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Bacille Calmette-Guérin (BCG) vaccination at birth and antibody responses to childhood vaccines. A randomised clinical trial

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ABSTRACT

Introduction: BCG vaccination has been associated with beneficial non-specific effects on child health. Some immunological studies have reported heterologous effects of vaccines on antibody responses to heterologous vaccines. Within a randomised clinical trial of Bacille Calmette-Guérin (BCG) vaccination at birth, The Danish Calmette Study, we investigated the effect of BCG at birth on the antibody response to the three routine vaccines against DiTeKiPol/Act-Hib and Prevenar 13 in a subgroup of participants.
Methods: Within 7 days after birth, children were randomised 1:1 to BCG vaccination or to the control group (no intervention). After three routine vaccinations given at age 3, 5 and 12 months, antibodies against DiTeKiPol/Act-Hib and Prevenar 13 (*Streptococcus pneumoniae* serotype type 4, 6B, 9V, 14, 18C, 19F and 23F) were measured 4 weeks after the third vaccine dose.
Results: Among the 300 included children (178 BCG; 122 controls), almost all children (>96%) had antibody responses above the protective levels. Overall BCG vaccination at birth did not affect the antibody level. When stratifying by 'age at randomisation' we found a possible inducing effect of BCG on antibodies against B. pertussis and all pneumococcal serotypes, when BCG was given after the first day of life. Girls had significantly higher antibody levels for Haemophilus influenza type b and pneumococcus than boys.
Conclusions and relevance: Three routine vaccinations with DiTeKiPol/Act-Hib and Prevenar 13 induced sero-protective levels in almost all children. No overall effect of neonatal BCG vaccination was observed.

1. Introduction

Bacille Calmette-Guérin (BCG) is among the most widely used vaccines worldwide. Since it was introduced in the 1920s, BCG has been the only vaccine used for the prevention of tuberculosis (TB) [1,2]. The most significant protection is seen against the more severe forms of TB such as milliary TB and TB meningitis [3,4].

BCG may protect not only against TB, but also against other potentially fatal infections [5–7]. In a recent review of the evidence for non-specific effects (NSE) of vaccines, BCG was found to reduce the overall mortality much more than can be explained solely by the specific protection against TB [8]. Other studies have recently investigated the NSEs of BCG on diabetes and eczema, and supported that BCG has broader effects [9–12]. As a consequence, WHO recommends further research into NSEs of BCG and other vaccines [13].

Several immunological studies have focused on the possible underlying biological mechanisms of NSEs of vaccines, including heterologous T-cell immunity and trained innate immunity [14]. BCG vaccination has been found to alter *in vitro* cytokine responses to non-related antigens and mitogens in both low- and high-income countries [15–17]. This has been linked to epigenetic reprogramming of monocytes, which also translated into increased protection against a heterologous infectious challenge in mice [18].

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An Australian study of the effect of BCG on antibody response from routine vaccines given during childhood found that BCG was associated with significantly higher response of several subclasses of the pneumococcus conjugate vaccine (PCV) [19].

From 2012 to 2015 The Danish Calmette Study investigated the effect of BCG vaccination at birth on childhood morbidity; the study was sized to examine the risk of all cause hospitalisation and atopic dermatitis as the main outcomes. In the present substudy we investigated the effect of BCG on antibody responses to routine vaccines given during the first year of life.

2. Methods

2.1. Study design and population

The Danish Calmette Study is a randomised clinical trial investigating the effect of neonatal BCG vaccination on childhood morbidity at three study sites. From October 2012 to November 2013, 4262 children were randomised to receive either BCG or no BCG; no placebo was used. All children were randomised within the first 7 days of life. Children were followed by telephone interviews and clinical examinations at the recruitment sites at 3 and 13 months of age. The study is described in detail elswhere [20].

The present antibody study was conducted at "Hvidovre Hospital" from April 2014 to December 2014. It was based on blood samples from two subpopulations. The first subpopulation (cohort I) consisted of infants born from May to November 2013 enrolled in a sub-study on cytokine responses in whole blood stimulated at age 4 days, 3 and 13 months (Nissen et al. 2016). The 13-months blood samples were analysed for antibody levels for the present study. To achieve sufficient power, a second subpopulation (cohort II) of infants seen for 13-months follow-up in the period from April to December 2014, were enrolled at age 13 months.

At recruitment (cohort I) or at the 13-months telephone interview (cohort II), families were asked for consent to participate in the present study. Blood samples were collected at the 13-month clinical examination at the hospital; this examination was scheduled 4 weeks after the third dose of DiTeKiPol/Act-Hib (Statens Serum Institut) and Prevenar 13 (Pfizer Limited) (*Streptococcus pneumoniae* serotype type 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F and 23F).

2.2. Blood collection

Blood was collected in 4 mL Z Serum Separator, VACUETTE® TUBE, Greinor Bio-One. Serum was aliquoted into Thermo Scientific 1 mL cryotubes and stored at −80 °C until analysis.

2.3. Intervention

The BCG Danish strain 1331 (Statens Serum Institut) was used in The Danish Calmette Study. A recommended dose of 0.05 mL of the vaccine suspension was applied intradermally in the left upper arm.

2.4. Routine vaccinations

According to the Danish vaccination programme children are vaccinated at 3, 5, and 12 months with DiTeKiPol/Act-Hib (*Corynebacterium diphtheria* toxoid (DT), *Clostridium tetanus* toxoid (TT), *Bordetella pertussis* toxoid (PT), Polio virus type 1–3, *Haemophilus influenzae* type b polysaccharide (Hib)), and Prevenar 13.

During some of the recruitment period, DiTeKiPol/Act-Hib was not available due to production problems and was temporarily replaced by 1–3 doses of Infanrixhexa (GlaxoSmithKline). The main differences between DiTeKiPol/Act-Hib and Infanrixhexa are HBsAg, the *B. pertussis* component and the aluminium content (Supplementary Table 1).

2.5. Antibody assay

IgG antibodies directed against *B. pertussis, C. diphtheria, C. tetani, H. influenzae* type b and *S. pneumoniae* were measured using a fluorescent bead-based multiplex immuno-assay (Luminex xMAP technology). All plates (Millipore, Amsterdam, The Netherlands) contained a reference, controls and blanks. Analyses were performed with a Bio-Plex 200 in combination with Bio-Plex manager software (Bio-Rad Laboratories, Hercules, CA, USA).

For measurement of antibodies against *B. pertussis*, diphtheria and tetanus, the samples were diluted 1/200 and 1/4000 in phosphate-buffered saline (Tritium, Eindhoven, The Netherlands) containing 0.1% Tween-20 (Merck Millipore, Billerica, MA) and 3% bovine serum albumin (Sigma-Aldrich, St. Louis, MO). Serum values for *B. pertussis* were assigned in IU/ml as the used reference was calibrated against the U.S. Reference Pertussis Anti-serum Human lot 3 (Ptx and FHA) and lot 4 (Prn) (CBER/FDA). For diphtheria and tetanus, serum values were expressed in IU/ml as the reference serum was calibrated against the international standards (NIBSC code Di-03 and NIBSC code TE-03) [21–24].

For measurement of antibodies against *H. influenzae* type b, the samples were diluted 1/100 in 50% antibody depleted human serum (ADHS, Valley Biomedical). Serum values were expressed in μg/ml as the reference serum was calibrated against the international reference serum Lot1983 (CBER/FDA) [24].

For measurement of antibodies against 7 serotypes *S. pneumoniae* (4, 6B, 9V, 14, 18C, 19F and 23F), the samples were diluted 1/1000 in 5% ADHS containing 15 μg/ml multi CWPS (Statens Serum Institut). Serum values were expressed in μg/ml as the reference serum was calibrated against the international reference serum lot 89S (FDA) [25].

2.6. Statistical analysis

To detect a difference of 10% in antibody levels, 300 infants (150 from each group) needed to be included (α: 0.05, β: 0.10)[26].

Before unblinding, a statistical analysis plan was deposit with the Danish Calmette Study Data Safety and Monitoring Bard. In accordance with the statistical analysis plan, in each randomisation group we report the proportion of children with a non-protective level of antibodies according to standard cut-off values for each vaccine. Antibody levels are presented as geometric mean concentrations (GMC). The effect of BCG vaccination is presented as a geometric mean ratio (GMR) with 95% confidence interval (CI) obtained as the anti-logged coefficient from a linear regression with log-concentrations as outcome and randomisation group as co-variate. A 5%-significance level is used. We had pre-specified to test the following factors for effect modification: maternal BCG, sex, 'age at randomisation' (which was pre-specified as 0–1 day versus 2–7 days, as most children were enrolled during the first days of life and there was limited power to analyse the effect on subsequent days), age at bleeding, time from third pentavalent vaccine to bleeding, and doses of Infanrixhexa. All statistical analyses were performed using STATA 13.1 (Statacorp LP, College Station, TX, USA).

2.7. Ethics

The trial was approved by the Committee of Biomedical Research Ethics (J.no. H-3-2010-087), the Danish Data Protection Board (J.no. 2009-41-4141), and the Danish Medicines Agency (J.no. 2612-4356. EudraCT 2010-021979-85. Protocol 2009-323). The trial was registered at www.ClinicalTrials.gov (NCT01694108)

and monitored by the Good Clinical Practice Unit and an independent Data Safety Monitoring Board.

3. Results

Of the 304 children recruited for the study, two controls were excluded because they sought BCG vaccination outside the study and two (1 BCG; 1 control) were excluded from the analysis because they were bled before their third pentavalent vaccine. The remaining 300 children all received three doses of DiTeKiPol/Act-Hib or Infanrixhexa and three doses of Prevenar 13 before bleeding. Included in the analysis 142 of the children (75 BCG; 67 controls) were recruited as part of cohort I; 158 of the children (103 BCG; 55 controls) belonged to cohort II. Thus, 178 BCG vaccinated children and 122 control children were included in the final analysis.

For most of the clinical parameters, the two groups had similar background characteristics (Table 1). However, in the BCG group 22% of the mothers were BCG vaccinated compared to 12% in the control group (p-value 0.04); this was due to a difference in cohort II (Supplementary Table 2). Also 25% in the BCG group had 1–3 doses of Infanrixhexa compared to 42% in the control group (p-value 0.002), this was due to a difference in cohort I (Supplementary Table 2).

3.1. Vaccination programme

Parents were asked at the 13-months interview which vaccines their child had received. Due to a temporary shift from DiTeKiPol/Act-Hib to Infanrixhexa in the Danish vaccination programme for some children one to three doses of the intended DiTeKiPol/Act-Hib were replaced with Infanrixhexa. The change of vaccines caused no delay in the timing of vaccination at any of the three time points (Table 1). According to protocol we only examined antibody levels against diseases present in both vaccines. When analysing the agreement between parent-reported information on doses of Infanrixhexa (1–3 or none), and antibodies against components present only in this vaccine, we found that 26% (54/205) children registered as having received no Infanrixhexa, had in fact antibodies against Pertactin (Prn), indicating that they received at least one dose of Infanrixhexa. Classifying these children as having received 1–3 doses of Infanrixhexa did not change the estimates where this co-variable was included in the analysis.

3.2. The effect of BCG on achieving protective antibody levels

The routine vaccination was effective; four weeks post-vaccination almost all children had antibody levels above the protection limit. BCG did not affect the probability of having titres above the protective level (Table 2).

3.3. The effect of BCG on antibody levels

There was no difference between BCG and controls with respect to antibody levels, neither overall, nor when adjusted for the background factors (maternal BCG and Infanrixhexa), which varied between the groups (Table 3, Fig. 1).

3.4. Effect modification by 'age at randomisation'

BCG was associated with higher GMC of anti-PT and anti-Hib IgG among children randomised on day 2–7, and lower GMC among children randomised on day 0–1. The interaction between BCG and age of randomisation reached statistical significance for anti-PT IgG with a GMR of 0.91 (95% CI 0.70–1.18) in the group randomised on day 0–1 compared with a GMR of 1.47 (95% CI 1.08–2.00) in the group randomised on day 2–7 (p-value = 0.02 for interaction between BCG and 'age at randomisation'). Though not significant in the interaction test there was a similar tendency for anti-Hib IgG; the group randomised on day 0–1 had a GMR of 0.64 (95% CI 0.40–1.05) compared to a GMR of 1.2 (95% CI 0.68–2.12) in the group randomised on day 2–7 (p-value = 0.10 for interaction between BCG and 'age at randomisation') (Fig. 2).

Also, the GMC of pneumococcal IgG serotypes tended to be higher in the BCG group for children randomised on day 2–7 whereas the GMC was lower in the BCG group for children

Table 1
Background characteristics of study population.

	BCG N = 178 n (%)	Control N = 122 n (%)	p-value
Age in days at randomisation (median (IQR))	1 (1–3)	1 (1–3)	0.54
Age group at randomisation			
0–1 days	103 (58)	71 (58)	0.95
2–7 days	75 (42)	51 (42)	
Sex (male)	107 (60)	66 (54)	0.30
Gestational age (GA) in weeks (median (IQR))	40.3 (39.2–40.9)	40.6 (39.4–41.1)	0.34
Weight in kg (median (IQR))	3.51 (3.26–3.84)	3.55 (3.19–3.88)	0.80
Caesarean section	31 (17)	26 (21)	0.53
Maternal BCG[a]	38 (22)	15 (12)	0.04
At least one parent of non-Danish ethnicity	30 (17)	19 (16)	0. 47
Maternal smoking during pregnancy	15 (8)	9 (7)	0.74
Level of maternal education			
Basic schooling and non-theoretical education	31 (18)	29 (24)	0.29
Theoretical education incl. BA level	87 (49)	50 (41)	
Master level or more	59 (33)	42 (35)	
Siblings	71 (40)	40 (33)	0.21
Atopic predisposition	118 (66)	72 (59)	0.20
Age in days at routine vaccination (median (IQR))			
- 3 months	93 (91–99)	92 (92–99)	0.97
- 5 months	154 (153–161)	155 (153–161)	0.89
- 12 months	372 (366–380)	369 (362–378)	0.27
Time in days from third pentavalent vaccine to bleeding (median (IQR))	34 (30–40)	35 (30–41)	0.96
Infanrixhexa 1–3 doses	44 (25)	51 (42)	0.002

[a] Two mothers with unknown BCG status excluded from the analysis.

Table 2
Proportion of children with an antibody responses above the limit of protection – comparing BCG vaccinated and controls.

	Protection limit	All N = 300 % (n)	BCG N = 178 % (n)	Control N = 122 % (n)	p-value[a]
Pertussis	20 IU/ml	99 (297)	99.4 (177)	98.4 (120)	0.36
Diphtheria	0.1 IU/mL	99.7 (299)	99.4 (177)	100 (122)	0.40
Tetanus	0.1 IU/mL	100 (300)	100 (178)	100 (122)	1
Haemophilus influenza type b	0.15 μg/mL	97 (291)	97.2 (173)	96.7 (118)	0.82
Pneumococcus					
Serotype 4	0.35 μg/mL	99 (297)	98.9 (176)	99.2 (121)	0.79
Serotype 6b	0.35 μg/mL	98 (294)	98.9 (176)	96.7 (118)	0.19
Serotype 9 v	0.35 μg/mL	99 (297)	98.9 (176)	99.2 (121)	0.79
Serotype 14	0.35 μg/mL	99.7 (299)	100 (178)	99.2 (121)	0.23
Serotype 18c	0.35 μg/mL	99.3 (298)	99.4 (177)	99.2 (121)	0.79
Serotype 19f	0.35 μg/mL	99.7 (299)	100 (178)	99.2 (121)	0.23
Serotype 23f	0.35 μg/mL	98.3 (295)	98.3 (175)	98.4 (120)	0.98

IU: International Units.
[a] Chi2 test for differences between groups.

Table 3
Geometric mean concentration (GMC) and geometric mean ratio (GMR) of IgG comparing BCG vaccinated and controls.

	BCG (N = 178)		Control (N = 122)		Unadjusted (N = 300)	p-value	Adjusted[a] (N = 300)	p-value
	GMC	95% CI	GMC	95% CI	GMR (95% CI)		GMR (95% CI)	
Pertussis	180	(158–205)	162	(139–189)	1.11 (0.91–1.36)	0.31	1.13 (0.92–1.39)	0.25
Diphtheria	1.66	(1.47–1.86)	1.71	(1.49–1.97)	0.97 (0.81–1.16)	0.72	0.98 (0.81–1.19)	0.86
Tetanus	1.87	(1.65–2.11)	2.02	(1.74–2.35)	0.92 (0.76–1.12)	0.43	0.95 (0.78–1.16)	0.64
Haemophilus influenza type b	6.58	(5.20–8.34)	7.88	(5.92–10.5)	0.84 (0.58–1.21)	0.34	0.78 (0.53–1.13)	0.19
Pneumococcus								
Serotype 4	9.21	(8.02–10.6)	9.51	(8.05–11.2)	0.97 (0.78–1.20)	0.77	0.96 (0.77–1.20)	0.72
Serotype 6b	10.3	(8.65–12.3)	9.58	(7.73–11.9)	1.08 (0.82–1.43)	0.59	1.07 (0.81–1.43)	0.62
Serotype 9v	9.46	(8.28–10.8)	9.70	(8.25–11.4)	0.98 (0.79–1.20)	0.82	0.99 (0.80–1.23)	0.93
Serotype 14	11.2	(9.79–12.8)	10.4	(8.81–12.2)	1.08 (0.88–1.33)	0.47	1.12 (0.91–1.39)	0.29
Serotype 18c	10.9	(9.26–12.8)	9.14	(7.30–11.5)	1.15 (0.93–1.42)	0.20	1.16 (0.93–1.44)	0.19
Serotype 19f	53.1	(45.2–62.4)	53.3	(42.7–66.6)	1.00 (0.80–1.24)	0.99	0.99 (0.79–1.23)	0.90
Serotype 23f	11.8	(9.77–14.2)	10.8	(8.38–14.0)	1.05 (0.82–1.35)	0.71	1.06 (0.82–1.37)	0.67

[a] Adjusted for maternal BCG and Infanrixhexa.

randomised on day 0–1. This interaction was statistically significant for *serotype 9v* (p-value = 0.005); *serotype 18c* (p-value = 0.02); and *serotype 19f* (p-value = 0.007) (Fig. 3).

3.5. Effect modification by sex and maternal BCG

Girls responded with significantly higher antibody levels than boys for *Haemophilus influenza* type b and pneumococcus (Supplementary Table 3). Most infants achieved protective levels, and there was a tendency towards more girls than boys had antibody levels above the protection limit. (Supplementary Table 4). No difference in the effect of BCG by sex (data not shown) or effect modification by maternal BCG status on antibody responses (Supplementary Table 5) was observed.

Analyses by age at bleeding, time from 12-month vaccine to bleeding and doses of Infanrixhexa did not show any modifying effect of BCG (data not shown).

4. Discussion

This is the first study in a high-income setting with a randomised design to investigate the effect of BCG vaccination at birth on antibody response from pentavalent vaccines given during the first year of life. Almost all children (>96%) had antibody responses above the protective levels, and we were not able to detect any additional improvement by administering BCG vaccine at birth. Stratifying for 'age at randomisation', we found a possible enhancing effect of BCG vaccination on antibody levels against *B. pertussis* and pneumococcal serotypes when BCG vaccine was administered after the first days of life. Girls had higher antibody responses towards *Haemophilus influenza* type b and pneumococcus than boys.

4.1. Strengths and limitations

The study is a randomised design with allocation to BCG or no BCG, and the study was conducted in a country with a high adherence to the national vaccination programme.

The two groups were comparable with regard to age at 12-month vaccination and time from vaccination to bleeding. Unfortunately, there was an uneven distribution of children in the BCG group (N = 178) and the control group (N = 122). Some children were included from another sub-study (Nissen 2016) in which parents consented before the randomisation (75 BCG; 67 controls); the rest of the children were included at the 13-month telephone interview (103 BCG; 55 controls). This unbalanced recruitment of participants of the antibody cohort was a result of a decreased willingness of participants randomised to the control group to participate, as we invited children by random without knowledge about their randomisation status. Thus, parents of BCG-vaccinated children were more prone to participate in our study, including bleeding of the child, when contacted at the 13-month telephone interview [27]. To show a significant difference of 10% between the BCG vaccinated and controls we needed 150 children in each group. The uneven distribution, in addition to the higher standard deviations in our data compared to the data

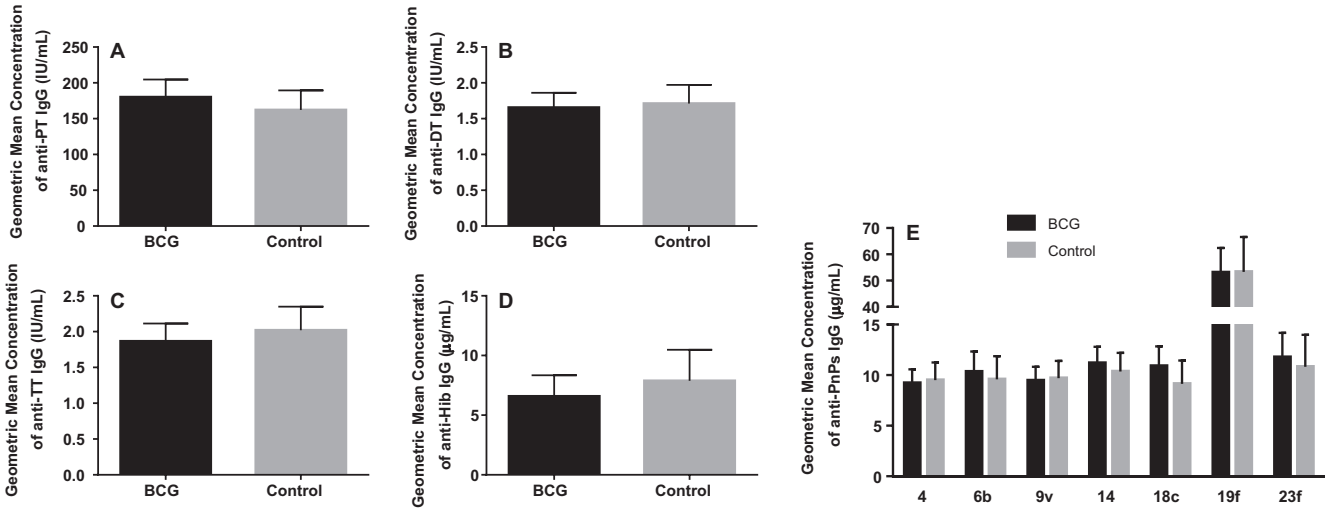

Fig. 1. Geometric mean concentrations (GMCs) of antibodies against A: anti-pertussis toxin (anti-PT), B: anti-diphtheria toxin (anti-DT), C: anti-tetanus toxin (anti-TT), D: anti-haemophilus influenzae type b, E: pneumococcal capsular polysaccharide antigens (anti-Pn Ps IgG) for each pneumococcal serotype. Error bars depict 95% CI.

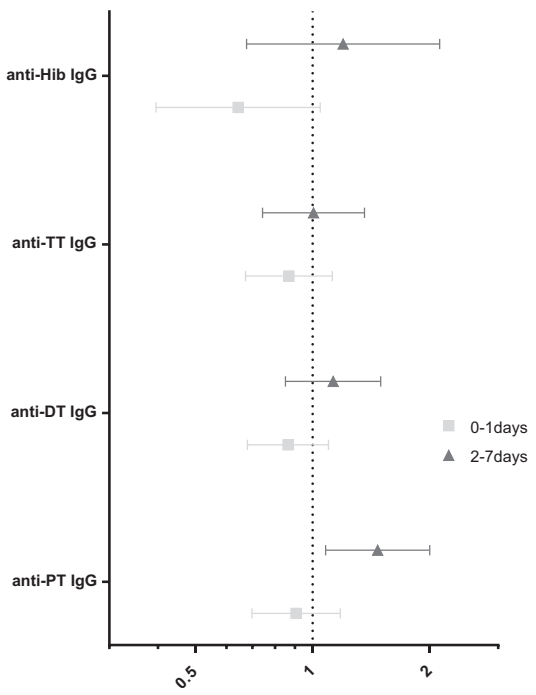

Fig. 2. Geometric mean ratios (GMRs) of the effect of BCG on antibodies against: anti-PT IgG, anti-DT IgG, anti-TT IgG, anti-Hib IgG. Dots display all children, squares display children randomised 0–1 days after birth, triangles display children randomised 2–7 days after birth. Error bars depict 95% CI.

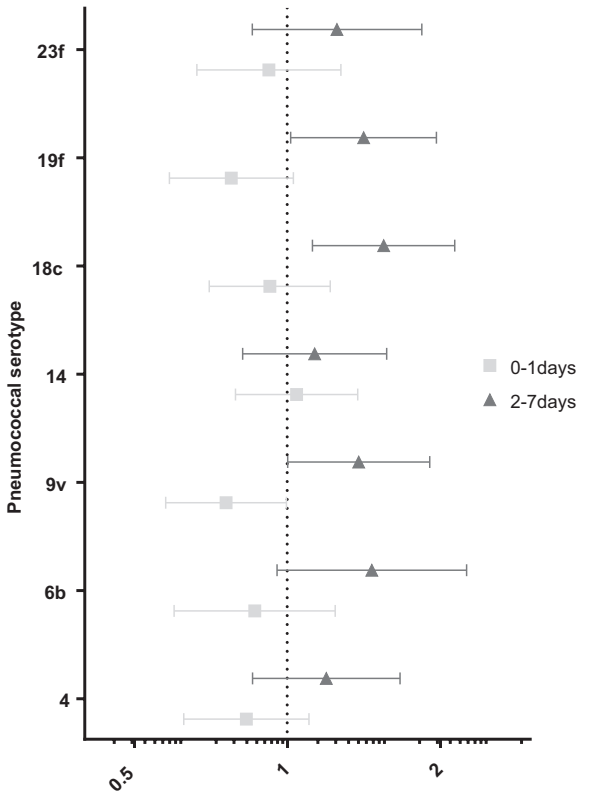

Fig. 3. Geometric mean ratios (GMRs) of effect of BCG on antibodies against pneumococcal capsular polysaccharide antigens (anti-Pn Ps IgG) for each pneumococcal serotype. Dots display all children, squares display children randomised 0–1 days after birth, triangles display children randomised 2–7 days after birth. Error bars depict 95% CI.

the sample size was based on, poses a risk for type-2 errors, but the difference seems unlikely to result in systematic bias.

Despite the fact that almost all children adhered to the Danish Vaccination Programme, the children received different combinations of childhood vaccines. This was due to a sudden temporary shift insupply from Statens Serum Institut leading to replacement of DiTeKiPol/Act-Hib with Infanrixhexa, which was unfortunate for this study, but impossible to predict or influence. We are confident that the parental reporting of number of childhood vaccines is accurate, but parental distinction between DiTeKiPol/Act-Hib vaccine and Infanrixhexa may be uncertain, as witnessed by the high proportion of children who were reported to have received the "usual" DiTeKiPol/Act-Hib vaccine, but who had nonetheless antibodies against the Prn component present in Infanrixhexa. However, it made no difference to the conclusions whether we classified children according to parental recall or by antibody responses to Prn, and importantly, the change in vaccination type was not associated with any delays in the timing of vaccination, which could have confounded the analysis.

4.2. Comparison with other studies

In The Gambia, Ota et al. measured antibodies at 4.5 months of age after receiving diphtheria-tetanus-pertussis (DTP) vaccine at 2, 3 and 4 months. They found no effect of BCG at birth on anti-TT nor anti-PT comparing children BCG vaccinated at birth with children not yet BCG vaccinated at the time of bleeding, but BCG-at-birth was associated with higher antibody response to Hepatitis B vaccination at birth, 2 and 4 months of age. They also studied the effect of BCG at 2 months of age, comparing again with children not yet BCG vaccinated, and found that BCG at 2 months of age, but not BCG at birth, was associated with higher antibody responses to oral polio vaccine [16].

A more recent study from Australia measured vaccine antibodies in 7-month-old children, 4 weeks after the last infant vaccination. BCG-vaccinated children were recruited from a related study randomising children with increased risk of TB to vaccination with BCG-Denmark, BCG-Japan or BCG-Russia [28]. Control children were recruited at the vaccination clinic. BCG was given at birth, and both the BCG group and the control group had Infanrixhexa, Prevenar7 and RotaTeq at 2, 4 and 6 months. The study found no effect of BCG on anti-TT and anti-Hib, but an inhibitory effect on antibodies against Hepatitis B. In the BCG group, children had higher antibody levels against *S. pneumococcus* serotypes compared to the control group. The most significant effect was seen for serotype 6B, 9V and 18C [19].

There are some significant differences between these two studies and the present study. All three studies have different vaccination programs in terms of vaccines and schedule. Also the times of bleeding were different although both the Australian study and the present study were measuring antibodies 4 weeks after the last vaccine. We did not measure antibodies against Hepatitis B, as this vaccine is not part of the standard vaccination programme in Denmark, but only offered to children of Hepatitis B-infected mothers (only one child in this study).

Despite some important differences between the studies, our observation that BCG may have an effect on the antibody response against *S. pneumococcal* serotypes in children randomised at day 2–7 supports previous findings of the Australian study on the same serotypes [19]. This indicates that BCG may have NSEs that increase antibody responses to routine vaccines, but the timing of BCG vaccination may be important. As a possible explanation the development and changes in the neonatal immune system during the first days of life could result in a varying response to BCG vaccination [29–32].

The proportion of children with antibody levels above the limit of protection was comparable with other studies for both BCG vaccinated and the control group [33–36]. Few children had antibody levels below the limit of protection. Overall, antibody levels against Hib and all Pneumococcus serotypes were lower among boys compared to girls. Some studies have reported sex differences of antibody levels against DT among adults [37], but we found no differences for DT and TT. For PT on the other hand, boys in our study tended to have a higher response compared to girls. Other studies found higher antibody responses in females than males [38–42]. An explanation could be genetic differences as the X-chromosome has several genes with potential influence on immunocompetence [43].

The BCG effect in other studies has varied by sex [17,44,45], but we observe no such differences by sex. Maternal BCG status did not modify the effect of BCG in the present study, although we have found that maternal BCG may influence the effect of neonatal BCG in regard to the risk of hospitalisation for infection (in submission).

Antibody response to routine vaccinations in our population was almost complete since nearly all children had protective levels. However, this absence of effect of BCG in a setting of good vaccine responsiveness should not detract from future studies in which BCG should be tested in situations in which basal vaccine responses are poorer: such examples can be vaccination with less effective vaccines, or in individuals with poor reactivity. In line with this, a recent randomised trial in adults showed improved antibody titres and seroconversion from 60% to 80% against H1N1 influenza by BCG [46], while in the present study all seroconversions were above 96% even in the absence of BCG.

5. Conclusion

In conclusion, we did not find an overall effect of BCG vaccination on antibody levels after pentavalent vaccines given during the first year of life. Age at BCG vaccination may be a modifying factor, as BCG vaccination 2–7 days after birth might have an enhancing effect on antibody responses. This would be important to assess in future studies of possible non-specific effects of BCG on antibody responses. This study adds to a growing number of studies showing that girls mount higher antibody responses than boys.

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Conflict of interest

All authors have completed the Unified Competing Interest form at www.icmje.org/coi_disclosure.pdf (available on request from the corresponding author) and all authors declare to have no potential conflict of interest.

Authors' contribution

CB, LGS, OP and PA conceived the idea; TNN, OP, CB and FK designed the experiments. CB, LGS, DLJ, PEK, TH and OP supervised the collection of data in the overall Danish Calmette Study. JK, GP and LMT participated in the overall data collection of The Danish Calmette Study. TNN and NMB collected the data for this sub study. GS performed and FK supervised the Luminex analysis. TNN carried out data management, and analysed the data with help from MGN, CB, AA, OP. AA supervised the statistical analyses. TNN drafted the manuscript. All authors read and approved the final manuscript.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.vaccine.2017.02.048.

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