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Causes of variation in BCG vaccine efficacy: Examining evidence from the BCG REVAC cluster randomized trial to explore the masking and the blocking hypotheses☆

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ABSTRACT

BCG protection varies and in some places (nearest the equator) is low or absent. Understanding this variation can inform the efforts to develop new vaccines against tuberculosis. Two main hypotheses are used to explain this variation: under masking, new vaccines are unlikely to increase protection; under blocking new vaccines have a greater potential to be effective when BCG is not. We conducted a cluster randomized trial to explored the masking and blocking hypotheses by studying BCG vaccine efficacy of neonatal vaccination and when administered for the first or a second (revaccination) time at school age in two sites (Manaus close and Salvador further south from the equator). Seven hundred and sixty three state schools were matched on socio economic characteristics of the neighborhood and 239,934 children were randomized to vaccine (BCG vaccination at school age) or control group. Protection by first BCG vaccination at school age was high in Salvador (34%, 95% CI 7–53%, $p = 0.017$) but low in Manaus (8%, 95% CI t_0 39–40%, $p = 0.686$). For revaccination at school age, protection was modest in Salvador (19%, 95% CI 3–33%, $p = 0.022$) and absent in Manaus (1%, 95% CI to 27–23%, $p = 0.932$). Vaccine efficacy for neonatal vaccination was similar in Salvador (40%, 95% CI 22–54%, $p < 0.001$) and Manaus (36%, 95% CI 11–53%, $p = 0.008$). Variation in BCG efficacy was marked when vaccine was given at school age but absent at birth, which points towards blocking as the dominant mechanism. New tuberculosis vaccines that overcome or by pass this blocking effect could confer protection in situations where BCG is not protective.

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1. Introduction

Tuberculosis (TB) causes an estimated 1.7 million deaths a year [1]. BCG (Bacillus Calmette-Guerin), a live vaccine derived from *Mycobacterium bovis*, is the only licensed vaccine against TB [2]. BCG provides consistently high protection against TB meningitis and miliary disease when given in infancy [3]. However, protection against pulmonary TB in adolescents and adults, the majority of TB cases, is highly variable ranging from 0% to 80% [3] and tends to be lowest in areas closer to the equator [4].

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Development of a new vaccine against TB is an international research priority. A suitable candidate should offer good protection in situations where BCG is not protective while also providing protection in situations where BCG is protective. Currently, dozens of vaccine candidates are at various stages of development and numerous candidates have entered clinical trials [5]. Understanding the reason for the variation in BCG efficacy is important for the development of effective new vaccines and selection of appropriate vaccination schedules [6,7]. A promising vaccine candidate, MVA85A, has recently been evaluated in BCG-vaccinated healthy infants aged 4–6 month, in the first double-blinded, randomized, placebo-controlled phase 2b trial of a new tuberculosis vaccine. The trial found no protection [8] and led to a lively discussion as to the reasons and implications for this lack of protection [8–10]. Of particular timeliness, a better knowledge of the mechanism that causes variation in BCG vaccine efficacy might help to explain why MVA85A failed to add to BCG effectiveness in its first trial [8,9].

The variation in BCG-induced protection cannot be explained by differences in the vaccines strains used or by ethnicity [11,12]. In fact, different BCG vaccines consistently provide efficient protection from TB in laboratory raised, immunological naive animals [13]. The traditional interpretation is that BCG confers high protection against miliary and meningeal disease but variable protection against pulmonary forms. An alternative interpretation is that BCG protection is consistently high when given at birth, and variable when given at school age – most studies of protection against meningitis were conducted in settings where BCG is given at birth [3,14] and the largest public health trials with long follow-up periods showing low protection for pulmonary disease excluded infants [15,16].

There has been much debate on what is behind the variation of BCG-induced protection when given later in childhood and why it seems to depend on latitude. For some time the favourite explanation was that exposure to non-tuberculosis mycobacteriae (NTM) affects the BCG-specific immune response of the host through cross reactivity with BCG and a degree of variation in prevalence with latitude. As we learn more about NTM, this explanation has become more problematic and most of the previous assumptions behind that explanation had to be revised. There are hundreds of NTM and it would no longer be accurate to say that prevalence of NTM decreases with latitude as different NTM have different geographical distributions [21]. Also, the cross reactivity of different NTM with BCG varies [17], as does their effect on in animal models [18–20]. So, although NTMs may still be part of the explanation, it is clear that the picture is more complex than previously assumed. In addition, variation in BCG efficacy is much less marked when BCG is given at birth and more marked, or possibly only present when BCG is given later in life. Under this perspective, the geographical variation could be a consequence of an unknown immunological effect that varies with latitude and with the age at vaccination. Let us call this environmentally dependent mechanism behind the variation in BCG protection "environmental sensitization" (ES) [22].

Two potential explanations of how "ES" leads to the geographical variation in BCG-induced protection have been postulated – masking and blocking. Although these theories had been developed with NTM in mind, they are in fact explanations of how "ES" works, independent of what causes "ES". The masking hypothesis postulates that "ES" confers substantive protective immunity against TB [23]. Since BCG-induced protection is measured by comparing TB incidence in those with and without BCG vaccination, higher levels of "ES" in unvaccinated subjects result in lower measured BCG-induced protection. This is because "ES" in unvaccinated subjects induces a protection similar to BCG while BCG-induced protection remains high [4]. Masking further postulates that "ES" occurs at different rates in different environments, and as the proportion exposed to "ES" increases with age, the geographical heterogeneity in BCG-induced protection increases with time since vaccination. It is irrelevant if "ES" occurs before or after BCG vaccination. Under this scenario, a more effective TB vaccine must induce a protection greater than BCG and hence greater than the protection induced by "ES". Since in situations where BCG works well BCG induced protection can be as high as 80% [24], the additional protection conferred by such a new vaccine would only be marginal [22].

In contrast, the blocking hypothesis postulates that "ES" does not confer immunity against TB, but when "ES" occurs before BCG vaccination, it prevents BCG from providing protection [4,22]. It has been suggested that "ES" prevents the multiplication of the BCG vaccine [25] or primes the host immune system against mycobacterial antigens shared with BCG leading to an accelerated clearance of BCG [19]. In reality, our understanding of the modulatory effect of "ES" on the protective efficacy of BCG is very limited. Nevertheless, under this hypothesis a new vaccine would not have to be more efficacious than BCG but only to overcome or by pass the suppressive immunological blocking effect of previous "ES" [18].

The masking and blocking hypotheses make predictions that can be empirically tested. When planning a trial to evaluate the protection of BCG revaccination (BCG-REVAC trial) [26], we chose two distinct environments: Manaus, by the Amazon River and close to the equator; and Salvador, by the Atlantic Ocean and further south from the equator, at a higher latitude. This paper explores the masking and blocking hypothesis by comparing neonatal BCG-VE in the two cities to BCG-VE when administered at school age for the first time [27] or as a revaccination [26,28]. Under blocking, the protection of neonatal BCG (given before "ES") would be similar in Salvador and Manaus, and remain similar with time; under masking, neonatal BCG would be more protective in Salvador than in Manaus, as in Manaus a higher proportion of individuals would have acquired protection from "ES" with time from vaccination to age of diagnosis.

2. Methods

We conducted a cluster-randomized trial involving more than 200,000 school-aged children from Salvador and Manaus (BCG-REVAC). The trial tested BCG-VE of a second BCG dose when administered at school age. The trial also allowed studying BCG-VE of a single dose BCG vaccination when administered to individuals at different age groups who reside in different ecological settings. Salvador and Manaus were chosen to explore the effect of different environments on BCG-VE. A full description of the study design [26,29], validity of scar reading as a measure of previous BCG vaccination [30,31], parallel immunological studies [32,33], and the frequency of adverse events [34] were presented elsewhere. The trial has ethical committee approval from the Universidade Federal da Bahia, Brazil, and the London School of Hygiene and Tropical Medicine. Parents wishing to withdraw their child from the trial could sign an "opt out" form.

The cities were chosen in part to test the hypothesis of whether in the long term the protective effect of vaccination differs between the two sites. Salvador was the primary site. The second site, Manaus, was chosen because it is environmentally very different from Salvador with a presumably higher degree of "ES" and to estimate BCG revaccination efficacy against leprosy, prevalent in Manaus [35]. Manaus is situated at a latitude of 03°06′07″ south, in the Amazon Region, with a humid equatorial climate with annual mean humidity around 80% and a mean temperature of 27 °C, and often surpassing 35 °C during the summer. Salvador, situated at a latitude of 12°58′16″ south and east from Manaus, is on the Atlantic coast with a hot tropical climate. The mean annual humidity is close to Manaus; the mean temperature is 25.5 °C, relatively stable around the year.

Table 1
Characteristics of populations for school age (first and second dose) and neonatal BCG vaccination, separately for Salvador and Manaus.

	I1: Second dose, school age (first dose neonatal)	I2: First dose, school age	C1: Firstdose, neonatal	C2: No dose (controls)
Salvador (low level of environmental sensitization)				
Age at randomization, mean (SD)	11.6 (2.1)	12.0 (2.1)	11.4 (2.1)	11.7 (2.1)
Sex (% males)	48.3	45.8	48.5	47.1
% of children from families with monthly income				
0–25% below minimum wage	3.1	2.1	3.5	2.7
26–50%below minimum wage	10.5	8.9	12.0	10.3
51–75%below minimum wage	34.9	29.0	29.1	30.4
>75% below minimum wage	51.5	60.0	55.4	56.6
Manaus (high level of environmental sensitization)				
Age at randomization, mean (SD)	11.2 (2.0)	11.8 (2.0)	11.3 (2.1)	11.9 (2.0)
Sex (% Males)	48.9	47.0	48.9	47.2
% of children from areas with				
low tuberculosis incidence (≤110/100,000)	43.8	42.8	35.6	35.2
high tuberculosis incidence (>110/100,000)	56.2	57.2	64.4	64.8
% of children from areas with				
Low leprosy incidence (≤6/10,000)	26.4	26.0	29.1	27.9
high leprosy incidence (>6/10,000)	73.6	74.0	70.9	72.1

We classified 763 schools into strata according to TB and leprosy incidence (Manaus) and income (Salvador) (Table 1). The two stratification criteria were chosen as leprosy incidence data was not available in Salvador but income was found to be associated with TB rates [29]. In each stratum pairs of schools were randomly allocated to intervention or control group (Fig. 1). The study population consisted of school children aged 7–14 years at entry and all children were visited at school to confirm their identification details. School age vaccination status was obtained from school and vaccination records and neonatal vaccination status was confirmed by BCG scar reading. Recruitment into the trial was done during 1996 and 1997 in Salvador and during 1998 in Manaus. Follow-up started in August 1997 in Salvador and January 1999 in Manaus.

Neither allocation nor intervention was concealed, i.e., the study was not double blinded. Children from the intervention group were intradermaly vaccinated at schools with the Moreaux strain (Fundação Ataulpho de Paiva, Rio de Janeiro), which is highly protective against tuberculous meningitis when given at birth [36–38]. Following recommendations by WHO [39] and the Brazilian Tuberculosis Programme (TCP) [40], children in the trial were not tuberculin tested prior to BCG vaccination.

TB cases were ascertained passively through the TCP. All cases diagnosed and treated are registered in the TCP as only the TCP can release medication for TB treatment. Cases in the first follow-up were validated independently by two senior academic chest physicians. We linked cases, blinded to vaccination status, to the study population.

The study windows were synchronized to a 9 years follow-up for both cities; until July 2006 in Salvador and until December 2007 in Manaus; this period started at the study implementation and therefore did not include incidence in the first years of life for the neonatal study. We estimated the protective effect of revaccination, vaccination at school age and neonatal vaccination separately for each city. To compensate for the loss in statistical power due to this stratification, we are presenting effect estimates for pulmonary and non-pulmonary forms combined.

2.1. Neonatal vaccination

BCG-VE was estimated in the 115,594 children in the control arm (C1+ C2) (Fig. 1), by comparing incidence of TB in 97,087 children with a BCG scar (C1) to the incidence in 18,507 children without a BCG scar (C2), during the 9-year follow-up. Since children were vaccinated at birth and recruited into the study aged 7–14 years, this is BCG-VE of neonatal BCG from 7 to 23 years after vaccination. An earlier estimate based on a shorter follow-up was published [41].

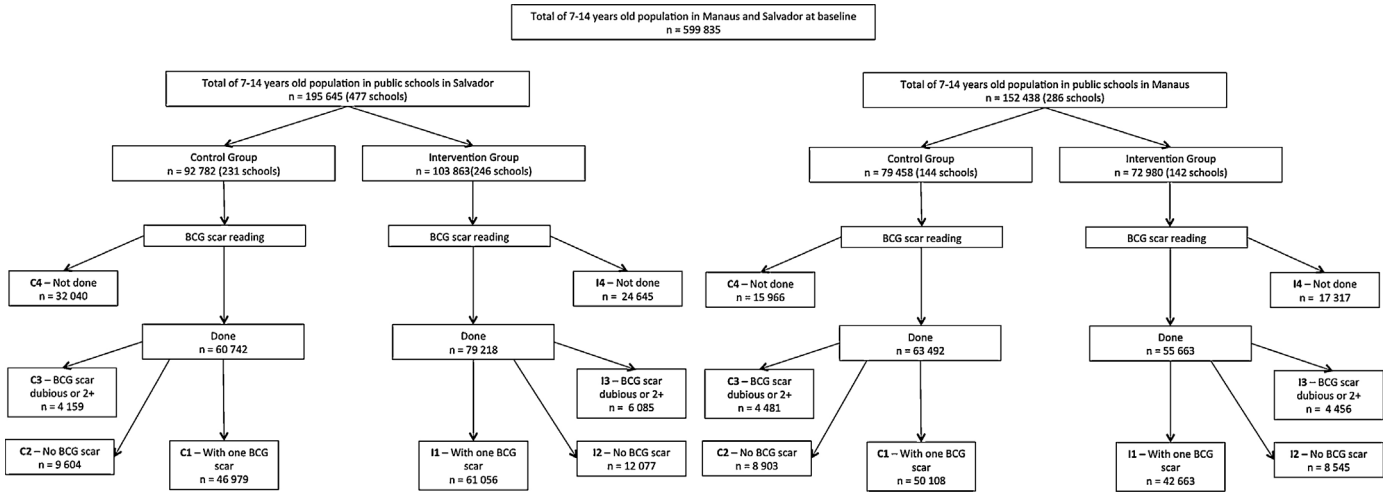

Fig. 1. Trial profile.

Table 2
BCG-VE from TB for school age (first and second dose) and neonatal vaccination, separately for Salvador and Manaus.

	Vaccinated	Cases in vaccinated	Crude incidence rate per 100,000 person years in vaccinated (95% CI)	Not vaccinated	Cases in not vaccinated	Crude incidence rate per 100,000 person in not vaccinated years (95% CI)	VE (95% CI)	*p* value
First dose, neonatal								
Salvador	46,762	217	5.13 (4.49–5.86)	9530	74	8.56 (6.81–10.75)	40% (22–54%)	<0.001
Manaus	49,938	170	3.77 (3.24–4.38)	8856	47	5.86 (4.41–7.81)	36% (11–53%)	0.008
First dose, school age								
Salvador	12,015	62	5.71 (4.44–7.31)	9530	74	8.56 (6.81–10.75)	34% (7–53%)	0.017
Manaus	8505	40	5.21 (3.81–7.09)	8856	47	5.86 (4.41–7.81)	8% (−39 to 40%)	0.686
Second doses, school age (first dose neonatal)								
Salvador	60,820	236	4.29 (3.78–4.88)	46,762	217	5.13 (4.49–5.86)	19% (3–33%)	0.022
Manaus	42,520	142	3.69 (3.14–4.36)	49,520	170	3.77 (3.25–4.38)	1% (−27 to 23%)	0.932

Estimate of vaccine efficacy (VE) adjusted for age, sex, socio-economic status (in Salvador), incidence of leprosy and incidence of tuberculosis before the start of the trial in Manaus.

2.2. First dose at school age

BCG-VE was estimated as an intervention study. The study population was the 39,129 children in the trial who did not have a BCG scar (C2+ I2). We compared the incidence of TB in the 18,507 children without a BCG scar who were allocated to the unvaccinated control group (C2) with the incidence in the 20,622 children without a BCG scar who were allocated to the intervention group (I2) and who received a first BCG vaccination at school age. Results were published [27].

2.3. Second dose at school age

BCG-VE for revaccination, compared with a single dose at birth, was estimated as an intervention study. The study population consisted of 200,805 children in the trial who had a BCG scar (C1+ I1). We estimated BCG-VE of revaccination by comparing TB incidence in 103,718 children who had one BCG scar from neonatal vaccination and who received a second dose at school age (I1), with the TB incidence in 97,087 children who had only one BCG scar from neonatal BCG (C1). Results were published [26,28].

Since follow-up was passive, we estimated person-years at risk for the study children assuming that participants remained in the study area until the end of the ascertainment period. For the calculation of the incidence rate, we used a generalized-estimation-equations (GEE) method suitable for overdispersed Poisson data [42]. The analyses were stratified by city and accounted for the clustered nature of the data. Additionally, we adjusted for characteristics at the cluster level (socio-economic condition, past incidence of TB and leprosy) and at the individual level (sex, age at vaccination, and age at diagnosis). Age at diagnosis was modelled as a time-dependent variable in 5 categories (up to 10, 11–12, 13–14, 15–16, 17 years and more). Interaction terms were calculated to estimate the effect of city on vaccine efficacy. BCG-VE was estimated as 1- (TB rate in vaccinated/TB rate in unvaccinated) × 100. We used the statistical software package STATA (version 11.1, STATA Corporation, College Station, TX, USA) for all analyses.

3. Role of the funding source

The trial was funded by grants from the Department of International Development, UK (DFID) and the National Health Foundation, Brazil (FUNASA). No funding bodies had any role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

4. Results

Table 1 shows the characteristics of the study population. At baseline, the distribution of age, sex and socioeconomic indicators was similar in both groups in both cities (Table 1). Table 2 shows BCG-induced protection against TB over the 9-year follow-up period, stratified by city and age of vaccination.

4.1. Neonatal vaccination

In Salvador, the crude TB incidence rate per 100,000 was 8.56 (95% CI 6.81–10.75) in the control group and 5.13 (95% CI 4.49–5.86) in the intervention group. In Manaus, the crude TB incidence rate per 100,000 was 5.86 (95% CI 4.41–7.81) in the control group and 3.77 (95% CI 3.24–4.38) in the intervention group. BCG-VE – 7–23 years after neonatal BCG vaccination – was similar in Salvador (40%, 95% CI 22–54%, $p < 0.001$) and Manaus (36%, 95% CI 11–53%, $p = 0.008$) (Table 2) with no evidence for a difference between the two cities (interaction term 1.07, 95% CI 0.77–1.61, $p = 0.751$).

4.2. First dose school age

In Salvador, the crude TB incidence rate per 100,000 was 8.56 (95% CI 6.81–10.75) in the control group and 5.71 (95% CI 4.44–7.31) in the intervention group. In Manaus, the crude TB incidence rate per 100,000 was 5.86 (95% CI 4.41–7.81) in the control group and 5.21 (95% CI 3.81–7.09) in the intervention group. In contrast to neonatal vaccination, BCG-VE for a first BCG dose given at school age and measured on average 9 years after vaccination, was higher in Salvador (34%, 95% CI 7–53%, $p = 0.017$) than in Manaus (8%, 95% CI −39 to 40%, $p = 0.686$) (Table 2) (interaction term 1.33, 95% CI 0.77–2.23, $p = 0.313$).

4.3. Second dose at school age

In Salvador, the crude TB incidence rate per 100,000 was 5.13 (95% CI 4.49–5.86) in the control group and 4.29 (95% CI 3.78–4.88) in the intervention group. In Manaus, the crude TB incidence rate per 100,000 was 3.77 (95% CI 3.25–4.38) in the control group and 3.69 (95% CI 3.14–4.36) in the intervention group. BCG-VE of a second BCG dose given at school age, after an average 9 of years after the first vaccination, was modest in Salvador (19%, 95% CI 3–33%, $p = 0.022$) and absent in Manaus (1%, 95% CI −27% to 23%, $p = 0.932$) (Table 2) (interaction term 1.19, 95% CI 0.87–1.63, $p = 0.262$).

5. Discussion

Our analyses show similar levels of neonatal BCG-VE in Salvador and Manaus. “ES” up to 23 years after vaccination in the first year of life did not lead to differences in protection between the two cities. Vaccination or revaccination at school age (after a proportion of subjects had the opportunity to be sensitized) conferred protection in Salvador but not in Manaus. These findings show that “ES”

occurred at different levels in Salvador as compared to Manaus, but only affected efficacy if the vaccine was not given in the first year of life blocking appears more plausible than masking as the dominant mechanism underlying this geographical variation in BCG-induced protection.

Bearing the limited statistical power in mind, a careful interpretation of our findings is possible. BCG-VE at school age when given as a first dose or as a second dose is higher in Salvador than in Manaus. This renders a chance finding less likely and points towards a variation in BCG-VE when BCG is given at school age between the two sites. In contrast, no difference in vaccine efficacy between Salvador and Manaus for neonatal vaccination was observed. Also, in Manaus, at almost the same number of cases, neonatal vaccination induced protection while school age vaccination did not. These observations are in line with neonatal vaccination (measured 7–23 years after vaccination) inducing similar levels of protection in Salvador and Manaus. The dual observation that BCG-VE depends on the study site and whether vaccination is administered prior to or post "ES" is consistent with the blocking mechanism. If masking had occurred, neonatal BCG-VE measured from 7 to 23 years after vaccination would also been much lower in Manaus than in Salvador.

The fact that our results are in line with previous studies adds to the plausibility of our findings. Our study is consistent with results from previous trials showing that vaccination later in life confers variable protection against pulmonary TB, with lower protection closer to the equator [24]. Protection induced by school age, vaccination or revaccination, was higher in Salvador (distant from the equator) than in Manaus (close to the equator). Protection induced by neonatal vaccination was similar in the two study sites but lower than in previous studies with shorter follow-up [14] suggesting that waning of protection had occurred at the same rate in both study populations.

This is the first study in a human population to provide field-derived evidence in favour of the blocking hypothesis. Most previous studies supporting the masking hypothesis had immunological outcomes or made use of animal models [23,43,44]. Although it is possible that masking could play a substantive role in other settings, in this extensive analysis of the BCG-REVAC trial, we do not find evidence supporting a major masking effect. While these findings need to be replicated, it adds to the existing evidence suggesting that in populations where BCG confers low protection, vaccine candidates are unlikely to substantially increase protection from TB if they do not overcome the possibly environmental-dependent blocking effect causing BCG-VE variation.

Masking has been suggested as an explanation for why the MVA86A vaccine, in addition to neonatal BCG, failed to increase BCG vaccine efficacy in young children [9]. Given the short time since birth and diagnosis, the results are more consistent with blocking. In this light it is worth noting that new vaccines, even when failing to increase protection in young children [8], could still be important for the prevention of tuberculosis in young adults [9]. Longer follow-up periods are needed to explore the full potential of such new vaccines and vaccination outside of infancy should be considered. In fact, school-age vaccination can be a cost-effective means to reach un-vaccinated populations [27] and if vaccines like MVA86A can override the blocking effect of "ES", its impact on reducing pulmonary tuberculosis could be considerable.

In animal models it has been possible to bypass the modulatory effect of "ES" before BCG vaccination. In guinea pigs and calves a modified BCG vaccine confers protection against TB when the traditional BCG vaccine does not [45,46]. Also, a recombinant BCG vaccine expressing the more virulent RD1 antigen can override an "ES" induced by NTM [19]. To date, several vaccine candidates that intend to replace BCG or boost BCG have entered clinical trials [5]. It is estimated that if these new vaccines are protective (we would say if they are less susceptible to blocking by "ES") they could halve the TB incidence by 2050 [47].

6. Conclusion

Our study supports the view that the dominating mechanism behind geographical variation of BCG-induced protection is more likely to be blocking than masking. While counteracting masking is difficult, new vaccines against TB that overcome a blocking effect may be protective in populations where BCG is not. Such vaccines may be particularly effective in the reduction of adult tuberculosis in regions away from the tropics.

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Contributors. LCR, MLB designed the study, supervised field work, participated in the analysis, interpreted the results and edited the manuscript; DP and BG conducted the statistical analysis; DP interpreted the results and drafted the manuscript; BG, SMP, SSC, MAH, MYI were involved in field work, interpretation and editing; AAC, CS'A were involved in clinical supervision, interpretation of results and editing. All authors had access to all data in the study and held final responsibility for the decision to submit for publication.
Conflict of interest: The authors declare no conflicts of interest.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.vaccine.2014.05.042.

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