

# Nonspecific effect of BCG vaccination at birth on early childhood infections: a randomized, clinical multicenter trial

Jesper Kjærgaard<sup>1</sup>, Nina M. Birk<sup>2</sup>, Thomas N. Nissen<sup>2</sup>, Lisbeth M. Thøstesen<sup>3</sup>, Gitte T. Pihl<sup>3</sup>, Christine S. Benn<sup>4</sup>, Dorthe L. Jeppesen<sup>2</sup>, Ole Pryds<sup>2</sup>, Poul-Erik Kofoed<sup>3</sup>, Peter Aaby<sup>5</sup>, Gorm Greisen<sup>6</sup> and Lone G. Stensballe<sup>1</sup>

**BACKGROUND:** Childhood infections are common and Bacillus Calmette-Guérin (BCG) vaccination at birth may prevent these via nonspecific effects.

**METHODS:** A randomized, clinical multicenter trial. All women planning to give birth ( $n = 16,521$ ) at the three study sites were invited during the recruitment period. Participating children were randomized to receive BCG within 7 d of birth or to a no intervention control group. Parent-reported infections (events) were collected using telephone interviews at 3 and 13 mo. Data collectors were blinded to allocation.

**RESULTS:** The analyses included 4,224/4,262 (99%) and 4,192/4,262 (98%) children at 3 and 13 mo. From 0 to 3 mo, there were 291 events in the BCG group vs. 336 events in the control group, incidence rate ratio (IRR) = 0.87 (95% confidence interval (CI): 0.72 to 1.05). In this age group, the IRR was 0.62 (95% CI: 0.39 to 0.98) if the mother was BCG vaccinated. From 3 to 13 mo, there were 7,028 vs. 6,791 events, IRR = 1.02 (95% CI: 0.97 to 1.07).

**CONCLUSION:** This study did not find a nonspecific public health benefit of BCG on parent-reported infections. BCG may have reduced the incidence of infections in children of BCG-vaccinated mothers during the first 3 mo.

The Bacillus Calmette-Guérin (BCG) vaccine is used to protect against tuberculosis and is one of the most widely used vaccines in the world. WHO recommends BCG as part of the childhood vaccination program if tuberculosis is prevalent (1). There is mounting clinical evidence that BCG has a positive nonspecific effect on nontuberculous mortality (2–6) and infectious diseases (7) in low-income countries, as well as an ecological study pointing to a positive effect on hospitalizations in a high-income country (8), resulting in a public health benefit that may extend beyond protection against tuberculosis. Several immunological mechanisms that might explain these effects have been proposed (9–11). It has not previously been examined in a randomized trial whether BCG has public health benefits on nontuberculous infectious disease in

high-income countries. BCG is not part of the childhood vaccination program in Denmark. Therefore, it was possible to conduct The Danish Calmette Study—a randomized, clinical trial designed to examine whether BCG at birth could reduce the number of all-cause hospitalizations from birth to age 15 mo (12).

The present study reports on a prespecified secondary outcome of The Danish Calmette Study. The purpose was to examine if BCG at birth can reduce the incidence of parent-reported infectious diseases in the first year of life.

## RESULTS

Between October 2012 and November 2013, we enrolled and randomized 4,262 children, 2,129 to receive BCG and 2,133 to the control group. Randomization baseline characteristics have been presented elsewhere (13). Baseline characteristics were distributed evenly except non-Danish ethnicity and paternal smoking during pregnancy, both of which were more frequent in the control group. First follow-up was 99% (4,224/4,262) complete and second follow-up was 98% (4,192/4,262) complete. Table 1 summarizes follow-up characteristics. There were 627 infectious illness episodes and 2,173 GP visits from birth to first follow-up at 3 mo of age and 13,819 infectious illness episodes and 10,563 GP visits from first to second follow-up at 13 mo of age. Hence, the distribution of disease episodes and GP visits was significantly different in the first and the second period. In the youngest age group, there were 3.5 times more GP visits than episodes of infectious illness reported by the parents (2,173/627). In contrast, between 3 and 13 mo of age, there were 31% more infectious illness episodes than GP visits reported (13,819/10,563) ( $\chi^2 = 1,200$ ,  $P < 0.001$ ).

The exposure time was 11,151 mo from birth to first follow-up and 40,802 mo from first to second follow-up in the entire cohort. Significantly, more parents (46.3% = 973/2,102) in the BCG-group than in the control group (39.6% = 822/2,074) had used a diary to aid memory of episodes of illness ( $\chi^2 = 19.36$ ,  $P < 0.001$ ), but there was no statistically significant difference in the number of events reported by users of a diary compared

<sup>1</sup>The Department of Paediatrics and Adolescent Medicine, Juliane Marie Center, Rigshospitalet, Copenhagen University Hospital, Copenhagen, Denmark;

<sup>2</sup>Department of Paediatrics 460, Copenhagen University Hospital, Hvidovre, Denmark; <sup>3</sup>Department of Paediatrics, Kolding Hospital, University of Southern Denmark, Kolding, Denmark; <sup>4</sup>Research Center for Vitamins and Vaccines (CVIVA), Statens Serum Institut and OPEN, Institute of Clinical Research, University of Southern Denmark/Odense University Hospital, Copenhagen, Denmark; <sup>5</sup>Bandim Health Project, Statens Serum Institut, Copenhagen, Denmark; <sup>6</sup>The Neonatal Department, Juliane Marie Centret, Rigshospitalet, Copenhagen University Hospital, Copenhagen, Denmark. Correspondence: Jesper Kjærgaard (Jesper.Kjaergaard@regionh.dk)

Received 2 March 2016; accepted 17 May 2016; advance online publication 17 August 2016. doi:10.1038/pr.2016.142

to nonusers (IRR: 0.95, 95% confidence interval (CI): 0.91 to 1.01,  $P = 0.08$ ). There were fewer visits to the GP reported by users of a diary compared to nonusers (IRR 0.90, 95% CI: 0.85 to 0.95,  $P = 0.001$ ).

**Table 1.** Characteristics at follow-up of 4,262 children in The Danish Calmette Study by allocation group

	BCG ( <i>n</i> = 2,129)	Control ( <i>n</i> = 2,133)	
Characteristics at follow-up			
Age at first follow-up ( <i>n</i> = 4,224), days	79 (7)	79 (6)	0.34 <sup>a</sup>
DTaP-IPV-Hib vaccination prior to first follow-up (unknown, <i>n</i> = 318)	150 (7.5)	187 (9.6)	0.03 <sup>b</sup>
Age at second follow-up ( <i>n</i> = 4,192), days	375 (9)	375 (10)	0.04 <sup>a</sup>
Child enrolled in day care at second follow-up (unknown, <i>n</i> = 75)	1,829 (86.2)	1,772 (85.3)	0.18 <sup>b</sup>
Parental use of notes to aid memory (unknown, <i>n</i> = 82)	974 (46.3)	822 (39.6)	<0.001 <sup>b</sup>

<sup>a</sup>Mann-Whitney *U*-test. <sup>b</sup>χ<sup>2</sup> test.

BCG, Bacillus Calmette-Guérin vaccine; DTaP-IPV-Hib, Diphtheria, tetanus, acellular pertussis, inactivated polio, *H. influenza* combined vaccine; GA, gestational age; IQR, interquartile range.

## Main Outcomes

There was no statistically significant difference in the number of infectious illnesses from birth to 3 mo or from 3 to 13 mo (Table 2). The effect did not differ significantly between first and second follow-up (test for no interaction;  $P = 0.09$ ).

## Secondary Outcomes and Subgroups

Comparing children randomized to BCG with control children, there was a difference in the number of visits to the GP at first follow-up at 3 mo of age, the IRR being 0.88 (95% CI: 0.79 to 0.98), but not at the second follow-up at 13 mo of age (IRR = 1.03, 95% CI: 0.97 to 1.09). Hence, the effect of BCG differed significantly between the two age groups (test for no interaction:  $P = 0.01$ ). There was no statistically significant effect of BCG on the number of episodes in the different infectious disease categories (Table 3). There were no differences in the BCG effect between premature and mature children or between boys and girls (Figure 1 and Supplementary Table S1 online). In the analysis stratified by maternal BCG status, BCG was associated with reduced number of infectious illness episodes from 0 to 3 mo if the mother was BCG vaccinated, IRR = 0.62 (95% CI: 0.39 to 0.98), corresponding to a number needed to vaccinate of 14, but the effect was not seen at 13-mo follow-up (test

**Table 2.** The effect of BCG vaccination on parent-reported infectious illness<sup>a</sup> in children aged 3 and 13 mo

	BCG group ( <i>n</i> = 2,129)		Control group ( <i>n</i> = 2,133)		IRR <sup>b</sup> (95% CI), BCG vs. control
	<i>n</i>	Rate (events/months)	<i>n</i>	Rate (events/months)	
Episodes of infectious illness from birth to 3 mo <sup>c</sup>	2,113	0.05 (291/5,580)	2,099	0.06 (336/5,571)	0.87 (0.72 to 1.05)
Episodes of infectious illness from 3 to 13 mo <sup>d</sup>	2,104	0.34 (7,028/20,526)	2,072	0.33 (6,791/20,276)	1.02 (0.97 to 1.07)

<sup>a</sup>Parent-reported episodes of cold, pneumonia, fever, otitis media and diarrhea. <sup>b</sup>Incidence rate ratio; negative binomial regression adjusted for prematurity with months at risk as exposure. <sup>c</sup>Missing (*n* = 50): no follow-up (*n* = 38), no data on total number of parent reported infectious illness episodes (*n* = 12). <sup>d</sup>Missing (*n* = 86): no follow-up (*n* = 70), no data on total number of parent reported infectious illness episodes (*n* = 12), no data on time from first to second follow-up (*n* = 4).

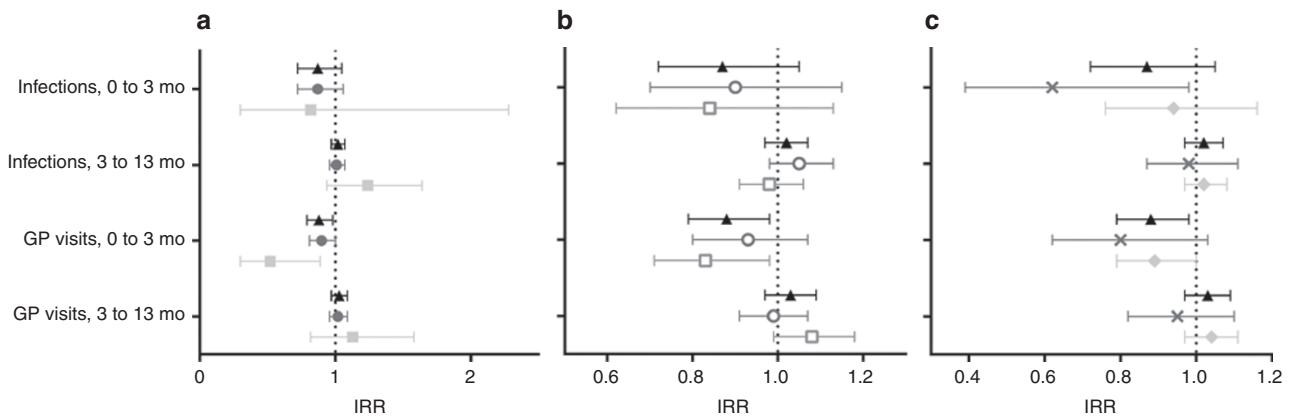
BCG, Bacillus Calmette-Guérin vaccine; IRR, incidence rate ratio. 95% CI, 95% confidence interval.

**Table 3.** The effect of BCG vaccination at birth on parent-reported types of infectious illness and visits to general practitioner

	<i>n</i> (missing)	BCG group ( <i>n</i> = 2,129) rate (events/months)	Control group ( <i>n</i> = 2,133) rate (events/months)	IRR <sup>a</sup> (95% CI) BCG vs. control	<i>P</i> value	Holland- corrected <i>P</i> -value
Three-month follow-up, <i>n</i> = 4,224						
Cold	4,209 (15)	0.029 (163/5,571)	0.032 (180/5,571)	0.91 (0.71 to 1.16)	0.46	0.96
Pneumonia	4,212 (12)	0.001 (5/5,580)	0.002 (10/5,571)	0.50 (0.17 to 1.46)	0.20	0.87
Fever	4,211 (13)	0.012 (66/5,580)	0.016 (91/5,568)	0.73 (0.52 to 1.03)	0.07	0.52
Diarrhea	4,212 (12)	0.010 (53/5,580)	0.008 (47/5,571)	1.13 (0.75 to 1.70)	0.56	0.96
AOM	4,212 (12)	0.001 (4/5,580)	0.001 (8/5,571)	0.50 (0.15 to 1.66)	0.26	0.91
GP visits	4,205 (18)	0.183 (1,017/5,569)	0.208 (1,156/5,563)	0.88 (0.79 to 0.98)	0.02	0.22
Thirteen-month follow-up, <i>n</i> = 4,192						
Cold	4,167 (25)	0.196 (3,990/20,407)	0.195 (3,928/20,186)	1.00 (0.94 to 1.07)	0.87	0.98
Pneumonia	4,170 (22)	0.010 (207/20,447)	0.008 (162/20,177)	1.26 (0.99 to 1.60)	0.06	0.52
Fever	4,167 (25)	0.068 (1,385/20,428)	0.065 (1,302/20,167)	1.05 (0.95 to 1.16)	0.33	0.94
Diarrhea	4,174 (18)	0.042 (870/20,476)	0.042 (845/20,186)	1.02 (0.92 to 1.13)	0.75	0.98
AOM	4,170 (22)	0.029 (594/20,430)	0.029 (591/20,197)	0.99 (0.83 to 1.19)	0.94	0.98
GP visits	4,165 (27)	0.263 (5,384/20,438)	0.257 (5,179/20,136)	1.03 (0.97 to 1.09)	0.41	0.96

<sup>a</sup>Incidence rate ratio; negative binomial regression adjusted for prematurity, age in days at 3 mo interview and age in days between 3 and 13 mo interview as exposure time.

AOM, acute otitis media; BCG, Bacillus Calmette-Guérin vaccine; GP, general practitioner.



**Figure 1.** Effect of neonatal BCG on parent-reported infections and visits to the general practitioner from birth to 13 mo in subgroups. (a) Effect of neonatal BCG by prematurity. (b) Effect of neonatal BCG by child sex. (c) Effect of neonatal BCG by maternal BCG vaccination status. ▲: All, ● Term children (GA  $\geq$  37 wk), ■ Premature children (GA < 37 wk), ○ Boys, □ Girls, × Maternal BCG vaccination, ♦ No maternal BCG vaccination. BCG, Bacille Calmette-Guérin vaccine; GA, gestational age; GP, general Practitioner; IRR, incidence rate ratio.

for no interaction:  $P = 0.03$ ) (Figure 1 and Supplementary Table S1 online).

### Sensitivity Analyses

Sensitivity analyses excluding those children that were followed-up later than specified in the standard operating procedure at first and second follow-up, those that did not follow the allocation (per-protocol analysis) and those that received DTaP-IPV-Hib prior to first follow-up did not change the estimates significantly. Statistical analyses that accounted for twins ( $n = 152$  children) and triplets ( $n = 3$  children) did not change the estimates. Adjustment for the use of infectious illness-diary did not change the estimates (Supplementary Table S2 online), nor did a *post-hoc* analysis restricted to children where the parents had used an infectious disease diary (data not shown).

### DISCUSSION

BCG given at birth does not seem to reduce the number of parent reported episodes of infectious illness from birth to 13 mo of age in a high-income country. Consistent with what has been seen for hospital admissions among the children participating in the Danish Calmette Study where we observed a protective effect of BCG on hospitalization for infection from birth to 15 mo if the mother was BCG vaccinated too, a stratified analysis revealed an effect from 0 to 3 mo if the mother of the child was BCG vaccinated (IRR = 0.62).

This randomized trial is large, with almost complete follow-up and enough events to detect an effect on the primary outcome. Data collection in this study covered a subset of the most common childhood infectious diseases and some diseases were narrowly defined (such as diarrhea only being recorded if there was coincident loose stools and vomiting) and the investigators were blinded during data collection.

Our study has limitations, a major one being that parents could not be blinded. Some parents were disappointed when they were randomized to the control group (14). Therefore, we suspect lower attachment to the study data collection in this

group, possibly resulting in under-reporting of events, thus attenuating the positive effect of BCG seen at first follow-up. However, adjusting the analysis for use of a diary did not modify the results (Supplementary Table S2 online). Furthermore, we asked the parents for number of episodes and thus we cannot establish whether BCG has an effect on the length of individual episodes. We observed a high incidence of visits to the GP compared to the actual number of reported infectious illness episodes, particularly so at the first follow-up. This may be explained by our choice of diseases and definitions of these possibly combined with a low threshold for parents of newborns to consult a GP in a country where health care is supplied free of charge and is readily available. Thus, visits to the GP may reflect parental concern and easy access more than severity of illness and actual need for treatment. However, the two measures of morbidity—infectious disease episodes and GP visits—gave essentially identical estimates for the effect of BCG in both age groups.

The Danish Calmette Study is the first major trial studying nonspecific effects of BCG in a high-income country. Previously, in Guinea-Bissau, BCG has been shown to have a positive nonspecific effect most pronounced during the first months after vaccination in low-birth-weight children (2), and it has also been shown that nonspecific effects of vaccines can be modulated by subsequent vaccines (2,15). Several clinical studies have suggested a transgenerational effect of maternal exposure to BCG or *Mycobacterium tuberculosis* that modulates the immune response to BCG in the newborn (16,17). In Guinea-Bissau, where the positive nonspecific effect of BCG has been most consistently found (2–6), BCG has been part of the childhood vaccination program for decades, thus most of the mothers in those studies would be BCG vaccinated or exposed to mycobacteria.

This study did not find a decreased risk of infectious illness in the first year of life among BCG-vaccinated children, but we did find a 38% reduction in the number of infectious illnesses up to 3 mo of age if the mother had received BCG (Figure 1 and Supplementary Table S1 online), corresponding to a number needed to treat of 14. This is consistent with

the another secondary outcome of The Danish Calmette Study, where fewer hospitalizations for infection were found if the mother was BCG vaccinated (13). If maternal BCG vaccination is a prerequisite for a nonspecific effect of BCG, a possible explanation for the lack of overall effect in this study could be that only 17.7% (740/4,184) of the mothers were BCG vaccinated.

This study does not support using BCG to prevent infectious illnesses in a high-income setting, even though our data indicates that there could be a positive effect if the mothers are covered by BCG. The beneficial effect disappeared after 3 mo of follow-up, possibly being offset by the vaccines given at age 3 mo and onwards. Hence, the present study may support previous studies in indicating that the sequence of vaccines is important for morbidity patterns (2,15,18). In the present trial, BCG was administered alone versus no vaccine. A randomized trial in Guinea-Bissau documented that oral polio vaccine coadministered with BCG versus BCG only had an additional beneficial nonspecific effect on infant survival (19), suggesting that the addition of other vaccines may affect the overall effect of BCG. Observational studies have also suggested that coadministration of BCG and inactivated diphtheria-tetanus-pertussis vaccine had marked beneficial nonspecific effects compared to receiving inactivated diphtheria-tetanus-pertussis vaccine after BCG (20,21). Hence, the present study may not predict what would happen in a high-income setting where another vaccine, e.g., hepatitis B vaccine, was also administered at birth.

Thus, in line with previous research on materno-fetal immunity to mycobacteria (16,17), The Danish Calmette Study indicates a positive nonspecific effect of BCG in infancy on number of infectious disease episodes and hospitalizations for infectious diseases among children whose mother was also BCG-vaccinated. It would be interesting to pursue if the nonspecific effect described in low-income settings is dependent on the BCG vaccination status of the mother and whether previous exposure to tuberculosis may similarly provide priming which enhances the benefit of BCG for the offspring. In any event, these observations, as well as previous observations on the benefit of being measles vaccinated in the presence of maternal antibodies (22), suggest that maternal immunity may influence the child's response to vaccines. This could mean that maternal immunity has a larger role in priming the immune system of the newborns than has been previously recognized.

## Conclusion

This study found no overall beneficial impact of BCG vaccination on morbidity up to 13 mo of age. Hence, there is no support for the use of BCG to reduce the burden of infectious diseases in high-income settings in which the mothers have not had BCG themselves.

## METHODS

The methods of The Danish Calmette Study are described in detail elsewhere (12). In brief, pregnant women planning to give birth at three Danish university hospitals from October 2012 to November 2013 received a letter in the second or third trimester with an invitation to

participate in the study. Study staff telephoned the women to answer potential questions about the study and conduct a baseline interview with those who wished to participate. Within 7 d of birth, study staff randomized children to receive BCG (SSI Danish strain 1,331) at the standard dose of 0.05 ml intradermally on the left shoulder or to a no-intervention control group. Parents were not blinded to allocation since the local inflammatory reaction caused by BCG vaccination could not be mimicked. Randomization was stratified by prematurity (gestational age (GA) < or ≥ 37 wk) and children were allocated 1:1 in permuting blocks of 2-4-6 using an online system. Exclusion criteria were GA < 32 wk, birth weight < 1,000 g, critically ill child, no Danish-speaking parent, known immune deficiency, or maternal use of immune modulating drugs during pregnancy.

## Follow-up for This Study

Following randomization, parents were asked to take notes of their child's illness and were given a diary for this purpose. Follow-up at age 3 and 13 mo was chosen because previous studies have indicated that the nonspecific effects of BCG is altered by other vaccines (20). In Denmark, the first vaccines in the childhood vaccination program are diphtheria, tetanus, acellular pertussis, polio, *Haemophilus influenzae* type b (DTaP-IPV-Hib), and *Streptococcus pneumoniae* conjugate (PCV) vaccine. The two vaccines are given at 3, 5, and 12 mo. Therefore, follow-up consisted of a period from birth to 3 mo of age in which the only vaccine given was BCG and a period from 3 to 13 mo where the children followed the vaccination program. Follow-up consisted of a structured telephone interview. Parents were asked at the beginning of the interview not to disclose if the child had received BCG to keep study staff blinded during data collection. At 3-mo follow-up, study staff asked parents the following questions: "How many visits have you had at your family doctor or at a doctor on call (collectively termed general practitioner (GP)) with your child due to suspected infectious illness?", "How many episodes of the common cold has your child had?" (only episodes where the parents suspected fever were recorded), "How many pneumonias has your child had?" (only pneumonias that were diagnosed by a doctor were recorded), "How many episodes of fever with no apparent cause has your child had?" (only episodes with a temperature > 38 °C were recorded), "How many episodes of diarrhea has your child had?" (only episodes with coincident loose stools and vomiting were recorded), "How many episodes of acute otitis media has your child had?" (only episodes with pus flowing from the ear were recorded). At the 13-mo follow-up, the same questions were asked, adding "since the last interview" and a supplementary question was asked: "Have you taken notes of your child's illness to aid your memory?"

The number of individual infectious illness episodes was summed as the total number of infectious illness episodes at 3 and 13 mo. BCG vaccination status of the mother was self-reported at the baseline interview.

## Outcomes

The primary outcome of the present study was total number of infectious illness episodes from 0 to 3 mo and from 3 to 13 mo. Secondary outcomes were number of visits to the GP and number of episodes of each type of infectious diseases. Subgroup analyses of child sex and prematurity were planned because previous studies have suggested differential effects of BCG in these groups (2,22). We also prespecified a subgroup analysis of maternal BCG vaccination status due to a previous study concluding that nonspecific effects of the measles vaccine are more pronounced when given in the presence of maternal antibodies (23). All outcomes and analyses were prespecified in analysis plans, which were deposited with the data and safety monitoring board prior to unblinding of the data, except if otherwise stated.

## Sample Size, Data Management, and Statistics

Sample size was determined by the primary outcome of The Danish Calmette Study—number of all-cause hospitalizations. With 3,972 children, a 20% reduction in all-cause hospitalization by 15 mo of age could be found with a power of 90% and a significance level of 0.05. We aimed to include 4,300 children. A large study of children's health in Denmark showed that 18.5% of infants in Denmark have had an episode of infectious illness within the previous 14 d and 7.3%



of parents have contacted their GP concerning their infant within the previous 14 d (24). Based on these figures, this study would be able to detect an incidence rate ratio (IRR) of 0.78 for parent-reported infectious illnesses with a power of 94% and an IRR of 0.68 for contacts to the GP with a power of 88% with a sample size of 4,300.

The standard operating procedure for the follow-up stated that it should be conducted between age 2 and 4 mo for the 3-mo follow-up and prior to 14 mo for the 13-mo follow-up. For children followed-up after the specified time slots, only episodes of illness that occurred up to the age of 4 mo (123 d) and up to the age of 14 mo (428 d) were recorded. In sensitivity analyses, children were excluded if follow-up did not take place within the timeframe specified, as were children who had received a DTaP-IPV-Hib and PCV vaccine prior to the 3-mo follow-up. Data were entered directly into an electronic case report form.

Secondary outcomes of the present study were subject to Holland correction (25), using the number of secondary outcome analyses as correction number. The number of infectious illnesses was analyzed using negative binomial regression. The number of months at risk from birth to first follow-up and from first to second follow-up was used as exposure time; results are presented as incidence rate ratios (IRR) with 95% CI. Number needed to treat was calculated as 1 divided by the absolute risk difference at follow-up. Ordered, non-normally distributed data were analyzed using Mann-Whitney *U* test and categorical data were analyzed using  $\chi^2$ . All analyses were performed using STATA 13.1 (StataCorp, College Station, TX).

### Ethics

The Calmette Study was approved by the Committees on 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) and was registered at [www.clinicaltrials.gov](http://www.clinicaltrials.gov) with trial registration number NCT01694108. The trial was supervised by the Good Clinical Practice Units of the Capital Region and the Region of Southern Denmark. The study was monitored by an independent data and safety monitoring board. All parents gave written informed consent prior to randomization.

### SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at <http://www.nature.com/pr>

### ACKNOWLEDGMENTS

We would like to thank all the study staff at the three study sites and the participating families in particular.

### STATEMENT OF FINANCIAL SUPPORT

This work was supported by Copenhagen University Hospital Rigshospitalet; Copenhagen University Hospital Hvidovre; Lillebaelt Hospital – Kolding Sygehus and by the Danish National Research Foundation (DNRF108). J.K. received grants from Rigshospitalets Forskningsfond; Dagmar Marshall fonden; Else og Mogens Wedell Wedellsborgs fond and Christian Larsen og Dommer Ellen Larsens Fond. The funders had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; and preparation, review, or approval of the manuscript.

Disclosure: None of the authors have any financial ties to the products used in this study. Vaccines were manufactured by Statens Serum Institute but were bought at regular market price for the study. Statens Serum Institute had no role in funding, conducting, or reporting the study.

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