nasal wash samples by Day 4 in most colonized subjects; the quantitative count then rose gradually and peaked on Day 11 (Figure 2A), with a substantial decrease on Days 15 and 16 following the commencement of azithromycin eradication therapy (see Supplementary Figure S3 for colonization densities, plotted individually). This was mirrored in the qPCR data (Figure 2B); *Bp* DNA was still detectable on Days 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* colonization; conventional culture was equally sensitive at detecting *Bp* colonization as qPCR of nasal wash samples (40 out of 48 samples, 83%; Table 3). Regarding pernasal swabbing, which is the conventional sampling procedure for a laboratory diagnosis of *Bp* infection, qPCR was more sensitive at detecting *Bp* colonization 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 ($P < .01$, with McNemar's test; Table 4). PCRs 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 1 throat swab was culture positive, and nasosorption fluid culture was never positive.

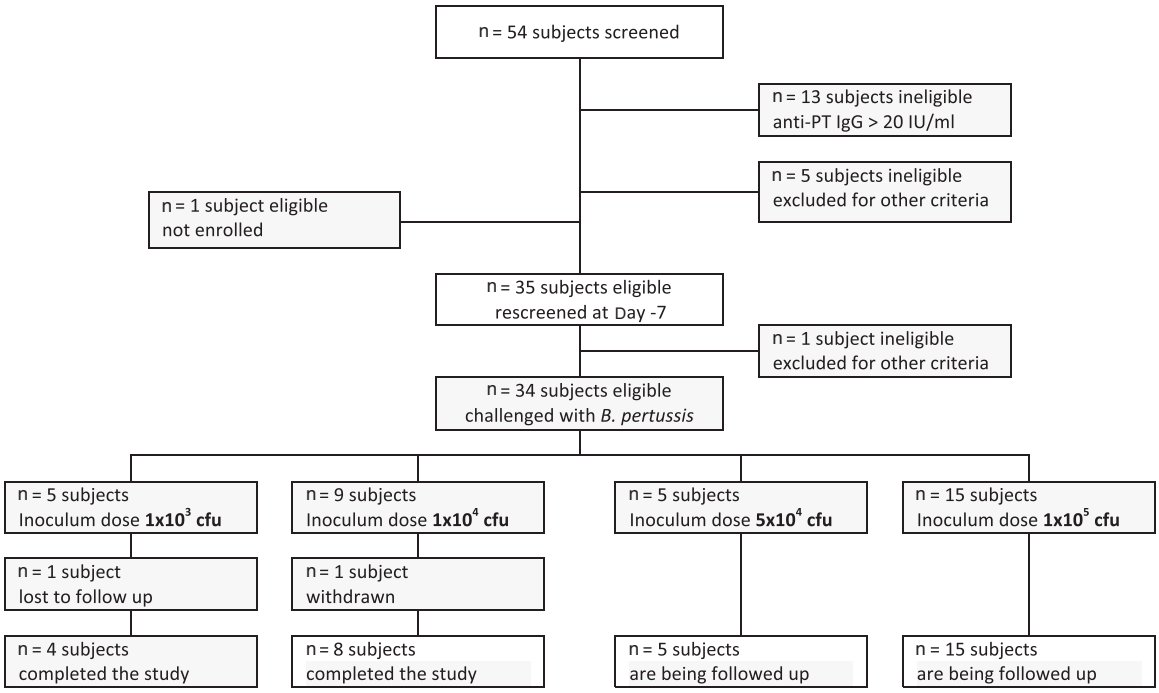

Figure 1. Subject flowchart. Abbreviations: *B. pertussis*, *Bordetella pertussis*; cfu, colony-forming units; IgG, immunoglobin G; IU, international units; PT, pertussis toxin.

Clearance and Eradication of Bordetella pertussis Colonization

Of the 19 participants who were successfully colonized with *Bp*, 3 cleared colonization prior to receiving azithromycin. Eradication therapy rendered all samples culture negative by 48 hours in 14 out of 16 subjects (88%). The remaining 2 volunteers who were still colonized 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 *Bordetella pertussis* 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 colonized group and the uncolonized group. Mild symptoms of cough, rhinorrhea, and nasal congestion were reported more frequently in the groups receiving higher inoculum doses (Supplementary Figures 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 Colonization

Serological Response Assessed by Multiplex Immune Assay

Serum antibody concentrations were measured against the following *Bp* antigens: PT, PRN, FHA, and FIM 2/3 on Day 0 and Day 28. Significant rises in serum IgG concentrations were found against PT, PRN, and FHA at the highest inoculum dose (Figure 3).

Table 2. Demographic Characteristics and Baseline Serum Immunoglobin G Concentrations Against *Bordetella pertussis* Antigens

	Excluded	Enrolled	Colonized	Uncolonized
	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, kg, median (IQR)	81 (73–95)	74 (65–83)	73.7 (65–83)	74.2 (65–83)
Height, cm, median (IQR)	177 (171–180)	176 (170–183)	175 (163–181)	177 (172–186)
Anti-PT IgG, IU/ml, median (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, IU/ml, median (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, AU/ml, median (IQR)	NA	5.2 (2.2–21.4)	2.7 (0.9–5.7)	12.3 (6.5–31)
Anti-FHA IgG, IU/ml, median (IQR)	NA	16.6 (8.4–32)	15.7 (6.1–26)	26.0 (8.4–50.8)

Abbreviations: AU, arbitrary units; FHA, filamentous hemaglutinin; FIM 2/3, fimbriae 2/3; IgG, immunoglobin G; IQR, interquartile range; IU, international units; NA, not available; PRN, pertactin; PT, pertussis toxin.

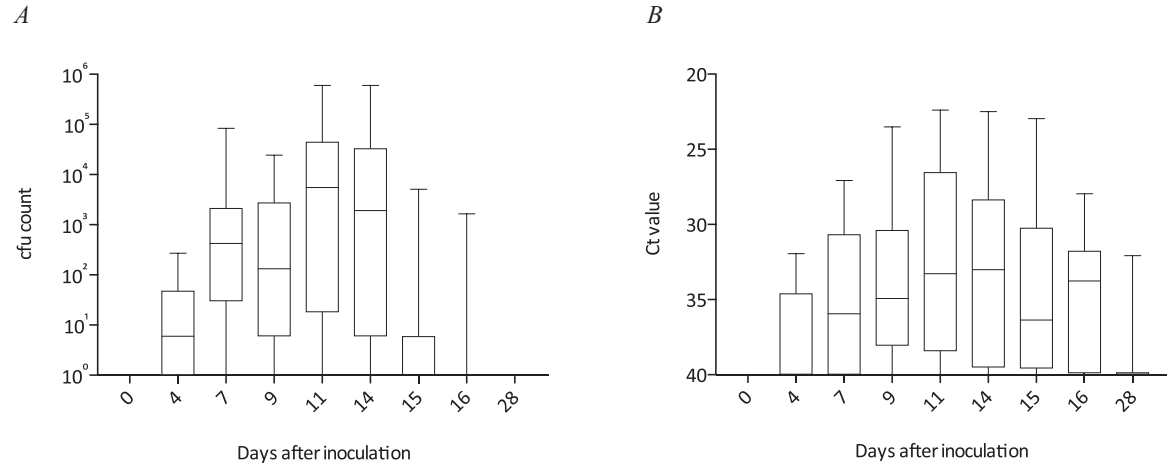

Figure 2. Colonization density in nasal wash samples of colonized subjects (n = 19) over time. *A*, Culture results in total cfu, measured by dilutional plating. *B*, Quantative polymerase chain reaction results, expressed as Ct value. Day 0 was the day of inoculation. Results are presented as box plots with medians and 25% and 75% interquartiles, and the whiskers represent the minimum and maximum values. Abbreviations: cfu, colony-forming units; Ct, cycle threshold.

Comparing colonized with uncolonized participants, 5 out of 12 of those colonized after inoculation with 10^5 cfu (n = 15) exhibited a 4-fold or more increase in the serum anti-PT IgG concentration. Conversely, none of the uncolonized subjects exhibited a rise in a serum anti-PT IgG concentration (Figure 4). All participants with a rise in an anti-PT concentration also had a rise in an anti-IgG concentration against other antigens (Table 6; Supplementary Table S4).

Detection of Antibody-secreting Plasma Cells Specific to *Bordetella pertussis* Antigens by ELISPOT

No antigen-specific IgG- or IgA-secreting cells were detected by ELISPOT in any of the subjects at Days 0 or 7, above background concentrations detected in the phosphate buffered saline 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 colonized participants, compared to Day 0 and Day 7, and compared to uncolonized participants. This increase was significant (≤0.05) for FHA-specific Ig-secreting cells (Figure 5C and D).

Environmental Sampling

In a controlled aerosolization experiment, a median of 17% (interquartile range 1–40%) of the *Bp* that had been aerosolized 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 cultures, no environmental shedding *of Bp* from colonized 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), and fingertip cultures (n = 442) all tested negative for *Bp*.

DISCUSSION

This first-in-human study has demonstrated that asymptomatic colonization can be induced safely by intranasal inoculation with wild-type *Bp*, and is associated with seroconversion, suggesting a true biological colonization of the host. The dose needed to induce colonization of approximately 80% of the exposed subjects is 10^5 cfu. Nasal washing was the most sensitive technique to

Table 3. Nasal Wash and Pernasal Swabs

		Nasal Wash Culture		
		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

Data to compare detection of *Bordetella pertussis* by qPCR and culture at Days 0–14. These data are derived from the 15 participants who were inoculated with 10^5 colony forming units of *Bordetella pertussis*. Pernasal swabs were taken on Days 3, 5, 7, 9, 11, and 14. Nasal wash samples were taken on Days 4, 7, 9, 11, and 14.
Abbreviation: qPCR, quantitative polymerase chain reaction.

Table 4. Culture Results of *Bordetella pertussis* in Nasal Wash and Pernasal Swab Samples

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

Data are from Day −7 to Day 16. These data are derived from the 34 participants who were inoculated with any dose of *B. pertussis*. Pernasal swabs and nasal washes were both taken on Days 7, 9, 11, 14, 15, and 16.

Table 5. ***Bordetella pertussis*** **Polymerase Chain Reaction Results**

		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	…	…	…

Data are of nasal wash, pernasal swab, and throat swab samples taken from Day −7 to Day 16. These data are derived from the 15 participants who were inoculated with 10^5 colony forming units of *Bordetella pertussis*.

detect colonization; pernasal swabbing, the conventional diagnostic technique, was even negative in 1 individual who seroconverted. Induced colonization causes a systemic immune response in the form of a rise in the antigen-specific serum IgG concentration and detectable, specific B cells in some, but not all, colonized individuals. Azithromycin clears carriage in most people by 48 hours.

This study adds *prima facie* evidence to support epidemiological and serological observations that suggest that asymptomatic *Bp* colonization is part of the natural life cycle of the organism.

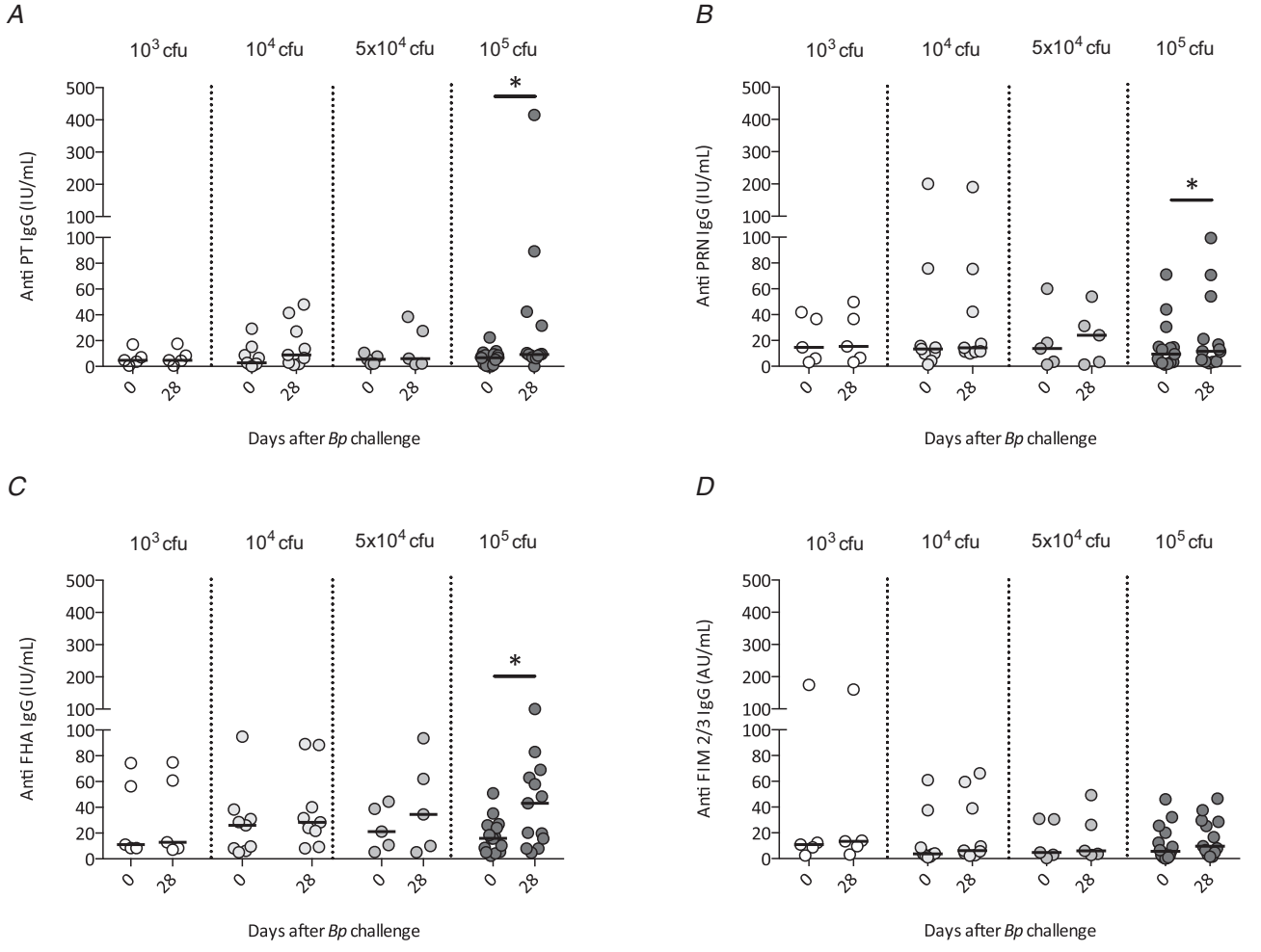

Figure 3. Antigen-specific serum IgG concentration after *Bp* exposure, comparing dose groups. *A*, Anti-PT. *B*, Anti-PRN. *C*, Anti-FHA. *D*, Anti-FIM 2/3. Infected with: ○ inoculum dose 10^3 cfu (n = 5), ○ inoculum dose 10^4 cfu (n = 9), ● inoculum dose $5x10^4$ cfu (n = 5), ● inoculum dose 10^5 cfu (n = 15). Day 0 was the day of inoculation. Results are presented as scatter plots with median values. *Significance between time points ($P < .05$), using the Wilcoxon test. Abbreviations: AU, arbitrary units; *Bp*, *Bordetella pertussis*; cfu, colony-forming units; FHA, filamentous hemaglutinin; FIM 2/3, fimbriae 2/3; IgG, immunoglobin G; IU, international units; PRN, pertactin; PT, pertussis toxin.

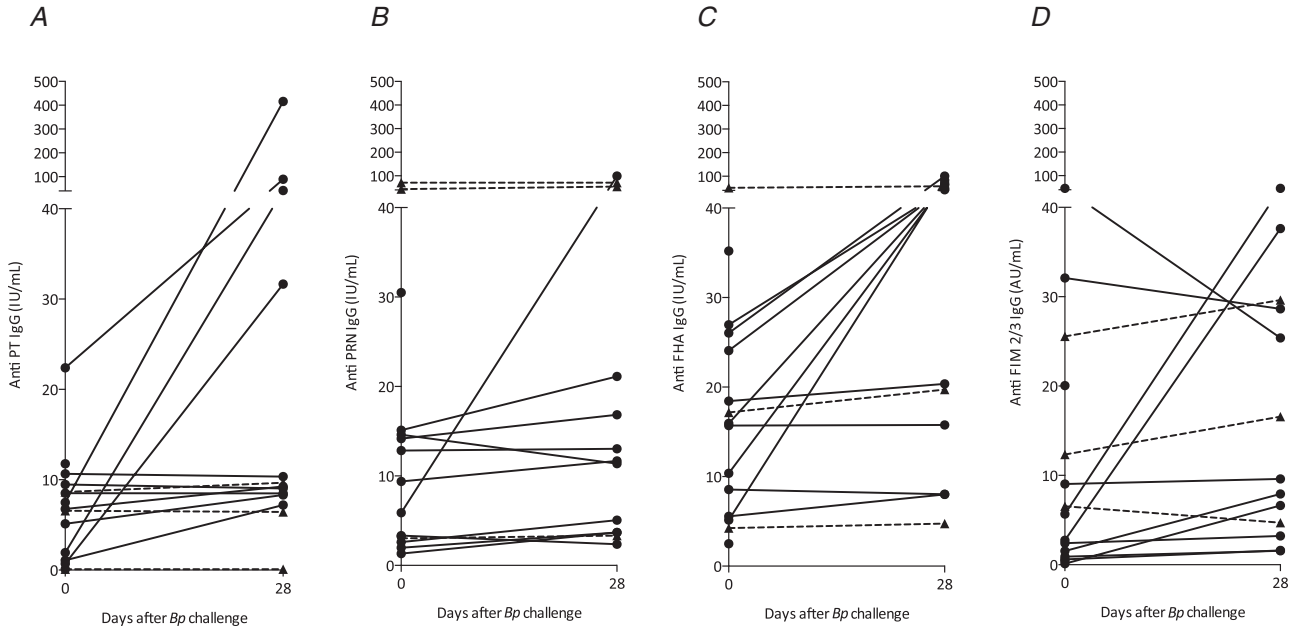

Figure 4. Serum IgG concentration against *Bp*-specific antigens after a challenge with 10^5 cfu of *Bp*. IgG concentration of n = 15 subjects exposed to 10^5 cfu *Bp*. *A*, Anti-PT. *B*, Anti –PRN. *C*, Anti-FHA. *D*, Anti-FIM 2/3. The black lines indicate colonized cases and the dashed lines indicate noncolonized cases. Day 0 was the day of inoculation. Abbreviations: AU, arbitrary units; *Bp*, *Bordetella pertussis*; cfu, colony-forming units; FHA, filamentous hemaglutinin; FIM 2/3, fimbriae 2/3; IgG, immunoglobin G; IU, international units; PRN, pertactin; PT, pertussis toxin.

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 colonization [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 pediatric study carried out in 1933, *Bp* disease was induced by exposing 2 presumably immunologically naive children to 140 cfu of *Bp* bacteria. No asymptomatic colonization was detected, as the participants developed symptoms at the same time as positive cultures were obtained from cough samples 1 week after exposure [19]. A Phase I trial of a live, genetically attenuated *Bp* intranasal vaccine has been reported. Asymptomatic colonization was demonstrated after inoculation with BPZE1, a *Bp* strain in which dermonecrotic toxin and tracheal cytotoxin are genetically deleted, and PT is genetically detoxified by 2 independent mutations, removing the toxic activity of PT without affecting its immunogenic properties [20]. In the BPZE1 study, colonization was detected in 1 out of 12 (8%) subjects inoculated with 10^3 cfu, 1 out of 12 (8%) inoculated with 10^5 cfu, and 5 out of 12 (42%)

Table 6. Baseline and Fold Changes in Serum Immunoglobin G Concentrations Against *Bordetella pertussis* Antigens, Comparing Day 0 and Day 28

Subject number	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 showed at least a 4-fold immunoglobin G concentration change against at least 1 antigen.
Abbreviations: AU, arbitrary units; cfu, colony-forming units; FHA, filamentous hemaglutinin; FIM 2/3, fimbriae 2/3; IU, international units; PRN, pertactin; PT, pertussis toxin.

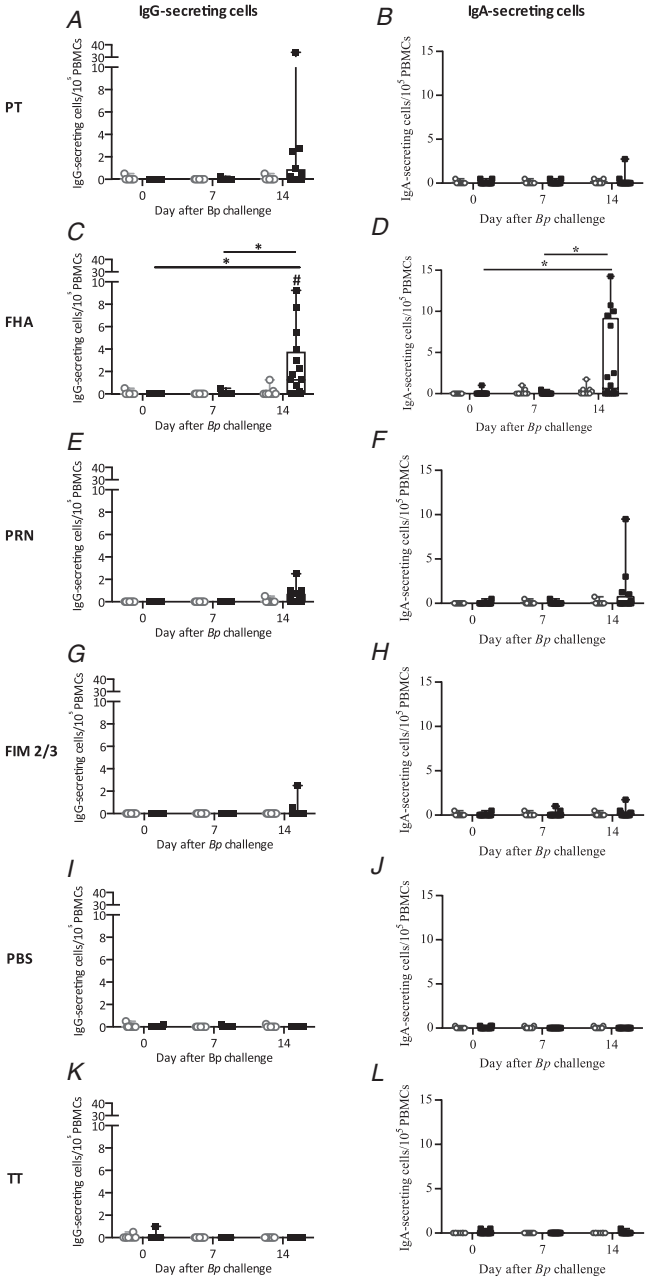

Figure 5. IgG- and IgA-secreting plasma B-cell responses to *Bp* challenge. Numbers of plasma B cells secreting IgG and IgA are specific for (*A–B*) PT, (*C–D*) FHA, (*E–F*) PRN, and (*G–H*) FIM 2/3 by ELISPOT. PBS (*I–J*) and TT (*K–L*) were used as a background control and negative control antigen, respectively. Results for volunteers who were uncolonized (○; n = 9) and colonized (■; n = 16) are shown as box plots representing the median, with 25% and 75% interquartile ranges, and whiskers representing minimum and maximum values. *Significance between time points ($P < .05$), using a Kruskel-Wallis test with Dunn's correction. #Significance between noncolonized and colonized responses ($P < .05$), using a Kruskel-Wallis test with Dunn's correction. Abbreviations: *Bp*, *Bordetella pertussis*; FHA, filamentous hemaglutinin; FIM 2/3, fimbriae 2/3; Ig, immunoglobin; PBMC, peripheral blood mononuclear cells; PBS, phosphate buffered saline; PRN, pertactin; PT, pertussis toxin; TT, tetanus toxoid.

inoculated with 10^7 cfu. These colonization 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 colonization. In addition, our study demonstrates that asymptomatic colonization can still occur in the presence of these potent toxins. Other nasal challenge studies, such as the *Streptococcus pneumoniae* challenge studies [21] and the *Neisseria lactamica* studies [22, 23], have shown similar increases in colonization fractions with increasing doses.

In the current study, subjects who were inoculated but not colonized exhibited no rise in anti-*Bp* antigen-specific IgG concentrations. A specific antibody was not produced in some colonized participants, unlike in other nasal challenge studies, in which all colonized volunteers exhibited seroconversion [21, 22]. This may reflect our protocol requirement to terminate colonization using azithromycin at 14 days, in order to avoid progression to a lengthy syndrome of cough [24]. All participants received a whole-cell pertussis vaccine in infancy, as the acellular pertussis vaccine was introduced into the United Kingdom in 2005. The increase in anti-*Bp* serum IgG concentrations seen in colonized subjects in our study is modest, compared to those 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 colonization 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 colonization in 88% of colonized subjects within 2 days, supporting current public health outbreak guidelines [27]. Although the sample used in our study was small, there was a trend suggesting that adults with higher anti-PRN and anti–FIM 2/3 antibody concentrations are protected against colonization, 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 colonization, 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 colonization 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 colonized 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.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted

materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

Author contributions. H. d,G., R. C. R., A. G., S. N. F., K. E. K., D. A. D., A. P., and A. M. B. contributed to the study design. H. d,G., R. C. R., A. G., D. A. D., M. I., A. R. H., A. T. V., G. A. M. B., and D. G. contributed to the data collection, analyses, and interpretation. H. d,G. and R. C. R. drafted and finalized 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. Individual participant data that underlie the results reported in this article (text, tables, figures, and appendices) will be available after de-identification for researchers who provide a methodologically sound proposal, in order to achieve aims in their proposal. This available data includes study protocol, statistical analysis plan, and analytic code. Proposals can be sent to the corresponding author beginning 3 months and ending 5 years following the article's publication. To gain access, data requestors will need to sign a data access agreement.

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