Safety and immunogenicity of the live attenuated intranasal pertussis vaccine BPZE1: a phase 1b, double-blind, randomised, placebo-controlled dose-escalation study

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Summary

Background Long-term protection and herd immunity induced by existing pertussis vaccines are imperfect, and a need therefore exists to develop new pertussis vaccines. This study aimed to investigate the safety, colonisation, and immunogenicity of the new, live attenuated pertussis vaccine, BPZE1, when given intranasally.

Methods This phase 1b, double-blind, randomised, placebo-controlled, dose-escalation study was done at the phase 1 unit, Karolinska Trial Alliance, Karolinska University Hospital, Stockholm, Sweden. Healthy adults (18–32 years) were screened and included sequentially into three groups of increasing BPZE1 dose strength (10^7 colony-forming units [CFU], 10^8 CFU, and 10^9 CFU), and were randomly assigned (3:1 within each group) to receive vaccine or placebo. Vaccine and placebo were administered in phosphate-buffered saline contained 5% saccharose as 0·4 mL in each nostril. The primary outcome was solicited and unsolicited adverse events between day 0 and day 28. The analysis included all randomised participants who received a vaccine dose. Colonisation with BPZE1 was determined by repeatedly culturing nasopharyngeal aspirates at day 4, day 7, day 11, day 14, day 21, and day 28 after vaccination. Immunogenicity, as serum IgG and IgA responses were assessed at day 0, day 7, day 14, day 21, day 28, 6 months, and 12 months after vaccination. This trial is registered at Clinicaltrials.gov, NCT02453048.

Findings Between Sept 1, 2015, and Feb 3, 2016, 120 participants were assessed for eligibility, 48 of whom were enrolled and randomly assigned (3:1) to receive vaccine or placebo, with 12 participants each in a low-dose, medium-dose, and high-dose vaccine group. Adverse events between day 0 and day 28 were reported by one (8%, 95% CI 0–39) of 12 participants in both the placebo and low-dose groups, and two (17%; 2–48) of 12 participants in both the medium-dose and high-dose groups, including cough of grade 2 or more, oropharyngeal pain, and rhinorrhoea and nasal congestion. During this time, none of the participants experienced any spasmodic cough, difficulties in breathing, or adverse events following immunisation concerning vital signs. The tested doses of BPZE1 or placebo were well tolerated, with no apparent difference in solicited or unsolicited adverse events following immunisation between groups. Colonisation at least once after vaccination was observed in 29 (81%; 68–93) of 36 vaccinated participants. The tested vaccine doses were immunogenic, with increases in serum IgG and IgA titres against the four *B pertussis* antigens from baseline to 12 months.

Interpretation The tested vaccine was safe, induced a high colonisation rate in an adult population, and was immunogenic at all doses. These findings justify further clinical development of BPZE1 to ultimately be used as a priming vaccine for neonates or a booster vaccine for adolescents and adults, or both.

Funding ILiAD Biotechnologies.

Introduction

Pertussis, or whooping cough, is a highly contagious respiratory disease caused by *Bordetella pertussis*.[1] The disease affects all age groups, but it is particularly severe and life-threatening in young infants.[2] Vaccination campaigns starting in the 1950s have greatly reduced the incidence worldwide, but, despite a global vaccination coverage of more than 85%,[3] the disease is not under control in any part of the world. In fact, in the past decade, it has made an alarming rebound in several countries, especially those that have switched from the whole-cell vaccines to acellular vaccines.[4] In the USA, epidemic peaks have reached annual incidences of nearly 16 per 100 000 people,[5] which had not been seen before the switch. However, pertussis incidence is also increasing in countries in which whole-cell vaccines are still in use.[6,7]

Several reasons could account for this resurgence, including pathogen adaptation to escape vaccine-induced immunity, rapid waning of immunity (especially after vaccination with acellular vaccines), and failure of current vaccines to prevent infection by and transmission of *B pertussis*.[8] Mathematical modelling studies suggest that the latter is the major driver of the current resurgence of pertussis in countries with high

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Research in context

Evidence before this study

Pertussis is a severe and life-threatening respiratory disease mainly caused by *Bordetella pertussis*. Two types of vaccine are currently available, the first-generation whole cell vaccine and the more recent acellular vaccine. However, despite global vaccination coverage of more than 85%, according to WHO, the disease has not been eliminated in any part of the world. Instead, incidence is rising in several countries, most probably because of rapid waning of vaccine-induced immunity and the failure of current vaccines to prevent infection by and transmission of *B pertussis*. Novel vaccines are therefore needed to prevent disease and infection. Since *B pertussis* is a strictly mucosal pathogen, mucosal vaccines might be more effective than the current injectable vaccines. We searched PubMed with the terms "pertussis", "mucosal vaccine", "intranasal", "live attenuated", and "whooping cough" for articles in any language up to Jan 10, 2020. Several combinations of these terms yielded up to 220 references. Some references related to novel mucosal vaccines, but none of these vaccines has yet reached clinical development, except for a first-in-man trial of the live attenuated vaccine BPZE1. Intranasal administration to human volunteers of low doses of this vaccine was well tolerated and resulted in low frequencies of vaccine take and seroconversion. In non-human primates, a single administration of BPZE1 protected them against both pertussis disease and infection by *B pertussis*, Therefore, BPZE1 is a promising candidate for an effective control of pertussis.

Added value of this study

This is, to our knowledge, the first clinical trial of a live attenuated pertussis vaccine delivered nasally to human adult volunteers at doses that result in seroconversion in 100% of vaccinated participants after a single administration. BPZE1 was well tolerated at doses of up to 10^9 colony-forming units. The frequencies and severity of adverse events following immunisation were similar in the placebo and vaccine groups. Transient colonisation of the respiratory tract by BPZE1 could be seen for more than 80% of the vaccine recipients. Serum antibody titres (both IgG and IgA) to the major protective *B pertussis* antigens increased in the vaccinated participants and remained high for up to 12 months after vaccination, when the study was terminated. At the highest dose tested, all participants responded to at least one of the tested antigens.

Implications of all the available evidence

This study defines the human dose for the nasal administration of the live attenuated pertussis vaccine BPZE1, which can be safely administered and resulted in seroconversion of 100% of the study participants. The safety and immunogenicity profile of BPZE1 supports the progression of this vaccine to larger efficacy trials. Together with preclinical studies, which show that, unlike currently available vaccines, BPZE1 can induce sterilising immunity, this trial shows that BPZE1 holds promise as an effective vaccine to protect against both pertussis disease and infection by *B pertussis*.

vaccination coverage.[9] Improved vaccines that protect against both pertussis disease and infection by *B pertussis* are therefore needed to finally bring whooping cough under control.[10]

By contrast with the action of current vaccines, infection by *B pertussis* prevents subsequent colonisation,[11] and immunity acquired by natural infection is longer lasting than that induced by vaccination.[12] Therefore, a live attenuated *B pertussis* vaccine, named BPZE1, was developed to be delivered intranasally, to mimic natural infection without causing disease. This vaccine strain was constructed by genetically removing dermonecrotic toxin, reducing tracheal cytotoxin to background levels, and inactivating pertussis toxin.[13] In a non-human primate model, a single nasal administration of BPZE1 was found to provide strong protection against both pertussis disease and infection, following a challenge by a highly virulent recent clinical *B pertussis* isolate.[14]

We previously reported the results of a first clinical evaluation in humans, in which BPZE1 was found to be safe up to a dose of 10^7 colony-forming units (CFU), and was able transiently to colonise the upper respiratory tract and induce antibody responses in young male participants.[15] However, even at the highest dose tested in that initial study, only five of 12 participants were colonised and produced antibodies to *B pertussis* antigens. This relatively low vaccine take might be because of several reasons. The dose or volume (100 µL/nostril) used in the previous study might have been too low for optimal immune induction, pre-existing immunity might have hampered vaccine take, or both. In this study, we aimed to investigate the safety, colonisation, and serum antibody responses of a subsequent phase 1b study in both men and women, in which the volume and dose of BPZE1 were increased.

Methods

Study design

This phase 1b, double-blind, randomised, placebo-controlled, dose-escalation study was done at the phase 1 unit, Karolinska Trial Alliance, Karolinska University Hospital, Stockholm, Sweden. The trial was done in accordance with the study protocol, International Conference on Harmonisation Good Clinical Practices standards, the Declaration of Helsinki, applicable regulatory requirements, and any applicable European and Swedish laws and regulations. The Swedish Medical Product Agency and the regional ethical review board in Stockholm approved the study protocol and later amendments. The full protocol is available in the appendix (pp 13–76).

See Online for appendix

Participants

Healthy adults aged 18–32 years were screened and included sequentially, respecting predefined minimum time intervals between the enrolment of individual participants as a safety principle (appendix pp 38–39). Most participants were born during the period 1979–96, when pertussis vaccination was suspended in Sweden. Participants were ineligible for enrolment if they had clinically or laboratory-verified pertussis during the preceding 10 years and had serum anti-pertussis toxin and anti-pertactin IgG levels of 20 IU/mL or more. Other exclusion criteria included elevated resting blood pressure, heart rate, or respiratory rate, asthma, use of corticosteroids in the respiratory tract, and any autoimmune or immune deficiency condition (appendix pp 1–2). Women of childbearing potential had to practice adequate contraception from 2 weeks preceding vaccination to 1 month after, and have a negative pregnancy test on the day of vaccination. All participants signed the informed consent form after receiving written and oral information, which was given before and during visit 1, to ensure that they had sufficient time to consider participation before vaccination. A new consent form was signed after protocol amendment to extend follow-up from the planned 6 months to 12 months.

Randomisation and masking

48 individuals were recruited into three consecutive cohorts of 16 participants. Within each group, participants were randomly assigned (3:1) to receive the BPZE1 vaccine or placebo, with vaccine doses of 10^7 CFU for group 1 (low dose), 10^8 CFU for group 2 (medium dose), and 10^9 CFU for group 3 (high dose). Vials containing vaccine or placebo were indistinguishable when frozen, and were coded by a number given by the manufacturer before shipment to the site. The randomisation list, which was provided centrally by the academic clinical trials unit (EUCLID/F-CRIN Clinical Trials Platform, Bordeaux, France), established the order in which the coded vials were allocated to the participants. A participant was considered to be randomly assigned when he or she was allocated a vial code on the day of vaccination (day 0, visit 2). Because vaccine and placebo might be distinguishable when thawed, the vaccine preparation and administration was done by a study nurse who was not involved in any other trial procedures, to ensure that masking of observers was maintained.

Throughout the trial, all personnel involved remained masked to the treatment assignment. Because the BPZE1 cultivation results could have led to treatment unblinding, access to these results was strictly controlled within the cultivating laboratory. The only exception was a statistician performing the interim analysis, who was not involved in any other study analysis.

Procedures

A liquid formulation of the three different BPZE1 batches (low, medium, and high dose) in phosphate-buffered saline containing 5% saccharose (manufactured by Q Biologicals, Gent, Belgium, for ILiAD Biotechnologies, in sequentially numbered vials) was used. Vaccine or placebo (phosphate-buffered saline containing 5% saccharose) was given as a single administration by nasal application of 0·4 mL (containing half the dose) in each nostril.

Participants were observed at the study site for at least 6 h after vaccination. Then, on-site visits took place at day 4, day 7, day 11, day 14, day 21, and day 28 (visits 3–8), and month 6 and month 12 (visits 9 and 10) after vaccination (appendix pp 7–8). During visits 3–10, the investigators did physical examinations (including vital signs) and sample collections, and asked questions concerning solicited general and local adverse events, and about other unsolicited adverse events. The participants were instructed to use a standardised paper diary with prewritten questions and space to record solicited and unsolicited adverse events between on-site visits up to day 28 after vaccination. The intensity of the adverse event was recorded as the maximum intensity observed and coded as mild and easily tolerated (grade 1), moderate and interfering with usual activity (grade 2), or severe with inability to do usual activity (grade 3), following the US Food and Drug Administration coding guidelines (appendix p 3).[16]

Nasopharyngeal aspirates were collected at each of the visits 3–8 to determine colonisation using a syringe aspiration kit (SAK-01, N-PAK, Baxter, MN, USA; appendix p 4). The aspirates were grown on charcoal agar plates as described in our previous paper.[15] If a participant was culture-positive for BPZE1/*B pertussis* at visit 8 (day 28), an additional visit was scheduled on day 45 to ensure clearing of the colonisation. Given that the recovery and quality of nasopharyngeal aspirates differed greatly between participants and timepoints, raising concerns about the reproducibility of exact quantitative assessments, the detection of bacteria was assessed qualitatively (detection of bacteria: yes *vs* no).

Venous blood was collected at screening 1–6 weeks before vaccination, on the day of vaccination, and at 1 week, 2 weeks, 3 weeks, 4 weeks, 6 months, and 12 months after vaccination (appendix pp 7–8). Serum IgG and IgA against pertussis toxin, pertactin, filamentous haemagglutinin (FHA), and fimbriae serotypes 2 and 3 (Fim2/3) were analysed by a standardized ELISA (appendix p 5).

Interim safety data reviews by an independent data safety monitoring board were done during the trial before enrolment of a higher-dose group. This board decided to remain masked (ie, they never requested review of unblinded data while the trial was ongoing). All interim analyses results remained confidential and were not shared with any investigators until after the data were locked.

Outcomes

Because the trial included live attenuated *B pertussis*, the main safety concern was potential symptoms of pertussis

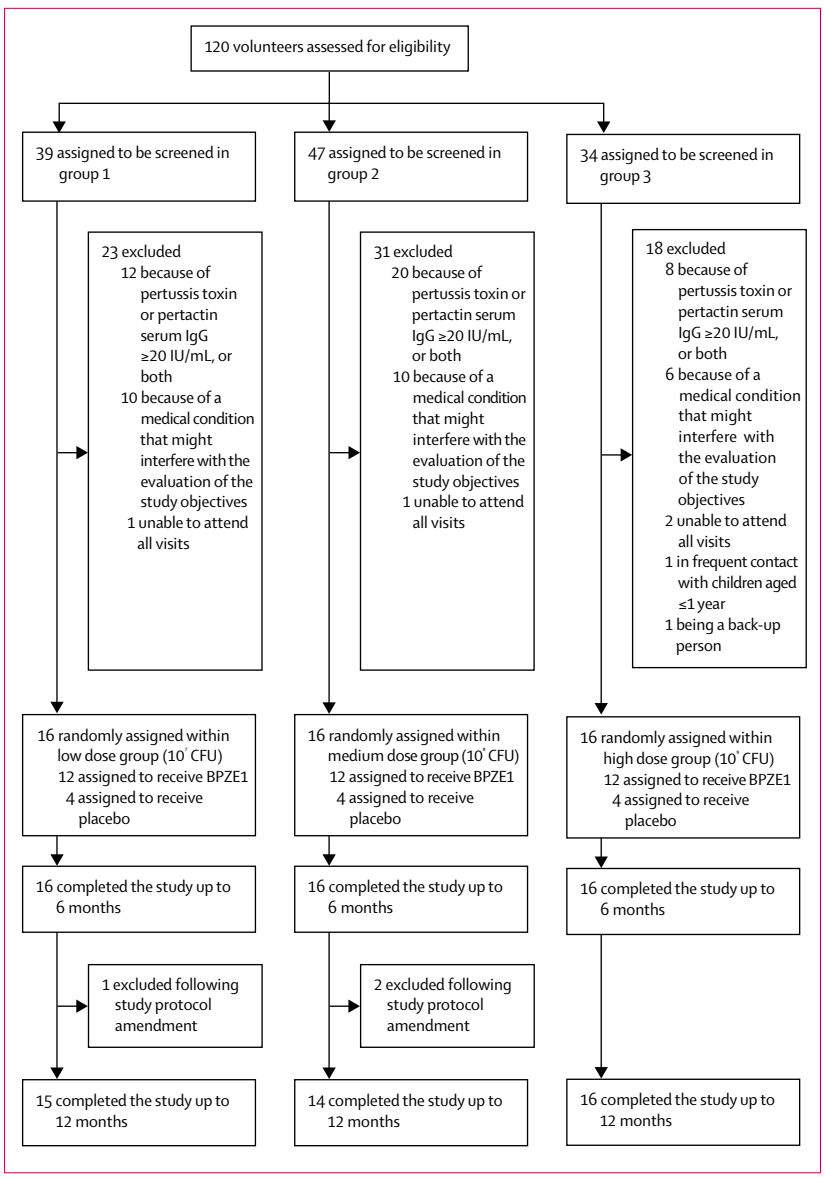


Figure 1: **Trial profile**
Participants were included in a stepwise manner until there were 16 participants in each dose group (ie, participants were assigned to group 1 until 16 eligible participants were found; participants were then assigned to group 2 until 16 eligible participants found; participants were then assigned to group 3; some back-up volunteers were eligible but not needed once 16 had been reached). CFU=colony-forming units.

infection in the participant. Therefore, the primary safety endpoint was the number and proportion of participants with at least one of the following adverse events between day 0 and day 28: cough or spasmodic cough of grade 2 or more; other respiratory tract adverse events related or possibly related to vaccination of grade 3 or more; or any other adverse event related or possibly related to vaccination of grade 3 or more. Secondary safety endpoints included additional adverse events in the respiratory tract, systemic adverse events, and severe adverse events (appendix p 6).

The frequency and duration of nasal colonisation, and serum IgG and IgA titres against pertussis toxin, FHA, pertactin, and Fim2/3 were further secondary endpoints. For each antigen, an antibody responder was defined as a participant with at least 100% increase of serum IgG or IgA levels from before vaccination to a given timepoint after vaccination and at least four times the minimum level of detection (appendix p 5), as defined previously.[17] Moreover, as a post-hoc exploratory endpoint, the positivity of the serological response evoked by BPZE1 by comparison with a natural *B pertussis* infection was defined by using a pertussis case definition,[18] meaning an IgG or IgA increase by two or more times between pre-vaccination and post-vaccination samples, for either pertussis toxin alone or a combination of at least two other antigens (ie, pertactin plus FHA, pertactin plus Fim2/3, or FHA plus Fim2/3).

Statistical analysis

12 participants receiving active vaccination per dose group constitutes a trade-off between detectable event rate and power in the context of a phase 1 trial.[19] A sample size of 12 participants allows the observation of at least one primary safety endpoint event with 80% power if the underlying event rate is at least 12·6%. If no primary safety endpoint event is observed among 12 participants, the upper bound of a two-sided 95% confidence interval for the event rate would be 26%. If no primary safety endpoint event is observed among 36 participants (pooled across groups 1–3), then the upper bound of the 95% confidence interval for the event rate would be 10%.

The main analysis was conducted by a modified intention-to-treat approach, which included all randomised participants who received a vaccine dose in the group to which they were initially assigned, using all of their data regardless of protocol deviations during the trial. The only exception from the modified intention-to-treat was the exclusion of the 12-month immunogenicity data of one participant who had received acellular pertussis vaccine shortly after the 6-month follow-up.

After assessment of baseline characteristics and antibody titres, not indicating any time trend in placebo observations between the consecutive dose groups, all placebo recipients from groups 1–3 were pooled for further analyses. Descriptive analyses were done using standard summary statistics for distributions per group. Antibody titres were described by their geometric mean and 95% confidence interval. The primary endpoint was described in terms of number, proportion, and exact binomial confidence interval of proportion. Stratified descriptive analyses for colonised and non-colonised participants were preplanned in the protocol. No statistical comparisons between groups were done because the trial was not designed for such comparisons.

Role of the funding source

The French public research institute, National Institute for Health and Medical Research (Inserm), was the legal

sponsor and responsible for the oversight of the trial. In a public–private partnership, ILiAD Biotechnologies provided funding and experimental products. All trial-related activities, including protocol development, trial set-up and conduct, data management and statistical analyses, were carried out by academic partners. ILiAD Biotechnologies and Inserm representatives were members of the trial steering committee and as such were involved in the study design, the overview of study conduct and analyses, the writing of the report, and the decision to submit the manuscript for publication. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Between Sept 1, 2015, and Feb 3, 2016, 120 participants were assessed for eligibility, 48 of whom were enrolled and randomly assigned (3:1) to receive vaccine or placebo, with 12 participants each in a low-dose, medium-dose, and high-dose vaccine group (figure 1). Group 1 participants were vaccinated between Sept 25 and Oct 1, 2015; group 2 participants were vaccinated between Nov 12 and Nov 18, 2015; and group 3 participants were vaccinated between Jan 28 and Feb 3, 2016. The final visit of the last participant was March 1, 2017. Men and women were evenly distributed, except for the medium-dose group, which had a higher number of men (table 1). During the trial, after reviewing the safety data, the data safety monitoring board recommended opening the consecutive dose cohorts to the enrolment of the higher dose groups as had been planned in the protocol.

All participants came to their scheduled appointments up to 6 months after vaccination. Three participants (one placebo, one low-dose recipient, and one medium-dose recipient) could not participate in the extended follow-up of 12 months after amendments were made to the protocol. The 12-month immunogenicity data for a fourth participant (in the low-dose group) were also excluded from the statistical analysis, although this participant completed the 12-month follow-up, because of vaccination with acellular pertussis vaccine shortly after the 6-month follow-up.

A specified adverse event, defined as the primary safety endpoint, between day 0 and day 28 was reported by one (8%) of 12 participants in both the placebo and low-dose groups, and two (17%) of 12 participants in both the medium-dose and high-dose groups (table 2). No immediate adverse events following immunisation were observed within the 6 h observation period at the investigational site. None of the participants experienced any spasmodic cough during the 28 days following immunisation, none reported difficulties in breathing, and no adverse events following immunisation were noted concerning vital signs during this period. Three participants (one medium-dose and two high-dose recipients) reported mild or moderate fever during the 28-day period (appendix p 9).

	Placebo (n=12)	Low-dose vaccine (n=12)	Medium-dose vaccine (n=12)	High-dose vaccine (n=12)
Women	6 (50%)	5 (42%)	2 (17%)	7 (58%)
Men	6 (50%)	7 (58%)	10 (83%)	5 (42%)
Age (years)	25 (22–27)	26 (25–30)	26 (25–28)	28 (24–30)
Height (cm)	174 (163–185)	177 (172–188)	180 (175–183)	172 (167–177)
Weight (kg)	69 (59–77)	71 (64–84)	74 (63–93)	69 (61–81)
BMI (kg/m^2)	22 (20–24)	22 (22–25)	22 (20–28)	23 (22–26)
Systolic blood pressure (mm Hg)	115 (109–124)	116 (110–130)	121 (117–126)	117 (115–122)
Diastolic blood pressure (mm Hg)	72 (69–76)	73 (72–78)	71 (69–74)	73 (69–76)
Heart rate (bpm)	65 (54–74)	70 (59–75)	63 (57–72)	69 (59–79)
Respiratory rate (breaths per min)	15 (13–17)	14 (13–15)	15 (13–17)	15 (14–16)
Oral temperature (°C)	36·5 (36·4–36·9)	36·8 (36·6–37·0)	36·6 (36·3–36·9)	36·6 (36·2–37·0)

Data are n (%) or median (IQR).

Table 1: **Baseline characteristics**

	Placebo (n=12)	Low-dose vaccine (n=12)	Medium-dose vaccine (n=12)	High-dose vaccine (n=12)
Spasmodic cough	0	0	0	0
Cough of grade 2 or more	1 (8%)	1 (8%)	1 (8%)	2 (17%)
Other respiratory tract adverse event related or possibly related to vaccination of grade 3 or more	0	0	1 (8%)*	1 (8%)†
Any other adverse event related or possibly related to vaccination of grade 3 or more	0	0	0	0
Composite primary endpoint: at least one of the events above (n [%, 95% CI])	1 (8%, 0–39)	1 (8%, 0–39)	2 (17%, 2–48)	2 (17%, 2–48)

Data are n (%) unless otherwise indicated. *Oropharyngeal pain. †Rhinorrhea and nasal congestion.

Table 2: **Primary safety endpoint and its components up to day 28 by participant per active dose group and placebo**

17 (35%) of 48 participants reported at least 1 day of cough after vaccination during the 28-day follow-up, without any marked difference between placebo and vaccine recipients. The highest number of cough events was reported during week 1 and week 2 after vaccination, including three (25%) of 12 placebo recipients, four (33%) of 12 low-dose vaccine recipients, six (50%) of 12 medium-dose vaccine recipients, and four (33%) of 12 high-dose recipients. Most events resolved within a week. Three cases of cough (but no cases of spasmodic cough) were reported during the first 3 days after vaccination, one in each group except for the high-dose group. In addition, four (33%) of 12 participants in the medium-dose group and one (8%) of 12 in the high-dose group reported a cough episode starting in week 3 or week 4. The reported cough was mild or moderate, except for one (8%) severe case in the high-dose group. Overall, the occurrence of cough was similar among the groups.

Half of the participants reported solicited general symptoms during the 28-day follow-up. The most frequent solicited symptoms were sneezing, fatigue, headache, rhinorrhoea, and nasal congestion. Most of the symptoms were mild or moderate. Many participants reported common cold during the 28-day follow-up. There were no apparent differences between the placebo group and any of the vaccination groups.

Two severe adverse events were reported, both of which started after day 28 and were judged as not related to vaccination. The first severe adverse event was pneumonia as a possible complication of suspected influenza in a

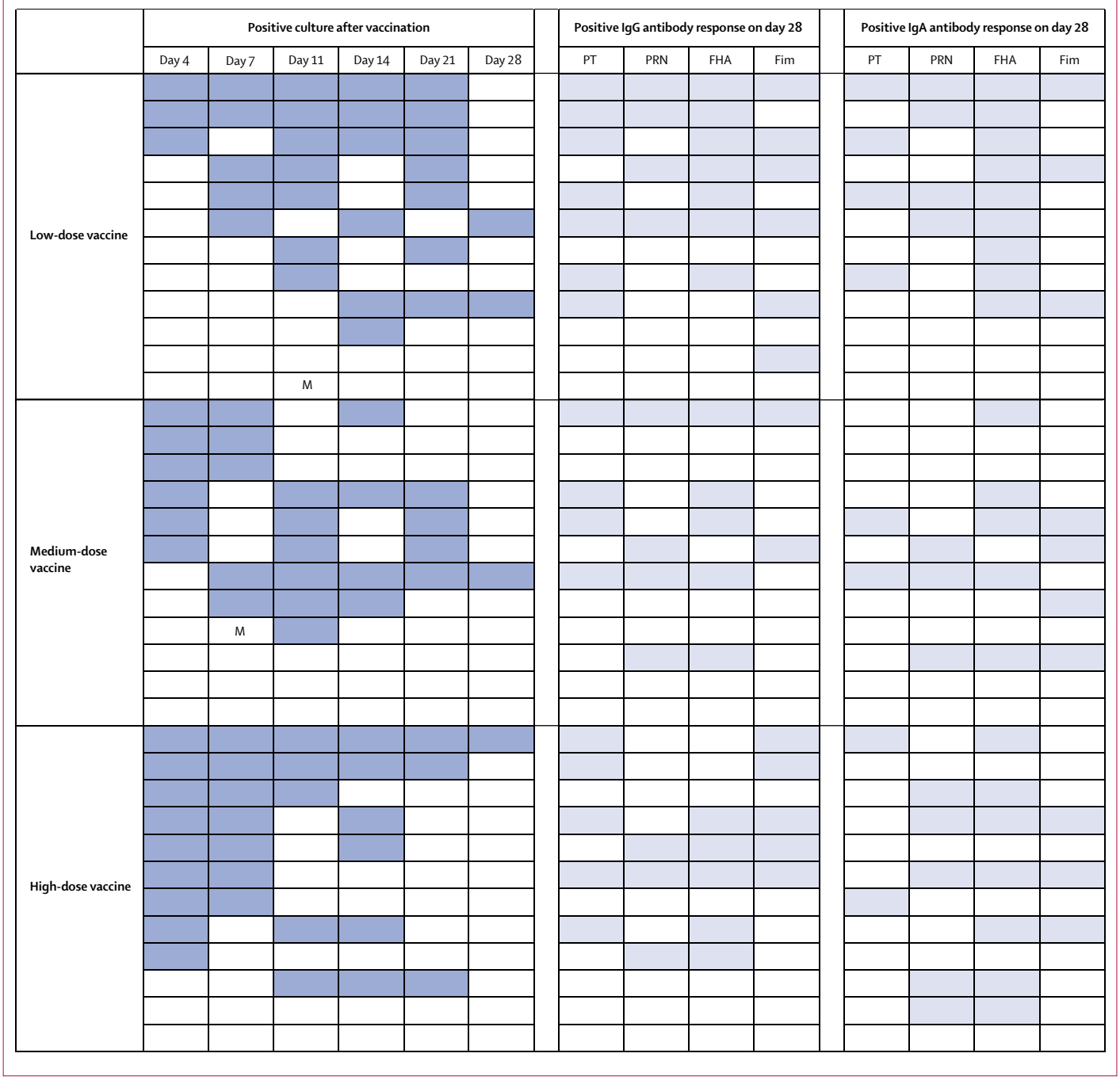
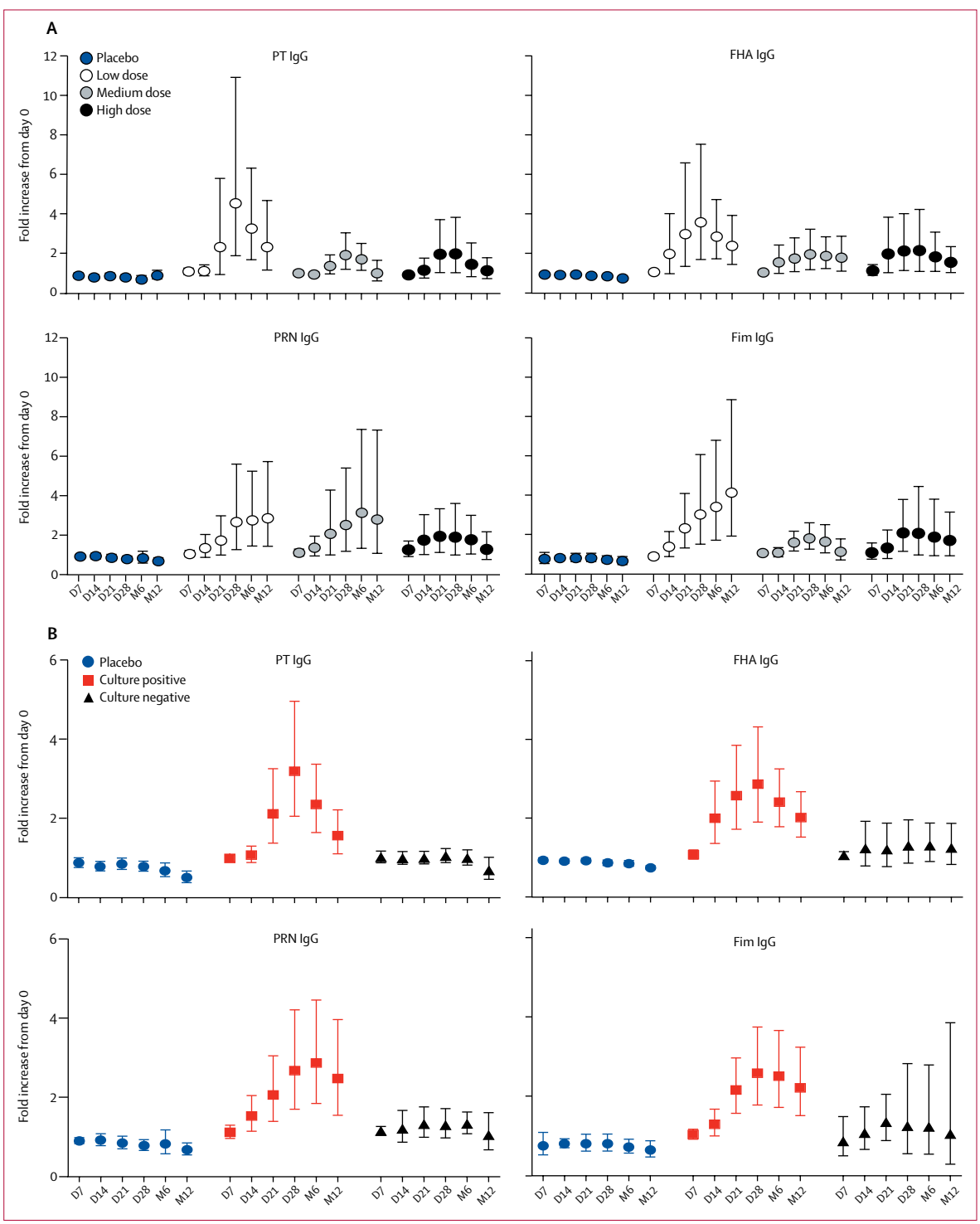


Figure 2: **Colonisation of nasopharyngeal mucosa from day 4 to day 28 after vaccination, and serum IgG and IgA responses on day 28**
Each row represents an individual participant within the assigned group. No individuals in the placebo group had colonisation or antibody response. Culture-positive samples are shown in dark blue and are listed after the time of the first positive sample. Light blue indicates samples with at least twice the serum IgG and IgA on day 28 compared with day 0 after vaccination and at least four times the minimum level of detection. M=missing sample. PT=pertussis toxin. PRN=pertactin. FHA=filamentous haemagglutinin. FIM=fimbriae 2/3.

participant 65 days after vaccination. The second severe adverse event concerned another participant who was hospitalised for acute renal failure with pathologically high serum creatinine, following the intake of a high dose of the non-steroidal anti-inflammatory drug naproxen after dental surgery, 82 days after vaccination. Both participants recovered uneventfully.

Regarding colonisation, BPZE1 was isolated from 29 (81%, 95% CI 68–93) of 36 vaccinated participants at least at one timepoint between day 4 and day 28. No



(Figure 3 continues on next page)

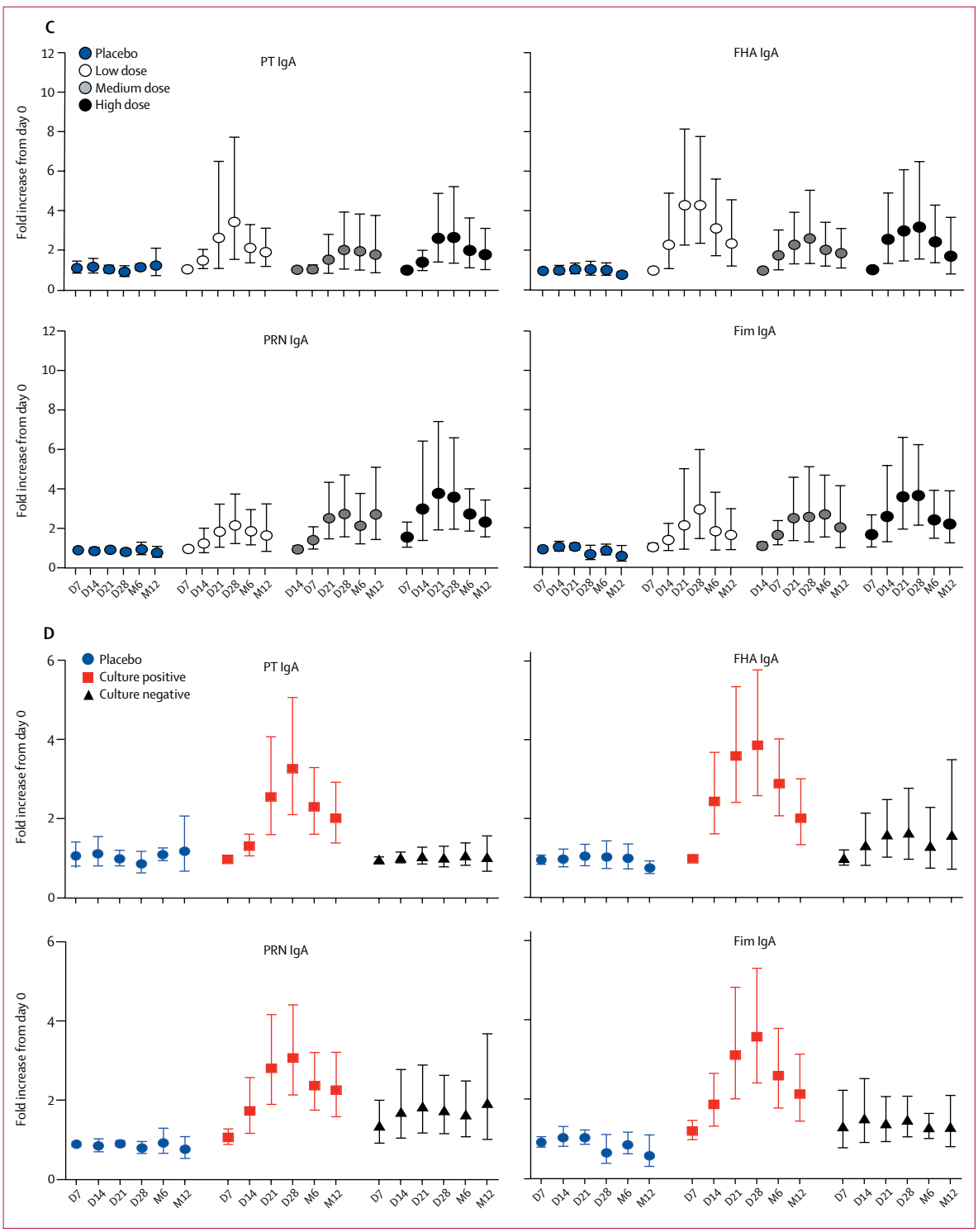


Figure 3: **Fold increase from day 0 of serum antibodies against pertussis toxin, pertactin, filamentous haemagglutinin, and fimbriae 2/3 per timepoint, per dose group for IgG (A) and IgA (C), and per colonisation status for IgG (B) and IgA (D)**

Figures show geometric means with the bars representing 95% confidence intervals. Culture positivity for a given participant is defined by at least one positive nasopharyngeal sample (at any timepoint). PT=pertussis toxin. PRN=pertactin. FHA=filamentous haemagglutinin. FIM=fimbriae 2/3.

placebo recipient was culture-positive at any time. Ten (83%) of 12 in the low-dose and high-dose groups and nine (75%) of 12 in the medium-dose group were culture-positive at least at one timepoint (figure 2). Colonisation profiles varied greatly between participants.

The duration of the nasal colonisation did not differ between the dose groups. However, BPZE1 was detected earlier with increasing dose levels with three (25%) participants in the low-dose group being culture-positive on day 4, six (50%) participants in the medium-dose group, and nine (75%) participants in the high-dose group. Four (11%) of 36 participants who were vaccinated were culture-positive at day 28, but new samples collected between day 43 and day 50 were negative. Cough occurrence was similar between colonised and non-colonised vaccine recipients.

Concerning immunogenicity in the different dose groups, the increase in serum IgG and IgA against pertussis toxin, pertactin, FHA, and Fim2/3 compared with before vaccination was measured at day 7, day 14, day 21, and day 28, and 6 months and 12 months after vaccination (figure 3). As expected, pre-vaccination antibody levels varied both within and between treatment groups (appendix pp 10–11). In general, increases in all four antigen-specific IgG levels were observed starting at day 14 after vaccination in all dose groups (figure 3). This increase usually peaked at day 28 and remained above the pre-vaccination level during 12 months of follow-up. Similar results were also found for IgA levels (figure 3).

22 (61%, 95% CI 45–77) of 36 vaccinated participants responded with serum IgG against at least one *B pertussis* antigen 4 weeks after vaccination. One of these participants was lost to follow-up at month 12, but out of the remaining 21 IgG responders, 20 (95%, 86–100) still responded after 12 months. For serum IgA, 24 (67%, 51–82) of 36 vaccinated participants responded to at least one *B pertussis* antigen 4 weeks after vaccination. Again, one of these participants was lost to follow-up at 12 months, and from the remaining 23 IgA responders, 17 (74%, 56–92) maintained their response at 12 months. At 4 weeks after vaccination, 28 (78%, 64–91) of 36 vaccinated participants had mounted IgG or IgA responses, or both, including 11 (92%) of 12 participants in the high-dose group. Of the 28 vaccinated participants that mounted IgG or IgA responses, 18 (64%, 47–82) responded with both IgG and IgA.

When the antibody responses were examined in relation to the colonisation status of the vaccine recipients, colonised participants (as defined by bacterial detection in at least one nasopharyngeal aspirate at any timepoint) had markedly higher increases in IgG and IgA titres than did non-colonised vaccine recipients or placebo recipients (figure 3). The individual profiles of IgG and IgA responses against the four *B pertussis* antigens on day 28 after vaccination in relation to colonisation are shown in figure 2, and the frequency of responders in each dose group can be found in the appendix (p 12). In the 29 colonised participants, the most frequently detected serum IgG responses were seen against pertussis toxin and FHA. Anti-FHA IgA was also the most frequent response detected, whereas, by contrast with IgG, anti-pertussis toxin IgA responses were seen with the lowest frequency amongst the participants.

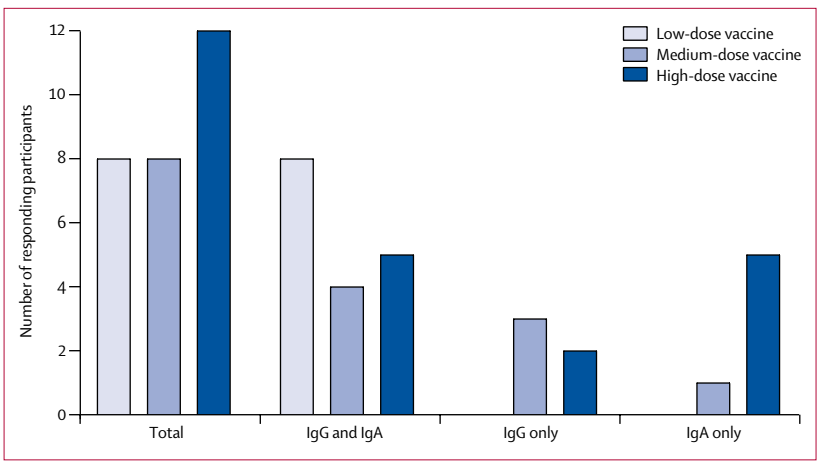


Figure 4: **Participants per dose group responding according to a pertussis case definition**
A participant was considered to have a positive response when the antibody level (IgG or IgA) increased with a fold change of two or more between pre-vaccination and post-vaccination samples for either pertussis toxin alone or for a combination of at least two other antigens (pertactin plus filamentous haemagglutinin, pertactin plus fimbriae 2/3, or filamentous haemagglutinin plus fimbriae 2/3).

Most of the culture-positive participants responded with either or both *B pertussis*-specific IgG and IgA. Only four (14%) of 29 culture-positive participants did not mount any detectable IgG or IgA response at day 28 after vaccination. However, one of them did show a rise in anti-pertussis toxin IgG level from day 21, but did not reach the responding criteria until 6 months after vaccination. Three (43%) of seven vaccinated culture-negative participants had positive IgG or IgA responses at day 28 after vaccination.

Investigating the positivity of the serological responses during the first 6 months after vaccination showed that 28 (78%) of 36 BPZE1-vaccinated participants responded according to the case definition,[18] including all 12 participants in the high-dose group. Of that 28, 17 (61%) responded with both IgG and IgA, whereas five (18%) responded with only IgG and six (21%) responded only with IgA (figure 4). None of the placebo recipients responded.

Discussion

This phase 1b, single-centre, double-blind, randomised, placebo-controlled, dose-escalation trial of the live, genetically attenuated *B pertussis* vaccine strain BPZE1 showed that the tested vaccine doses were well tolerated without any sign of differences in tolerance between the different active doses and placebo. Nasal colonisation was frequent in vaccine recipients in all dose groups, and no colonisation was observed in placebo recipients. Increases in serological responses against the four *B pertussis* antigens tested were seen in all dose groups

after intranasal vaccination, whereas no increase was apparent in placebo recipients.

This study follows the first-in-man trial reported earlier.[15] By contrast with the previous trial, this study escalated to higher doses, with the lowest dose in this trial corresponding to the highest dose used in the previous study. Furthermore, the volume was increased to 0·4 mL per nostril to optimise bacterial adherence to the nasal epithelium, and unlike in the previous study, women were also included. Finally, in this study participants with high serum IgG against both pertussis toxin and pertactin were excluded, whereas previously, participants were only excluded because of high serum anti-pertussis toxin IgG. The first study showed that high anti-pertactin IgG levels were associated with absence of colonisation. In the present study, which excluded people with either serum high anti-pertussis toxin or anti-pertactin IgG, and used higher vaccine volumes, colonisation rates with the 10^7 CFU dose were ten in 12 (compared with five in 12 in the previous study).

In this trial, the safety profile of BPZE1 showed no marked differences in the occurrence of solicited or unsolicited adverse events following immunisation between the three active dose groups and placebo. Reported symptoms were usually mild or moderate. Spasmodic cough was not reported by any participant. There was no sign of differences in cough occurrence between groups. However, as an inherent limitation of a phase 1 trial, the small numbers of participants included in this study mean that local tolerability and general safety need to be further investigated in future, larger-scale trials.

Overall, colonisation by the vaccine was observed in 29 (81%, 95% CI 68–93) of 36 vaccine recipients, without any apparent difference between dose groups. Culture positivity was examined between day 4 and day 28 after vaccination, with a highly variable duration of positivity between participants. Some participants were culture-positive at only one timepoint, others were continually positive at several timepoints, and some showed discontinuously detectable colonisation. These variations might be due to a suboptimal aspiration technique, because the nasopharyngeal samples varied greatly in volume and mucus content. Bacteria were detected somewhat earlier in the high-dose group than in the other groups. Increases in antibody responses to the tested antigens tended to be more frequent in colonised vaccine recipients than in non-colonised vaccine recipients, but cough occurrence was similar.

The increase of serum IgG against *B pertussis* antigens is often used for the evaluation of immunogenicity of pertussis vaccines,[20] although no serological correlate of protection has been widely accepted for pertussis. Nevertheless, correlations between post-vaccination antibody levels and protective efficacy against pertussis disease have been reported for anti-pertactin, anti-Fim2/3, and to lesser extent for anti-pertussis toxin IgG.[21,22] All three antibody types were induced by BPZE1 in most of the vaccinated participants.

Generally, antibody responses were related to colonisation. However, some culture-negative vaccinated participants showed increases in their IgG or IgA levels, or both, although typically at a lower level than the culture-positive participants. It cannot be excluded that these participants might nevertheless have been colonised, but at levels below the detection limit or before the first sampling day (day 4 after vaccination). Furthermore, suboptimal aspiration technique could possibly have rendered falsely negative colonisation results. In addition, although the doses were standardised and determined by CFU counting after thawing of the vaccine lot, it should be kept in mind that the vaccine suspension does not only contain live BPZE1, but also bacteria that died during the freeze–thaw cycle. It is therefore conceivable that the dead bacteria contributed to immunogenicity in a similar way to a killed whole-cell vaccine, especially in participants with a pre-existing immune response induced by previous exposure to *B pertussis*.

The serum IgG levels to the four tested antigens observed here were low in relation to those induced by injectable acellular pertussis vaccines.[23] This result is not unexpected, because acellular pertussis vaccines contain much larger amounts of these antigens than does BPZE1. However, we have recently shown that BPZE1 induces a much broader and functional Th1-type antibody response than acellular vaccines do.[24] In addition, the serological responses to the four tested antigens were evaluated using the same case definition as is used for patients with a natural infection, to see whether the BPZE1 vaccine could induce similar responses to those induced by natural infection.[18] This response was seen in most of the vaccinated participants, including all participants in the high-dose group.

BPZE1 induced notable increases in IgA levels against the four antigens. Given that *B pertussis* is a strictly mucosal pathogen and disseminated bordetellosis is very rare,[25] IgA is expected to play a protective role against *B pertussis* colonisation.[26] By contrast with whole-cell and acellular vaccines, which fail to prevent colonisation by *B pertussis*,[11] a single administration of BPZE1 has been shown to protect against both pertussis disease and *B pertussis* colonisation in baboons.[14] Recent studies in mice have confirmed the role of IgA in protection against nasal colonisation by *B pertussis*.[27] Therefore, in future clinical studies, it will be important to evaluate the nasal IgA responses induced by BPZE1. However, such an evaluation will require an improved and standardised method for the collection of nasopharyngeal specimens.

In summary, the three tested vaccine doses were safe, induced colonisation, and were immunogenic in an adult population, which justifies further clinical development of BPZE1. Currently, a larger-scale phase 2 study (NCT03942406) with 10^9 CFU of BPZE1 is underway, which should gather additional safety data. This study will also examine the effect of previous acellular pertussis

vaccination on BPZE1 take, as well as the booster effect of a second BPZE1 dose. If future studies confirm that BPZE1 can protect against infection by and transmission of *B pertussis*, in addition to protecting against pertussis disease, then this stand-alone pertussis vaccine might be useful for boosting adolescents and adults, and as an effective tool for cocoon vaccination. A limitation of the use of this vaccine might occur in people who are immunosuppressed, including during pregnancy, although studies in mice have shown that BPZE1 is safe, even in severely immune-compromised mice.[28]

Furthermore, if safety can be shown in neonates, BPZE1 might potentially be used as a priming vaccine in early life. This application might help to protect the most vulnerable population in conjunction with maternal vaccination. Maternal vaccination is not expected to interfere with BPZE1 take in neonates, because maternal antibodies are unlikely to prevent colonisation by *B pertussis*, as shown in the baboon model.[29] Moreover, we have recently shown that BPZE1 vaccination results in significant protection within days in a murine model.[28] This result is reminiscent of observations made with the live attenuated *Bordetella bronchiseptica* vaccine, shown to protect dogs against kennel cough as soon as 2 days after vaccination.[30] This evidence provides strong hope that live attenuated *Bordetella pertussis* vaccines might effectively and quickly protect neonates against pertussis and, in conjunction with maternal immunisation, might constitute a powerful tool to finally control the pertussis pandemic.

Contributors

MJ, LR, JS, MT, KS, KR, RT, and CL contributed to the study design. Na-T and JS contributed to clinical data collection, and MJ and RT contributed to laboratory data collection. LR, CC, and CB contributed to the statistical data analysis, and MJ, LR, JS, KS, KR, NM, RT, and CL contributed to the data interpretation. MJ, LR, JS, RT, and CL contributed to the manuscript writing and literature search, and MJ, LR, CC, CB, and RT contributed to the figures. LR, Na-T, JS, CC, CB, MT, KS, KR, NM, RT, and CL contributed to the manuscript review and critique. All authors had full access to the data, and reviewed, revised, and gave final approval of the manuscript before submission.

Declaration of interests

NM and CL hold patents on the BPZE1 vaccine, which is licensed to ILiAD Biotechnologies. MT, KS, and KR are employees of ILiAD Biotechnologies. All other authors declare no competing interests.

Data sharing

Individual participant data will not be made available; only group data will be made available after de-identification (text, table, figures, and appendices including the study report). Individual data currently requires conformity to the GDPR, which was not provided for the protocol and informed consent. Data will be made available to researchers who provide a methodologically sound proposal, to achieve the aims in the approved proposal, immediately after publication and ending 10 years following publication. Proposals should be directed to promoteur.inserm@inserm.fr; to gain access, data requestors will need to sign a data access agreement.

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