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Original article

Maternal pneumococcal nasopharyngeal carriage and risk factors for neonatal carriage after the introduction of pneumococcal conjugate vaccines in The Gambia

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ABSTRACT

Objectives: Pneumococcal nasopharyngeal carriage occurs early in life. However, the role of vertical transmission is not well understood. The aims of this study were to describe carriage among mothers and their newborns, and to assess for risk factors for neonatal carriage.

Methods: In a nested retrospective cohort study, we analysed data from the control arm of a randomized controlled trial conducted in The Gambia 2 to 3 years after introduction of pneumococcal conjugate vaccine (PCV) 13. Nasopharyngeal swabs were collected from 374 women and their newborns on the day of delivery, then 3, 6, 14 and 28 days later. Pneumococci were isolated and serotyped using conventional microbiologic methods.

Results: Carriage increased from 0.3% (1/373) at birth to 37.2% (139/374) at day 28 (p <0.001) among neonates and from 17.1% (64/374) to 24.3% (91/374) (p 0.015) among women. In both groups, PCV13 vaccine-type (VT) serotypes accounted for approximately one-third of the pneumococcal isolates, with serotype 19A being the most common VT. Maternal carriage (adjusted odds ratio (OR) = 2.82; 95% confidence interval (CI), 1.77–4.80), living with other children in the household (adjusted OR = 4.06; 95% CI, 1.90–8.86) and dry season (OR = 1.98; 95% CI, 1.15–3.43) were risk factors for neonatal carriage. Over half (62.6%) of the neonatal carriage was attributable to living with other children in the same household.

Conclusions: Three years after the introduction of PCV in The Gambia, newborns are still rapidly colonized with pneumococcus, including PCV13 VT. Current strategies for pneumococcal control in Africa do not protect this age group beyond the herd effect. **E. Usuf, Clin Microbiol Infect 2017;■:1**

Introduction

Streptococcus pneumoniae is a leading cause of morbidity and mortality in children under 2 years of age [1,2]. Generally, invasive pneumococcal disease (IPD) peaks in the second year of life in developed countries and in the first year of life in sub-Saharan Africa [3,4]. Nevertheless, a substantial burden of neonatal IPD has been reported across different regions worldwide, although data are lacking in the least developed countries [5–10]. In a recent systematic review, the global pooled neonatal IPD was estimated at 36 per 100 000 live births (95% confidence interval (CI), 20.0–64.7) [5]. From one study in The Gambia, it was estimated as 369.5 per 100 000 (95% CI, 119.2–1138.5) [3,5].

Pneumococcal conjugate vaccines (PCVs) are effective against IPD and nasopharyngeal carriage of vaccine serotypes (VT). The latter is important because carriage is a preliminary step towards disease and a measure of community transmission. The introduction of PCV has been shown to reduce acquisition of VT nasopharyngeal carriage among both vaccinated [11] and unvaccinated individuals [12]. However, it has also been shown to increase non-

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vaccine-type carriage such that the overall carriage prevalence remains similar [11].

Since 2009, three formulations of PCV targeting pneumococcal serotypes most commonly associated with IPD worldwide have been introduced into routine vaccination in sub-Saharan Africa, first PCV7, then PCV10 and PCV13. Although two randomized trials in Kenya and Papua New Guinea showed that PCVs are immunogenic and well tolerated when given at birth [13,14], the World Health Organization (WHO) recommends vaccination with PCVs from 6 weeks of age [15]. This means newborns until 6 weeks of age are only protected through indirect effects (i.e. maternal antibodies and herd effect) [16–18]. New strategies are required to reduce early infection and to minimize its long-term effects such as growth impairment and neurologic sequelae [19–21].

Risk factors for pneumococcal nasopharyngeal carriage are well known for African children [22–25] but not for newborns. In this study, we assessed the prevalence of pneumococcal carriage among women and their newborns in The Gambia and analyzed risk factors for neonatal carriage after the implementation of PCV through an expanded program on immunization.

Materials and Methods

Study site

The study was conducted at the Jammeh Foundation for Peace, a public health facility in Western Gambia. PCV7 was introduced in The Gambia in August 2009 and was replaced by PCV13 in May 2011. Coverage of two or more doses of PCV13 before 12 months of age was 94% among rural Gambian children born in the second half of 2013 [26].

Study design

This was a retrospective nested cohort study. The study cohort comprised mother–newborn pairs included in the placebo arm of a phase 3, double-blind, placebo-controlled, randomized trial in which women in labour were randomized to receive a single dose of 2 g of oral azithromycin or placebo (292.7 mg pregelatinized starch, 881.8 mg dibasic calcium phosphate and 11.5 mg magnesium stearate coated with 18 mg Opadry). Study women aged 18 to 45 years were recruited between April 2013 and April 2014 when attending prenatal clinic.

Nasopharyngeal swabs (NPS) were collected from mothers and their newborns during the 28 days of active follow-up. An NPS was collected from women in labour and from newborns within 6 hours of birth. Additional maternal and newborn NPS were collected at days 3, 6, 14 and 28 during household visits conducted by study nurses and field workers. Sample collection was discontinued if participants received antibiotics as part of standard care.

Epidemiologic data including demographic and other risk factor data were collected by trained field staff using questionnaires.

Sample collection

All NPS were collected in accordance with the WHO protocol for detecting *S. pneumoniae* in the upper respiratory tract [27] and as described previously [28].

Bacterial Isolation

Stored NPS were plated on appropriate agar for selective isolation of *S. pneumoniae* and *Staphylococcus aureus* [28]. All pneumococcal isolates were serotyped by using the latex agglutination test (Statens Serum Institute, Copenhagen, Denmark) [29].

Data management and statistical analysis

All data were double entered into Openclinica and analysed by Stata 14 (StataCorp, College Station, TX, USA). The analysis was restricted to mothers and newborns in the placebo arm of the trial that were not missing day 28 carriage data.

The prevalence of PCV13 VT carriage (i.e. carriage of serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F or 23F) and non-vaccine-type carriage was calculated for 0, 3, 6 and 14 days after birth where the latter included nontypeable isolates. We estimated the proportion of concordant pairs, defined as the proportion of mother–newborn carrier pairs where both mother and newborn carried the same serotype, either concurrently or at different times, during the neonatal period. The association between maternal carriage—defined as carriage at any time during the neonatal period—and neonatal carriage at day 28 was investigated by logistic regression. The following potential confounders were selected *a priori* for inclusion in the model [30]: birth weight, other children in the household, mother's age, mother's education and season.

The association between other children living in the household and neonatal carriage at day 28 was also investigated. The potential confounders listed above were included in this second model, except for 'other children in the household,' because this variable was already included as the exposure of interest. The model did not include maternal carriage because this may be on the causal pathway.

A similar analysis was conducted for the association between maternal carriage at day 14 and acquisition of carriage in the newborn between day 6 and day 14—i.e. carriage of any serotype at day 14 among noncarriers at day 6, and for the association between neonatal carriage at day 14 and acquisition of carriage in the mother over the same period.

The percentage of neonatal carriage attributable to each risk factor—i.e. maternal carriage and living with other children, the population-attributable fraction—was calculated using the 'punaf' command in Stata [31].

Ethical approval

The trial was approved by the joint Medical Research Council Group–Gambia Government Ethics Committee (ClinicalTrials.gov NCT01800942).

Results

Characteristics of mothers and newborns

There were 417 mother–newborn pairs in the placebo arm of the trial, and 374 (89.7%) were included in the analysis. As there were four sets of twins, only 370 women were sampled. The median age of the mothers was 26 years (interquartile range, 22–30 years). Approximately half of the women had less than 1 year of any schooling (184/359, 51.2%). Forty-seven percent (173/367) of newborns were girls. The median birth weight was 3.1 kg (interquartile range, 2.9–3.4 kg), and 22 (5.9%) of 373 were low birth weight (<2.5 kg). All the newborns were breastfed; only seven were not exclusively breastfed.

Maternal and newborn pneumococcal nasopharyngeal carriage

Among the mothers, carriage increased from 17.1% to 24.3% (p 0.015) between days 0 and 28, and among newborns it increased from 0.3% to 37.2% (p <0.001) (Fig. 1). PCV13 VT represented approximately one-third (28.2%) of all pneumococci isolates, 27.1%

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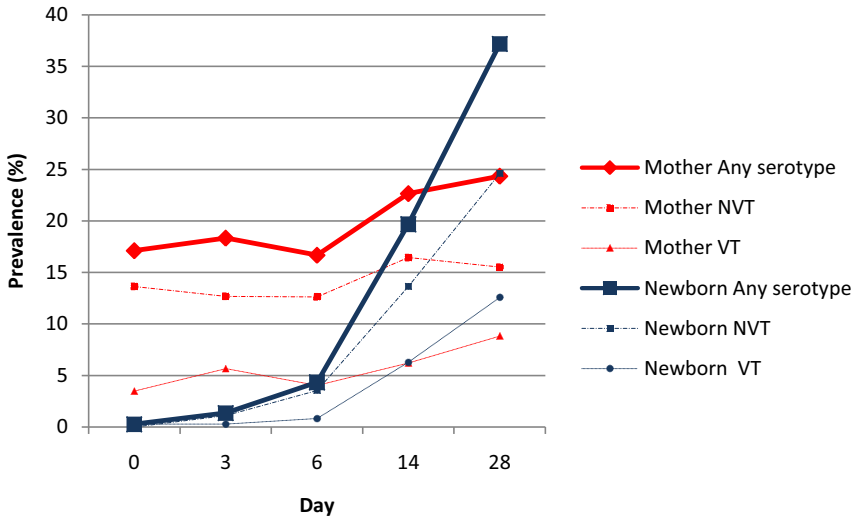

Fig. 1. Pneumococcal nasopharyngeal carriage in mothers and newborns. VT, vaccine type; NVT, non–vaccine type.

(99/366) for mothers and 29.9% (70/234) for newborns (Fig. 2). Serotype 19A was the most common serotype among newborns and the second most common among mothers. Nontypeable pneumococci were also common among both mothers (36/366, 9.8%) and newborns (14/234, 6.0%). Only five mothers and seven newborns carried multiple serotypes during the study period.

In 76 (20.3%) of the mother–newborn pairs, the mother was a carrier but the newborn was not, and in 56 pairs (15.0%) the newborn was a carrier but the mother was not. Among the 97 pairs (25.9%) where both the mother and newborn were carriers, 47 (48.5%; 95% CI, 38.2–58.9) were concordant (i.e. carried the same serotype). Of these, 31.9% (15/47) pairs were concordant with VT;

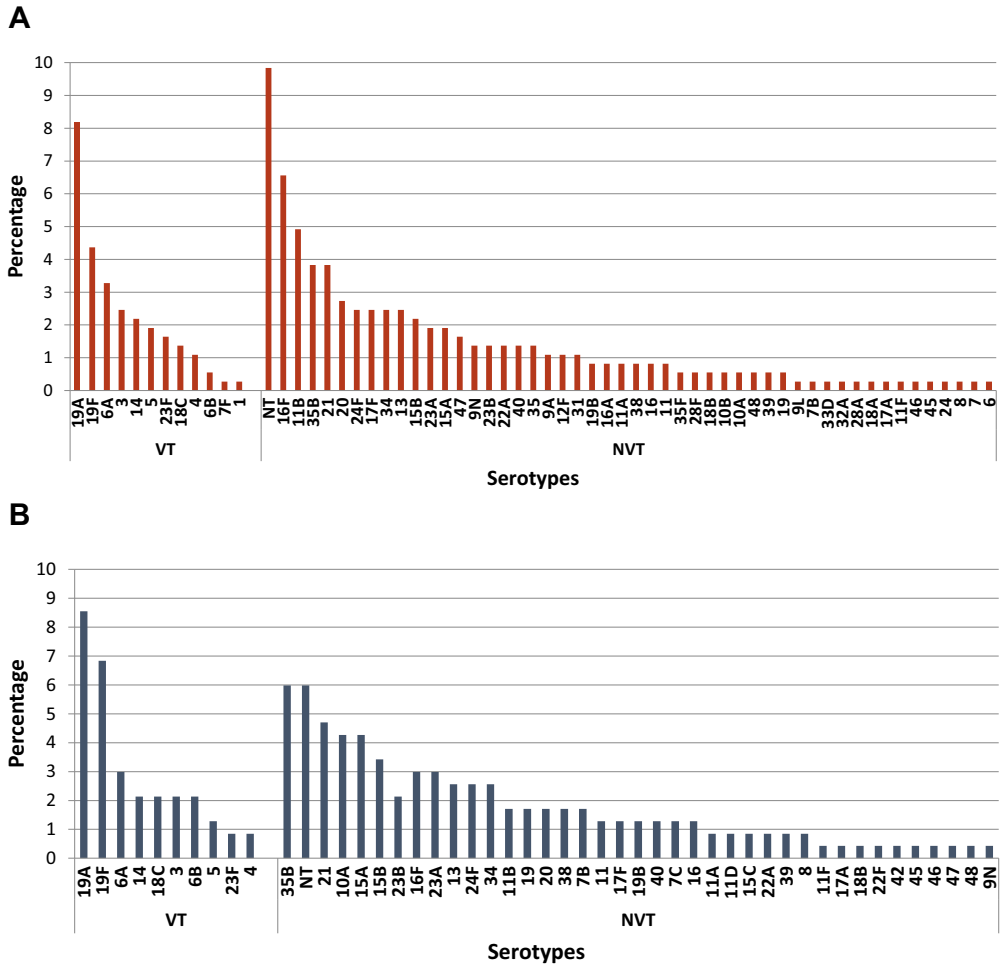

Fig. 2. Serotypes isolated from mothers and their babies during neonatal period. (A) Serotypes among mothers. (B) Serotypes among newborns.

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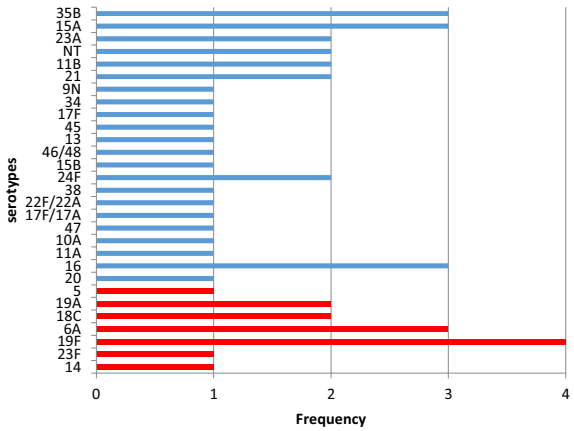

Fig. 3. Frequency of concordant serotypes among mother–newborn pairs. Red bars, vaccine type serotypes; blue bars, non–vaccine type.

19F was the most common, at 8.5% (4/47). There were four concordant cases among those who did not have other children in the household (serotypes 9N, 19F, 21 and 47) (Fig. 3).

Risk factors for pneumococcal nasopharyngeal carriage in newborns

In the univariable analysis, the prevalence of carriage at day 28 was higher in newborns born during the dry season (odds ratio (OR) = 1.84; 95% CI, 1.13–2.99), those whose mothers were carriers (OR = 2.63; 95% CI, 1.71–4.05), those living with other children (OR = 4.62; 95% CI, 2.28–9.36) and those with normal birth weight (OR = 4.00; 95% CI, 1.16–13.79) (Table 1).

Maternal carriage remained a risk factor for neonatal carriage after adjusting for birth weight, other children in household, mother's age, mother's education and season (adjusted OR = 2.69; 95% CI, 1.68–4.30; p <0.001). Living with other children in the household after adjusting for birth weight, mother's age, mother's education and season was also a risk factor for neonatal carriage at

Table 1
Risk factors for neonatal nasopharyngeal pneumococcal carriage at day 28 ($N = 374$)

Characteristic	Variable	Carriage, *n* (%)	Total (*N*)	Crude		Adjusted	p
				OR (95% CI)	p	OR	
Newborn							
Gender	Female	67 (38.7)	173	1			
	Male	72 (37.1)	194	0.93 (0.61–1.42)	0.750		
Birth weight	<2.5 kg	3 (13.6)	22	1		1	
	≥2.5 kg	136 (38.8)	351	4.00 (1.16–13.79)	0.028	3.461 (0.91–13.11)	0.068
Gestational age	<37 weeks	85 (38.8)	219	1			
	≥37 weeks	54 (35.5)	152	0.87 (0.56–1.33)	0.520		
Staphylococcus aureus carriage at day 28	No	87 (36.0)	242	1			
	Yes	52 (39.4)	132	1.15 (0.75–1.79)	0.510		
Maternal							
Mother carrier *Streptococcus pneumoniae* at day 28	No	94 (33.2)	283	1			
	Yes	45 (49.5)	91	1.97 (1.21–3.18)	0.006		
Mother carrier day 14 and carrier day 28	No	117 (35.1)	333	1			
	Yes	22 (53.7)	41	2.13 (1.11–4.11)	0.023		
Mother carrier day 28, noncarrier day 14	No	118 (36.2)	326	1			
	Yes	21 (43.8)	48	1.37 (0.74–2.53)	0.313		
Mother carrier any time during neonatal period	No	54 (26.9)	201	1		1	
	Yes	85 (49.1)	173	2.63 (1.71–4.05)	<0.001	2.82 (1.77–4.80)	<0.001
Mother *S. aureus* carrier at day 28	No	99 (36.0)	275	1			
	Yes	40 (40.4)	99	1.21 (0.75–1.93)	0.437		
Mother's age	<25 years	72 (34.0)	212	1			
	≥25 years	67 (41.6)	161	1.39 (0.91–2.11)	0.131		
Ethnicity	Mandinka	54 (32.7)	165	1	0.469		
	Wollof	17 (40.5)	42	1.40 (0.70–2.81)			
	Jola	22 (42.3)	52	1.51 (0.80–2.86)			
	Fula	25 (44.6)	56	1.66 (0.89–3.08)			
	Others	21 (35.6)	59	1.13 (0.61–2.12)			
Mother's years of schooling	None or <1 year	74 (40.2)	184	1			
	≥1 year	59 (33.7)	175	0.76 (0.49–1.16)	0.203		
Mother can read	No	99 (40.4)	245	1			
	Yes	40 (31.0)	129	0.66 (0.42–1.04)	0.075		
Mother can write	No	98 (40.2)	244	1			
	Yes	41 (31.5)	130	0.69 (0.44–1.08)	0.101		
Household							
Smoker in house	No	126 (36.7)	343	1			
	Yes	13 (41.9)	31	1.24 (0.59–2.62)	0.567		
Who bathes child	Mother	82 (40.4)	203	1			
	Other	53 (32.9)	161	0.72 (0.47–1.11)	0.143		
Other children in household	No	10 (13.9)	72	1		1	
	Yes	129 (42.7)	302	4.62 (2.28–9.36)	<0.001	4.06 (1.90–8.86)[a]	<0.001
Number of children at school	0	62 (34.3)	181	1			
	≥1	76 (39.8)	191	1.26 (0.83–1.93)	0.270		
Other factors							
Season	Rainy	30 (27.5)	109	1		1	
	Dry	109 (41.1)	265	1.84 (1.13–2.99)	0.014	1.98 (1.15–3.43)	0.014

CI, confidence interval; OR, odds ratio.
Adjusted OR adjusted for birth weight, other children living in household, mother's age, mother's education and season.
[a] Model did not include maternal carriage, thought to be on the causal pathway.

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day 28 (adjusted OR = 3.40; 95% CI, 1.55–7.50, p 0.002). The fraction of neonatal carriage at day 28 attributed to maternal carriage was 10.6% (95% CI, 2.5–18.0), and the fraction attributable to living with other children in the household was 62.6% (95% CI, 35.4–78.4).

Mothers who were not carriers at day 6 had a higher carriage at day 14 if their baby was a carrier at day 14, although this was not statistically significant (adjusted OR = 1.54; 95% CI, 0.75–3.18; p 0.326). Newborns who were not carriers at day 6 had a higher risk of being a carrier at day 14 if their mother was a carrier at day 14 (adjusted OR = 1.88; 95% CI, 1.01–3.48; p 0.045).

Discussion

The prevalence of pneumococcal nasopharyngeal carriage among Gambian women and their newborns during the 4 weeks after delivery was high. Although the study was conducted 2 to 3 years after routine PCV13 introduction (and 4 to 5 years after PCV7 introduction), one-third of the serotypes identified were PCV13 VT. Both maternal carriage and living with other children in the household were risk factors for neonatal carriage, with the latter accounting for almost two-thirds of neonatal carriage.

The prevalence of neonatal colonization (37.2%) was lower than in two previous studies conducted before PCV introduction in The Gambia. In both these studies, the prevalence was more than 50% by the age of 4 weeks [17,32]. One difference between these previous studies and ours is that the former were conducted in rural areas where most deliveries occurred at home, while the latter was conducted in a periurban health facility.

A striking finding in our study was the sharp increase in carriage in both mothers and newborns between days 6 and 14 (carriage in newborns continued to increase up to day 28). In The Gambia, newborns are given their name at day 7 after birth in a naming ceremony, and this is usually preceded by cooking and people moving around the compound. This may increase pneumococcal transmission during this period as people visit the mother and newborn. In line with our results, a previous longitudinal study in rural Gambia showed that maternal carriage doubled within the first 2 months after delivery and remained high until the end of 1 year, when follow-up ended [33].

In our study, a third of the isolates were PCV13 VT, a lower proportion than that observed in a pre-PCV study conducted in rural Gambia where 50% of isolates were PCV13 VT. Serotype 19A, included in PCV13 but not in PCV7, was one of the most common VT serotypes, representing approximately 9% of pneumococcal carriage. By comparison, in the United States, serotype 19A significantly decreased after PCV13 introduction, although it still contributed up to 5% of the total *S. pneumoniae* carriage 5 years later [34]. In South Africa, prevalence of serotype 19A in children was 2.7% two years after the introduction of PCV13 [35].

The prevalence of PCV13 VT among mothers in our study was 8.8% and among newborns was 11.8%. These estimates are consistent with the predictions of a mathematical model of carriage in The Gambia, which predicted that PCV13 VT carriage would decline to 7% three years after PCV13 [36]. Although it appears that VT have decreased, Gambian newborn babies are still unprotected against VT during a period when they are at high risk of infection, since the first dose in the Expanded Program on Immunization (EPI) schedule is given at 8 weeks of age in the national EPI.

Between 6% and 10% of isolates were not typeable, similar to earlier studies conducted after PCV [18,37], but different from the prevaccine estimates in The Gambia, which were about 2% [38]. An increase in nontypeable isolates after PCV has been related to serotypeable isolates that do not express their capsule [18].

Serotype concordance for mother–newborn carrier pairs was close to 50%. In Malawi, a lower concordance (9.1%) between mothers and their newborns was reported, but these infants were older (<3 months of age) and therefore more likely to acquire carriage from sources other than the mother [39]. The high concordance appears to be at odds with the low proportion of neonatal carriage attributable to maternal carriage (population-attributable fraction), observed both in our study (10.6%) and in a previous study in rural Gambia (9.5%) [33]. This discrepancy may suggest that other children in the household may be transmitting to both mothers and newborns. Compared to the proportion of neonatal carriage attributable to maternal carriage, the proportion attributable to living with other children was considerably higher (62.6%). This was both because the OR was higher and because carriage was more common in children than in mothers.

Although the association between other children living in the household and neonatal carriage was strong and has been observed in Asia and other parts of Africa [35,40–42], we cannot exclude the notion that it is due to differences in socioeconomic status between households with a small and large number of children.

Other factors associated with neonatal carriage were low birth weight and dry season. The decreased carriage risk with low birth weight was a surprising finding, though it has been reported in Indian and Dutch babies (https://repub.eur.nl/pub/22193/) [19] and may be due to behavioural factors, as these children are perhaps less exposed to other family members. The association with season was previously shown among older children in rural Gambia, and we therefore would expect the same pattern with neonates. Such an association may reflect greater social mixing during the dry season, when villagers spend less time on their farms [43].

Our analysis has a number of limitations. Firstly, the rates of carriage may have been underestimated because the women and their newborns received relatively more medical attention than the general local population during the study period. In addition, only healthy mothers and newborns were recruited into the study. Secondly, the analysis was restricted to mother–newborn pairs with both swabbed at day 28. Thirdly, the risk factors tested were not exhaustive. In particular, we did not assess for the presence of upper respiratory tract infections, which has been shown to increase the risk of carriage [44], and we were unable to explore the role of breastfeeding because the newborns were all breastfed. The effect of maternal carriage on newborn carriage may have been diluted, as about half of the mother–newborns pairs were discordant. Fourthly, we did not use molecular techniques [45], so we may have missed low-density carriers and cases of multiple carriage.

Conclusions

Gambian newborns are rapidly colonized by pneumococcus during the first month of life, and current strategies for pneumococcal control in Africa do not protect this age group beyond the herd effect. More than 2 years after PCV13 introduction (4 years after PCV7 introduction), PCV13 VT are still transmitted in the community, representing one-third of strains isolated from neonatal and maternal nasopharyngeal carriage. Living with other children was the major risk factor for neonatal carriage.

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Transparency Declaration

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.cmi.2017.07.018.

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