Biological sex influences antibody responses to routine vaccinations in the first year of life

Short title: The influence of early-life factors on vaccine responses

Petra Zimmermann[1,2,3,4], MD, Kirsten P. Perrett[5,6,7], FRACP, PhD, Nicole Ritz[2,8], MD, PhD, Katie L. Flanagan[9], FRACP, PhD, Roy Robins-Browne[2,10], FRACP, PhD, Fiona R. M. van der Klis[11], PhD, Nigel Curtis[1,2,3], FRCPCH, PhD and the MIS BAIR group*

Affiliations:

[1] Department of Paediatrics, The University of Melbourne, Parkville, Australia

[2] Infectious Diseases Research Group, Murdoch Children's Research Institute, Parkville, Australia

[3] Infectious Diseases Unit, The Royal Children's Hospital Melbourne, Parkville, Australia

[4] Department of Paediatrics, Fribourg Hospital HFR and Faculty of Science and Medicine, University of Fribourg, Switzerland

[5] Food Allergy Research Group and Melbourne Children's Trial Centre, Murdoch Children's Research Institute;

[6] Departments of Allergy and Immunology and General Medicine, Royal Children's Hospital; Melbourne;

[7] School of Population and Global Health, The University of Melbourne, Parkville, Australia

[8] Infectious Diseases Unit, University of Basel Children's Hospital, Basel, Switzerland

[9] University of Tasmania, Launceston and Monash University, Clayton, Australia

[10] Department of Microbiology and Immunology, The University of Melbourne, Parkville, Australia

[11] National Institute of Public Health and the Environment, Centre for Infectious Diseases, Bilthoven, the Netherlands

Address correspondence to: Prof Nigel Curtis, Department of Paediatrics, The University of Melbourne, Royal Children's Hospital Melbourne, Parkville, VIC 3052, Australia, Tel: +61 3 9345 6366, nigel.curtis@rch.org.au

** Veronica Abruzzo, Katie Allen, Rhian Bonnici, Dan Casalaz, Hannah Elborough, Bridget Freyne, Kaya Gardiner, Susie Germano, Tobias Kollmann, Nicole Messina, Clare Morrison, Helder Nakaya, Anne Louise Ponsonby, Frank Shann, Mike South*, *Peter Vuillermin*

Abstract

Aim: We investigated the effect of early-life factors, namely sex, delivery mode, feeding method and antibiotic exposure, on antibody responses to routine vaccinations administered during the first year of life.

Methods: One and seven months after the primary course of routine vaccines and one month after routine vaccines at 12 months of age, antibodies against 26 vaccine antigens were measured in 398 healthy infants. The geometric mean concentration (GMC) of antibodies (adjusted for effect modifiers with multiple linear regression) and the seroprotection rate for each vaccine were compared for each early-life factor.

Results: Sex had an influence on GMCs. Antibody concentrations were significantly lower at 7 months of age in females for tetanus and filamentous haemagglutinin and at 13 months of age for pertactin. In contrast, at 13 months of age, antibody concentrations were significantly higher in females for polio type 3, pneumococccal serotype 6A and measles. Sex did not have an influence on seroprotection rates. Delivery mode, feeding method and antibiotic exposure did not exert a substantial influence on vaccine antibody concentrations.

Conclusion: There is a difference between males and females in the humoral response to routine vaccinations in the first year of life.

Key Notes

- There are substantial differences between individuals in the immune response to vaccination.
- In this study, we found that there are differences between males and females in antibody responses to routine vaccinations in the first year of life.
- In contrast, delivery mode, feeding method and antibiotic exposure does not affect antibody responses.

Keywords: antibodies, humoral, immunoglobulin, titre, immunisation, infant

Introduction

Vaccination is one of the most cost-effective, life-saving medical interventions. However, there is substantial variation between individuals in the magnitude of the immune response to vaccination, which might have implications for both protective efficacy and duration of protection. At the age of six months, for example, antibody responses to pneumococcal and *Haemophilus influenzae* type b (Hib) vaccines may vary up to 40-fold (1).

A number of intrinsic host, perinatal and extrinsic factors are likely to contribute to the variation in vaccine responses (2). One of the intrinsic factors that has been most investigated is biological sex. But studies to date have almost exclusively been in adults. Other intrinsic factors include polymorphisms in genes encoding major histocompatibility complexes or pattern recognition receptors, such as Toll-like or RIG-like receptors (2). Perinatal factors that might influence vaccine responses include gestational age, birth weight (3, 4) and feeding method (formula vs breastfed). Potential extrinsic factors include exposure to probiotics and antibiotics. While, there is evidence for a beneficial effect of probiotics on vaccine responses (2), to date, only one study has investigated the effect of antibiotics in vaccine responses in humans. This study reported that administration of antibiotics before vaccination did not alter serum immunoglobulin (Ig) A concentrations to oral rotavirus vaccine (ORV) in adults (5).

Determining the influence of intrinsic and extrinsic factors on vaccine antibody responses has implications for optimising vaccine recommendations and individualising vaccine schedules.

In this study, we investigated the effect of biological sex, mode of delivery, feeding method and antibiotic exposure on antibody responses to routine vaccinations given in the first year of life.

Methods

Study design and population

Participants were a subset of 471 healthy infants from The Melbourne Infant Study: Bacille Calmette-Guérin (BCG) for Allergy and Infection Reduction (MIS BAIR) (http://misbair.org). In this trial infants were recruited at birth from 2013 to 2016 and randomised to receive neonatal BCG vaccination or no intervention to investigate whether BCG protects from childhood allergies. Inclusion criteria included the following: greater than 32 weeks gestation and birth weight greater than 1500 grams. Details about feeding and antibiotic exposure were prospectively collected by parent questionnaire.

From the subset of participants whose parent/guardian provided consent, blood samples were collected at study visits at 7 and / or 13 months of age (designed to be 4 weeks after the administration of routine scheduled vaccinations). Only participants who had a blood taken 28 ± 14 days after their 6-month and / or 12-month routine vaccinations were included in the analysis of immediate post-vaccination responses. For those in whom persistence of antibodies to their 6-month vaccines was measured blood was taken 7 months ± 23 days after their 6-month vaccinations.

Infant vaccination

All infants received routine vaccinations according to the Australian National Immunisation Program: at birth: intramuscular hepatitis B (HepB) vaccine (*H-B-Vax II Paediatric*®, bioCSL)); at 6 weeks, 4 months and 6 months of age: intramuscular combined diphtheria-tetanus-acellular pertussis (DTPa)-HepB-inactivated polio (IPV)-Hib vaccine (*Infanrix*® Hexa (GlaxoSmithKline)), intramuscular conjugate pneumococcal vaccine (PCV13) (*Prevenar13*® (Wyeth)) and ORV (*RotaTeq*® (Merck)); at 12 months of age: subcutaneous measles-mumps-rubella (MMR) vaccine (*Priorix*® (GlaxoSmithKline)) and intramuscular combined Hib and

conjugated meningococcal C vaccine (*Menitorix*® (GlaxoSmithKline)). Approximately half of the infants received intradermal BCG-Denmark (*Statens Serum Institut, Copenhagen*) shortly after birth as part of the MIS BAIR. Vaccine records were obtained from individual vaccination records and/or the Australian Immunisation Register.

Blood collection and antibody assay

Following collection (*S-monovette*® (Sarstedt)), plasma was stored at -80° C until analysis at the National Institute for Health and Environment, in Bilthoven, the Netherlands. IgG antibodies against 26 vaccine antigens (diphtheria, tetanus, pertussis (pertussis toxin (PT), filamentous haemagglutinin (FHA), pertactin (PRN)), polio (types 1, 2, 3), Hib, pneumococcus (serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F], meningococcus type C (MenC), measles, mumps and rubella) were measured using fluorescent bead-based multiplex immune-assays (*Luminex* xMAP technology). In all assays, international or in-house reference controls and blanks were included on each plate. All analyses were performed with a Bio-Plex 200 in combination with Bio-Plex manager software (Bio-Rad Laboratories, Hercules, CA).

Categorisation of participants

Infants were categorised by biological sex (male or female), delivery mode (vaginal vs Caesarean section), feeding method and antibiotic exposure using information collected from hospital records and parent questionnaires. Feeding method was categorised as follows: i) infants who did or did not receive formula before leaving their birth hospital; ii) infants who were or were not still receiving breast milk at one month of age; iii) infants who were or were not still receiving breast milk at six months of age. Antibiotic exposure was categorised as follows: i) infants whose mother did or did not receive antibiotics during delivery; ii) infants

who did or did not received systemic antibiotics before their first routine vaccinations scheduled at the age of six weeks (assessed individually).

Statistical analysis

In each group, the proportion of infants with an antibody concentration above the standard protective correlate value (seroprotection rate) was calculated for each vaccine.(6-8) The Clopper-Pearson method was used to estimate the 95% confidence intervals (CIs) of the seroprotection rates. The rates were compared between groups using Fisher's exact test and the 95% confidence interval (CI) for differences in proportions estimated. For FHA and PRN, no standard protective correlate value exists. In each group, the geometric mean concentration (GMC) for each vaccine antibody was calculated. The geometric mean ratio (GMR) with 95% confidence interval (CI) was obtained as the anti-logged coefficient from a linear regression with log-concentrations as outcome and early-life factors (biological sex, delivery mode, feeding choice and antibiotic exposure) as covariates (univariate regression). Effect modification was assessed using multiple linear regression with the following pre-specified factors: maternal age, maternal diphtheria-tetanus-pertussis (dTpa, *Boostrix*® (GlaxoSmithKline)) and trivalent influenza vaccination in pregnancy, birth weight, gestational age, infant BCG and MMR vaccination status, age at first DTPa-HepB-IPV-Hib / PCV13 vaccination, age at sampling and time interval between 6-month or 12-month vaccination and blood sampling. The GMR for each vaccine antigen was adjusted for those factors that had an effect on vaccine antigen response. As the Hib-MenC vaccine includes a tetanus toxoid (as carrier protein) and a Hib component, infants who had received this vaccine before blood sampling at 13 months of age were excluded from the analysis of persistence of antibodies against these two vaccine antigens. A 5%-significance level was used. All statistical analyses were done using R version 3.4.3.

Ethics

Informed consent was obtained from participants' parents or guardians. The study was approved by the Royal Children's Hospital Human Research Ethics Committee (HREC, authorisation, 38124A).

Results

Of the 471 participants, seven were excluded because they were a twin of another participant, five because extended parental consent to use the blood samples was not available, four because they were not vaccinated according to the routine schedule and one because vaccine records were not available (Figure 1). Of the remaining 454 participants, 365 had blood taken in the predefined time frame. At 7 months of age, 91 were included in the final analysis. At 13 months of age, 307 were included for measurement of persistence of antibodies against the primary course of vaccines ending at 6 months and 141 for antibody responses to the 12-month vaccines. The background characteristics of the 365 included participants are summarised in Table 1. The median gestational age was 39.3 weeks (interquartile range (IQR) 38.4-40.4) and the median birth weight 3.45 kg (IQR 3.13-3.76).

Of the 365 infants, 179 (49%) were male, 137 (37%) were born by Caesarean section and 100 (28%) had received formula milk before discharge from their birth hospital. At 1 month of age, 302 (83%) infants were still receiving breast milk, at 6 months of age this number was 263 (72%). In total, 70 (19%) mothers received antibiotics during delivery: 36 (51%) because they were colonised with group B streptococcus, 27 (39%) because of prolonged rupture of membranes, 5 (7%) because of Caesarean section delivery and for 2 (3%) the reason was not specified. The administered antibiotics were benzylpenicillin (n=30), amoxycillin (n=14), ampicillin (n=11), cephazolin (n=5), cefotaxime (n=1), metronidazole (n=1) and not specified (n=11).

Antibiotics were administered to 30 (8%) infants before their first routine vaccination schedule at six weeks of life. These infants received only one course of antibiotics each. The majority (n=22, 73%) received antibiotics within the first week of life (intravenous benzylpenicillin and gentamicin (n=18), intravenous benzylpenicillin, gentamicin and vancomycin (n=1), intravenous antibiotics not documented (n=1), oral cephalexin (n=1), and oral flucloxacillin (n=1)). The duration of administration varied between 2 and 7 days (mean 3, median 3). Of the remaining 8 infants, 4 were admitted to hospital for intravenous antibiotics (azithromycin (n=1) and antibiotics not documented (n=3)) between 2 and 5 weeks of age (mean 3.5, median 3.5). The duration of administration was less than 5 days (n=2 infants) and between 5 and 10 days (n=2 infants). The infant who received intravenous azithromycin was continued on oral azithromycin for 3 days. Additionally, two infants were continued on oral antibiotics (not documented), one for 5 days and one for more than 10 days. There were 4 infants who received oral antibiotics after the first week of life (age range 2 to 4, mean 3.8, median 4 weeks) (amoxicillin (n=2), cephalexin (n=2)). The duration of administration was 5 to 10 days for all.

Sex influences the magnitude of responses to certain vaccines

At 7 months of age, females had a lower seroprotection rate against Hib compared with male infants (66% (95% CI 51.6-79.6%) vs 86% (95% CI 72.1-94.7%); p=0.05). However, overall there were no statistically significant differences between males and females in seroprotection rates at 7 or 13 months of age (Table 2a and Figure 2). After adjustment for pre-specified factors, the female:male GMR was below one (indicating lower average antibody concentrations) in females for tetanus and FHA (Table 2b and Figure 2) at 7 months of age and for PRN at 13 months of age. These findings were statistically significant. At 13 months

of age, adjusted average antibody concentrations were statistically significantly higher in females for polio type 3, pneumococcus serotype 6A and measles (Table 2b and Figure 2).

No effect of delivery mode on vaccine responses

At 7 months of age, seroprotection rates were lower in infants born vaginally than in those born by Caesarean section for the majority of vaccine antigens. However, none of these differences reached statistical significance. At 13 months of age, there were no differences in seroprotection rates between infants born vaginally or by Caesarean section (Supplementary Table S1a and Figure S1). At 13 months of age, infants born vaginally had a higher average antibody concentration against Hib (Supplementary Table S1b and Figure S1).

No effect of feeding method on vaccine antibody responses

At 7 and 13 months of age, there were no statistically significant differences in seroprotection rates or adjusted average antibody concentrations between infants who received or did not receive formula milk in first few days of life or between infants who were still receiving breast milk at 1 or 6 months of age and those who were not (Supplementary Tables S2a-4b and Figures S2-4). The only exception was a statistically significant higher adjusted average antibody concentration (i.e. a GMR greater than one) against pneumococcus serotype 7F at 7 months of age in infants who did not receive formula milk during the first few days of life compared to those who did (Supplementary Table S2b and Figure S2), and higher antibody concentration for pneumococcus serotype 19F and MenC at 13 months of age in infants who were still receiving breast at 6 months of age (Supplementary Tables S4b and Figure S4).

Minimal effect of intrapartum or infant antibiotic exposure on vaccine responses

At 7 and 13 months of age, there were no differences in seroprotection rates between infants whose mothers did or did not receive antibiotics during delivery (Supplementary Table S5a and Figure S5). Infants whose mother received intrapartum antibiotics had a significantly higher adjusted average antibody concentration against PRN at 7 months and 13 months of age of age (Supplementary Tables S5b and Figure S5). Antibiotic exposure before their first routine vaccination scheduled at 6 weeks of age did not influence seroprotection rates or average antibody concentrations (Supplementary Table S6a-6b and Figure S6).

Discussion

This study is the first to investigate the effect of multiple different early-life factors on antibody responses to routine vaccinations in infancy. We found that infant biological sex had an impact on responses but delivery mode, feeding method, intrapartum antibiotics, and antibiotic exposure before the first routine immunisation did not.

Our finding that females had lower antibody responses against tetanus, and higher responses against polio (type 3) is consistent with previous studies in adults (9-11). Previous studies have also reported female adults to have higher antibody responses to dengue, HepA, HepB, rabies, smallpox, and trivalent influenza vaccines, while male adults have been reported to have higher responses to diphtheria, and conjugated meningococcus A vaccines (2). The only studies which have previously been done in infants, reported higher antibody responses in females to HepB, Hib and PCV13 (2). Aside from a higher average antibody concentration in females against one pneumococcal serotype (6A), we did not find sex differences in the response to pneumococcal vaccination. This contrasts with studies in adults, in which males have generally been reported to have higher antibody responses to pneumococcal vaccines (2).

In our study, we found that female infants had higher antibody levels against measles at 13 months of age than corresponding males. Previous studies investigating sex differences in responses to MMR vaccination report inconsistent findings. Some studies found higher antibody responses to measles, mumps, and rubella vaccination in females, whilst others found transiently lower GMCs to rubella and lower seroconversion rates to measles vaccination in females (2). Other studies did not find any sex differences in antibody responses to mumps and rubella, rubella vaccination. Notably, even though females are often reported to have higher antibody responses, faster waning of antibodies has been observed for antibodies against HepA and pneumococcus in females (2).

One mechanisms through which sex can affect vaccine responses is through hormones: oestradiol, progesterone and testosterone all influence the function of immune cells (12). However, as sex differences in vaccine responses also occur before puberty and after menopause, other factors might also be involved. Many genes for proteins involved in the immune responses are encoded on the X chromosome, and mutations and polymorphisms of X-linked genes therefore have a greater impact in males (13). A further mechanism through which sex differences in vaccine responses might occur, is through epigenetic programming, which is also influenced by sex hormones (12).

The microbiota, especially the intestinal microbiota, plays a crucial rule in the development and regulation of the immune system, and its composition affects how individuals respond to vaccinations (14). Sex hormones play a key role in bacterial-host interactions (15) and sex differences in the microbiota might therefore influence immune responses (16, 17). As delivery mode and feeding method strongly affect the composition of the infant microbiota (18), these factors might be expected to influence vaccine responses. However, in our study, we did not observe any significant impact of delivery mode or feeding method on vaccines responses.

Maternal antibodies may inhibit infant antibody responses to some vaccines (19). Although the majority of maternal antibody transfer is through the placenta, breast milk also contains antibodies that might interfere with immune responses. Small studies have reported that after routine vaccination, breast-fed infants have higher serum IgG levels to diphtheria (20), Hib (21, 22) and oral polio virus (OPV) (20, 23) vaccination, and higher salivary IgA levels to tetanus, diphtheria and OPV vaccination (20). For ORV vaccine, reduced seroconversion rates were observed in breast-fed infants compared with formula-fed infants (24, 25), however, this was not confirmed in subsequent studies (26). Infants with mixed feeding are sometimes classified as breast-fed and sometimes as non-breast-fed (26) and IgA levels, as well as the neutralising activity of IgA in breast-milk, may depend on geographic location (27). These factors could explain some of the inconsistency in results from different studies.

The strengths of our study include the ability to adjust for a large number of possible confounding factors, the standardisation in the vaccines given and the wide range of measured antibody responses. The limitations of the study are the sample size and risk of type-2 error. Furthermore, we did not analyse antibody responses to the HepB vaccine, as this antigen was not included in our multiplex assay and we had insufficient sample volume for alternative assays. We also did not evaluate the effect of antibiotics that might have been transferred through breastmilk on infant vaccine responses.

As most vaccines induce antibody concentrations above the protective threshold in the majority of infants, small differences in antibody concentrations between groups of individuals are unlikely to be clinically significant. However, differences in antibody concentrations might be relevant for the duration of protection. Nonetheless, when developing

new vaccines and designing vaccine schedules, recognising factors which might influence vaccine antibody responses, offers ways to optimise vaccine immunogenicity and efficacy.

Funding statement

This work was supported by the Australian National Health and Medical Research Council (NHMRC) (grants GNT1051228 and GNT1099680), the University of Melbourne (International Research Scholarship to PZ), and the European Society of Paediatric Infectious Diseases (ESPID) (fellowship to PZ).

Acknowledgements

We thank Hannah Elborough and Judith Spotswood for collection of blood samples, Nicole Messina and Rhian Bonnici for help with processing of blood samples, Casey Goodall for help with data entry and Kaya Gardiner for help with project co-ordination. We also thank Prof Andrew Pollard for his helpful comments on the manuscript

Competing interests

The authors declare that they have no competing interests.

Conflict of interest

The authors declare no conflict of interest.

Abbreviations

BCG – Bacille Calmette-Guérin

CI – confidence interval

DTPa – diphtheria-tetanus-acellular pertussis vaccine

FHA – filamentous haemagglutinin

GMC – geometric mean concentration

GMR – geometric mean ratio

HepA – hepatitis A

HepB – hepatitis B

Hib – *Haemophilus influenzae* type b

Ig – immunoglobulin

IPV – inactivated polio vaccine

IQR – interquartile range

MenC – meningococcus C

MIS BAIR – The Melbourne Infant Study: BCG for Allergy and Infection Reduction

ORV – oral rotavirus vaccine

PCV13 – 13-valent conjugate pneumococcal vaccine

Pn – pneumococcus serotype

PRN – pertactin

PT – pertussis toxin

References

1. Ritz N, Mui M, Balloch A, Curtis N. Non-specific effect of Bacille Calmette-Guerin vaccine on the immune response to routine immunisations. *Vaccine* 2013; 31: 3098-103.

2. Zimmermann P, Curtis N. Factors that influence the immune response to vaccination. *Clinical microbiology reviews* 2018; In press:

3. Han K, Shao X, Zheng H, Wu C, Zhu J, Zheng X, et al. Revaccination of non- and low-responders after a standard three dose hepatitis B vaccine schedule. *Human vaccines & immunotherapeutics* 2012; 8: 1845-9.

4. Omenaca F, Garcia-Sicilia J, Garcia-Corbeira P, Boceta R, Torres V. Antipolyribosyl ribitol phosphate response of premature infants to primary and booster vaccination with a combined diphtheria-tetanus-acellular pertussis-hepatitis B-inactivated polio virus/Haemophilus influenzae type b vaccine. *Pediatrics* 2007; 119: e179-85.

5. Harris VC, Haak BW, Handley SA, Jiang B, Velasquez DE, Hykes BL, Jr., et al. Effect of Antibiotic-Mediated Microbiome Modulation on Rotavirus Vaccine Immunogenicity: A Human, Randomized-Control Proof-of-Concept Trial. *Cell host & microbe* 2018; 24: 197-207.e4.

6. Plotkin SA. Correlates of protection induced by vaccination. *Clinical and vaccine immunology : CVI* 2010; 17: 1055-65.

7. Freidl GS, Tostmann A, Curvers M, Ruijs WLM, Smits G, Schepp R, et al. Immunity against measles, mumps, rubella, varicella, diphtheria, tetanus, polio, hepatitis A and hepatitis B among adult asylum seekers in the Netherlands, 2016. *Vaccine* 2018; 36: 1664-72.

8. Schepp RM, Berbers GA, Ferreira JA, Reimerink JH, van der Klis FR. A novel multiplex poliovirus binding inhibition assay applicable for large serosurveillance and vaccine studies, without the use of live poliovirus. *Journal of virological methods* 2017; 241: 15-23.

9. Stark K, Schonfeld C, Barg J, Molz B, Vornwald A, Bienzle U. Seroprevalence and determinants of diphtheria, tetanus and poliomyelitis antibodies among adults in Berlin, Germany. *Vaccine* 1999; 17: 844-50.

10. Hainz U, Jenewein B, Asch E, Pfeiffer KP, Berger P, Grubeck-Loebenstein B. Insufficient protection for healthy elderly adults by tetanus and TBE vaccines. *Vaccine* 2005; 23: 3232-5.

11. Gergen PJ, McQuillan GM, Kiely M, Ezzati-Rice TM, Sutter RW, Virella G. A population-based serologic survey of immunity to tetanus in the United States. *The New England journal of medicine* 1995; 332: 761-6.

12. Klein SL, Flanagan KL. Sex differences in immune responses. *Nature reviews Immunology* 2016; 16: 626-38.

13. Fish EN. The X-files in immunity: sex-based differences predispose immune responses. *Nature reviews Immunology* 2008; 8: 737-44.

14. Zimmermann P, Curtis N. The influence of the intestinal microbiome on vaccine responses. *Vaccine* 2018; 36: 4433-9.

15. Garcia-Gomez E, Gonzalez-Pedrajo B, Camacho-Arroyo I. Role of sex steroid hormones in bacterial-host interactions. *BioMed research international* 2013; 2013: 928290.

16. Fransen F, van Beek AA, Borghuis T, Meijer B, Hugenholtz F, van der Gaast-de Jongh C, et al. The Impact of Gut Microbiota on Gender-Specific Differences in Immunity. *Frontiers in immunology* 2017; 8: 754.

17. Vemuri R, Sylvia KE, Klein SL, Forster SC, Plebanski M, Eri R, et al. The microgenderome revealed: sex differences in bidirectional interactions between the microbiota, hormones, immunity and disease susceptibility. *Semin Immunopathol* 2018; :

18. Zimmermann P, Curtis N. Factors Influencing the Intestinal Microbiome During the First Year of Life. *Pediatr Infect Dis J* 2018; :

19. Voysey M, Kelly DF, Fanshawe TR, Sadarangani M, O'Brien KL, Perera R, et al. The Influence of Maternally Derived Antibody and Infant Age at Vaccination on Infant Vaccine Responses : An Individual Participant Meta-analysis. *JAMA pediatrics* 2017; 171: 637-46.

20. Pickering LK, Granoff DM, Erickson JR, Masor ML, Cordle CT, Schaller JP, et al. Modulation of the immune system by human milk and infant formula containing nucleotides. *Pediatrics* 1998; 101: 242-9.

21. Pabst HF, Spady DW. Effect of breast-feeding on antibody response to conjugate vaccine. *Lancet* 1990; 336: 269-70.

22. Silfverdal SA, Bodin L, Ulanova M, Hahn-Zoric M, Hanson LA, Olcen P. Long term enhancement of the IgG2 antibody response to Haemophilus influenzae type b by breast-feeding. *The Pediatric infectious disease journal* 2002; 21: 816-21.

23. Hahn-Zoric M, Fulconis F, Minoli I, Moro G, Carlsson B, Bottiger M, et al. Antibody responses to parenteral and oral vaccines are impaired by conventional and low protein formulas as compared to breast-feeding. *Acta paediatrica Scandinavica* 1990; 79: 1137-42.

24. Pichichero ME. Effect of breast-feeding on oral rhesus rotavirus vaccine seroconversion: a metaanalysis. *The Journal of infectious diseases* 1990; 162: 753-5.

25. Chilengi R, Simuyandi M, Beach L, Mwila K, Becker-Dreps S, Emperador DM, et al. Association of Maternal Immunity with Rotavirus Vaccine Immunogenicity in Zambian Infants. *PloS one* 2016; 11: e0150100.

26. Glass RI, Ing DJ, Stoll BJ, Ing RT. Immune response to rotavirus vaccines among breast-fed and nonbreast-fed children. *Advances in experimental medicine and biology* 1991; 310: 249-54.

27. Moon SS, Wang Y, Shane AL, Nguyen T, Ray P, Dennehy P, et al. Inhibitory effect of breast milk on infectivity of live oral rotavirus vaccines. *The Pediatric infectious disease journal* 2010; 29: 919-23.

Table 1 Characteristics of study participants

	Total cohort	Samples at 7 months of age for antibodies	Samples at 13 months of age for persistence of antibodies	Samples at 13 months of age for antibodies
		to primary course of vaccines ending at 6 months of age		to 12-month vaccines
	(n=365) n (%) or median (IQR)	(n=91) n (%) or median (IQR)	(n=307) n (% or IQR)	(n=144) n (% or IQR)
Sex (male)	179 (49)	43 (47)	149 (49)	71 (49)
Gestational age (weeks)	39.3 (38.4-40.4)	39.5 (38.6-40.5)	39.3 (38.4-40.3)	39.2 (38.4-40.4)
Birth weight (kg)	3.45 (3.13-3.76)	3.48 (3.10-3.78)	3.45 (3.13-3.76)	3.41 (3.13-3.67)
Caesarean section	136 (37)	35 (38)	115 (37)	53 (37)
Received formula milk in hospital in first week of life	100 (27)	25 (27)	84 (27)	37 (26)
Still receiving breast at 1 month of age	302 (83)	80 (88)	251 (82)	119 (83)
Still receiving breast at 6 months of age	263 (72)	71 (78)	219 (71)	107 (74)
Antibiotics during admission for delivery				
Intrapartum antibiotics	70 (19)	21 (23)	62 (20)	32 (22)
Antibiotics before first vaccination	30 (8)	4 (4)	29 (9)	13 (9)
BCG-vaccinated	198 (54)	45 (49)	166 (54)	76 (53)
Maternal dTpa vaccination in pregnancy	174 (48)	46 (51)	146 (48)	67 (47)
Maternal influenza vaccination in pregnancy	208 (57)	55 (60)	171 (56)	84 (58)
Age at routine vaccination (days)				
- 6-week vaccines	45 (43-494)[1]	45 (43-48)[1]	45 (43-49)	45 (43-48)
- 4-month vaccines	125 (120-131)	123 (117-127)	125 (121-132)	124 (120-131)
- 6-month vaccines	190 (184-202)	187 (181-193)	191 (185-204)	190 (185-201)
- 12-month vaccines	376 (369-383)	-	-	376 (369-383)
Interval between (days)				
- 6-month vaccines and 7-month blood sample	-	28 (21-37)	-	-
- 6-month vaccines and 13-month blood sample	-	-	209 (190-224)	-
- 12-month vaccines and 13-month blood sample	-	-	-	28 (22-36)
Age at blood sampling (days)	-	218 (208-227)	400 (389-418)	406 (397-414)

[1] age for one participant not available

BCG = Bacille Calmette-Guérin

dTpa = diphtheria-tetanus-acellular pertussis vaccine

Table 2a Seroprotection rates at 7 and 13 months of age in female and male infants

		Antibodies to primary course of vaccines ending at 6 months of age measured at 7 months of age				Antibodies to primary course of vaccines ending at 6 months of age measured at 13 months of age			
Vaccine antigen	**Protective correlate**	**Female** (n=48) **% (n); (95% CI)**	**Male** (n=43) **% (n); (95% CI)**	**Difference % (95% CI)**	**Two-sided p-value**	**Female** (n=158) **% (n); (95% CI)**	**Male** (n=149) **% (n); (95% CI)**	**Difference % (95% CI)**	**Two-sided p-value**
Diphtheria	0.1 IU/mL[1]	97.9 (47); (88.9, 99.9)	95.3 (41); (84.1, 99.4)	2.6 (-6.9, 13.7)	0.60	98.7 (156); (95.5, 99.8)	98.0 (146); (94.2, 99.6)	0.7 (-2.7, 4.6)	0.68
Tetanus	0.1 IU/mL[1]	100 (48); (92.6, 100)	100 (43); (91.8, 100)	0 (-7.5, 8.3)	-	100 (85); (96.8, 100)[2]	100 (78); (95.4, 100)[2]	0 (-4.3, 4.7)[2]	-
PT	25 IU/mL	89.6 (43); (77.3, 96.5)	90.7 (39); (77.9, 97.4)	-1.1 (-14.4, 12.7)	1	28.5 (45); (21.6, 36.2)	31.5 (47); (24.2, 39.7)	-3.1 (-13.3, 7.2)	0.62
Hib	0.15 µg/mL	66.7 (32); (51.6, 79.6)	86.0 (37); (72.1, 94.7)	-19.4 (-36.0, -1.7)	0.05	80.0 (68) (69.9, 87.9)[2]	73.1 (57); (61.8, 82.5)[2]	6.9 (-6.1, 20.1)[2]	0.36[2]
Polio type 1	0.23 IU/mL	100 (48); (92.6, 100)	100 (43); (91.8, 100)	0 (-7.5, 8.3)	-	100 (158); (97.7, 100)	100 (149); (97.6, 100)	0 (-2.4, 2.5)	-
Polio type 2	0.29 IU/mL	100 (48); (92.6, 100)	100 (43); (91.8, 100)	0 (-7.5, 8.3)	1	100 (158); (97.7, 100)	99.3 (148); (96.3, 100)	0.7 (-1.7, 3.7)	0.49
Polio type 3	0.12 IU/mL	100 (48); (92.6, 100)	100 (43); (91.8, 100)	0 (-7.5; 8.3)	-	100 (158); (97.7, 100)	100 (149); (97.6, 100)	0 (-2.4, 2.5)	-
Pn 1	0.35 µg/mL	95.8 (46); (85.7, 99.5)	97.7 (42); (87.7, 99.9)	-1.8 (-12.1, 8.4)	1	92.4 (146); (87.1, 96.0)	95.3 (142); (90.6, 98.1)	-2.9 (-8.7, 2.7)	0.35
Pn 3	0.35 µg/mL	97.9 (47); (88.9, 99.9)	100 (43); (91.8, 100)	-2.1 (-11.0, 6.3)	1	70.3 (111); (62.5, 77.3)	69.1 (103); (61.0, 76.4)	1.1 (-9.1, 11.4)	0.90
Pn 4	0.35 µg/mL	66.7 (32); (51.6, 79.6)	72.1 (31); (56.3, 84.7)	-5.4 (-24.0, 13.8)	0.65	24.1 (38); (17.6, 31.5)	21.5 (32); (15.2, 28.9)	2.6 (-6.9, 12.0)	0.68
Pn 5	0.35 µg/mL	95.8 (46); (86.7, 99.5)	100 (43); (85.6, 99.5)	-4.2 (-14.1, 4.3)	0.50	89.2 (141); (83.3, 93.6)	84.6 (126); (77.7, 90.0)	4.7 (-2.9, 12.5)	0.24
Pn 6A	0.35 µg/mL	97.9 (47); (88.9, 99.9)	97.7 (42); (87.7, 99.9)	0.2 (-8.9, 10.3)	1	88.0 (139); (81.9, 92.6)	85.2 (127); (78.5, 90.5)	2.7 (-5.0, 10.6)	0.50
Pn 6B	0.35 µg/mL	83.3 (40); (69.8, 92.5)	95.3 (41); (84.2, 99.4)	-12.0 (-25.8, 1.1)	0.10	68.4 (108); (60.5, 75.5)	59.7 (89); (51.4, 67.7)	8.6 (-2.1, 19.2)	0.12
Pn 7F	0.35 µg/mL	100 (48); (92.6, 100)	100 (43); (91.8, 100)	0 (-7.5, 8.3)	-	99.4 (157); (96.5, 100)	99.3 (148); (96.3, 100)	0 (-2.9, 3.1)	1
Pn 9V	0.35 µg/mL	97.9 (47); (88.9, 99.9)	97.7 (42); (87.7, 99.9)	0.2 (-8.9, 10.2)	1	79.1 (125); (71.9, 85.2)	72.5 (108); (64.6, 79.5)	6.6 (-3.0, 16.2)	0.18
Pn 14	0.35 µg/mL	91.7 (44); (80.0, 97.7)	90.7 (39); (77.8, 97.4)	1.0 (-11.8, 14.6)	1	84.8 (134); (78.2, 90.0)	87.9 (131); (81.2, 92.3)	-3.1 (-10.9, 4.7)	0.51
Pn 18C	0.35 µg/mL	95.8 (46); (85.7, 99.5)	95.3 (41); (84.2, 99.4)	0.5 (-10.0, 11.9)	1	78.5 (124); (71.2, 84.6)	75.2 (112); (67.4, 81.9)	3.3 (-6.2, 12.8)	0.50
Pn 19A	0.35 µg/mL	93.8 (45); (82.8, 98.7)	93.0 (40); (80.9, 98.5)	0.7 (-11, 13.3)	1	47.5 (75); (39.5, 55.6)	52.3 (78); (44.0, 60.6)	-4.9 (-15.9, 6.3)	0.42
Pn 19F	0.35 µg/mL	100 (46); (92.6, 100)	100 (43); (91.8, 100)	0 (-7.5; 8.3)	-	98.7 (156); (95.5, 99.8)	100 (149); (97.6, 100)	-1.3 (-4.5, 1.3)	0.50
Pn 23F	0.35 µg/mL	91.7 (44); (80.0, 97.7)	95 (41); (84.2, 99.4)	-3.7 (-15.7, 8.3)	0.68	62.7 (99); (54.6, 70.2)	59.7 (89); (51.4, 67.7)	2.9 (-8.0, 13.8)	0.64
						Antibodies to 12-month vaccines measured 13 months of age			
						Female (n=73) **% (n); (95% CI)**	**Male** (n=71) **% (n); (95% CI)**	**Difference % (95% CI)**	**Two-sided p-value**
Measles	0.12 IU/mL					100 (73); (95.1, 100)	95.8 (68); (88.1, 99.1)	4.2 (-0.9, 11.7)	0.12
Mumps	45 IU/mL					58.9 (43); (46.8, 70.3)	62.0 (44); (49.7, 73.2)	-3.1 (-18.8, 12.9)	0.74
Rubella	10 IU/mL					91.8 (67); (83.0, 96.9)	81.7 (58); (70.7, 89.9)	10.1 (-1.1, 21.8)	0.09
MenC	2 µg/mL					100 (73); (95.1, 100)	98.6 (70); (92.4, 100)	1.4 (-3.7, 7.6)	0.49
Hib	0.15 µg/mL					98.6 (72); (92.6, 100)	98.6 (70); (92.5, 100)	0 (-6.1, 6.4)	1
Tetanus	0.01 IU/mL					100 (73); (95.1, 100)	100 (71); (94.3, 100)	0 (-5.0, 5.1)	-

[1] 0.01 IU/mL at 13 months of age [2] includes only participants who have not had Hib-MenC

BCG = Bacille Calmette-Guérin, CI = confidence interval, Hib = *H. influenzae* type b, MenC = meningococcus C, Pn = pneumococcus serotype, PT = pertussis toxin

Table 2b Geometric mean antibody concentrations (GMCs) and geometric mean antibody ratios (GMRs) at 7 and 13 months of age in female and male infants

	Antibodies to primary course of vaccines ending at 6 months of age measured at 7 months of age						Antibodies to primary course of vaccines ending at 6 months of age measured at 13 months of age					
Vaccine antigen	**Female** (n=48) **GMC (95% CI)**	**Male** (n=43) **GMC (95% CI)**	**Unadjusted GMR (95% CI)**	**Two-sided p-value**	**Adjusted* GMR (95% CI)**	**Two-sided p-value**	**Female** (n=158) **GMC (95% CI)**	**Male** (n=149) **GMC (95% CI)**	**Unadjusted GMR (95% CI)**	**Two-sided p-value**	**Adjusted** GMR (95% CI)**	**Two-sided p-value**
Diphtheria[1]	0.38 (0.30, 0.48)	0.41 (0.33, 0.52)	0.92 (0.67, 1.27)	0.61	0.98 (0.72, 1.32)	0.88	0.08 (0.07, 0.09)	0.08 (0.06, 0.09)	1.05 (0.83, 1.32)	0.69	1.12 (0.91, 1.38)	0.29
Tetanus[1]	0.98 (0.82, 1.18)	1.27 (1.01, 1.60)	0.77 (0.58, 1.03)	0.08	**0.72 (0.54, 0.97)**	**0.03**	0.41 (0.32, 0.52)[3]	0.48 (0.37, 0.61)[3]	0.85 (0.60, 1.22)[3]	0.38[3]	0.78 (0.56, 1.10)[3]	0.16
PT[1]	63.68 (51.21, 79.18)	77.99 (60.10, 101.22)	0.82 (0.58, 1.13)	0.23	0.85 (0.62, 1.17)	0.31	16.67 (14.26, 19.47)	18.19 (15.37, 21.54)	0.92 (0.73, 1.15)	0.45	0.98 (0.80, 1.21)	0.87
FHA[1]	**52.41 (42.42, 64.76)**	**79.28 (60.59, 103.73)**	**0.66 (0.47, 0.92)**	**0.02**	**0.71 (0.51, 0.98)**	**0.04**	**16.96 (14.50, 19.83)**	**21.17 (18.28, 24.52)**	**0.80 (0.65, 0.99)**	**0.04**	0.85 (0.69, 1.04)	0.11
PRN[1]	54.00 (42.26, 69.00)	68.66 (49.36, 95.50)	0.79 (0.53, 1.17)	0.24	0.80 (0.53, 1.21)	0.29	**10.13 (8.39, 12.24)**	**14.24 (11.73, 17.28)**	**0.71 (0.54, 0.93)**	**0.02**	**0.73 (0.57, 0.95)**	**0.02**
Hib[2]	0.40 (0.25, 0.64)	0.68 (0.44, 1.06)	0.58 (0.30, 1.11)	0.10	0.66 (0.35, 1.26)	0.21	1.5 (0.80, 2.51)[3]	0.82 (0.51, 1.33)[3]	1.82 (0.90, 3.70)[3]	0.09[3]	1.51 (0.88, 2.61)[3]	0.14[3]
Polio type 1[1]	33.75 (24.34, 46.81)	35.59 (26.50, 47.80)	0.95 (0.61, 1.47)	0.81	1.04 (0.66, 1.65)	0.86	11.07 (9.28, 13.19)	11.32 (9.41, 13.61)	0.98 (0.76, 1.26)	0.86	1.03 (0.80, 1.32)	0.82
Polio type 2[1]	65.36 (49.45, 86.40)	60.43 (42.14, 86.66)	1.08 (0.69, 1.69)	0.73	1.14 (0.73, 1.78)	0.57	22.73 (18.74, 27.57)	20.46 (16.54, 25.31)	1.11 (0.83, 1.48)	0.47	1.18 (0.89, 1.56)	0.25
Polio type 3[1]	23.43 (17.03, 32.24)	19.41 (13.69, 27.52)	1.21 (0.76, 1.92)	0.42	1.24 (0.76, 2.01)	0.38	10.17 (8.29, 12.48)	7.71 (6.25, 9,53)	1.32 (0.98, 1.77)	0.06	**1.39 (1.04, 1.84)**	**0.03**
Pn 1[2]	3.97 (2.86, 5.50)	4.87 (3.70, 6.40)	0.82 (0.53, 1.25)	0.34	0.89 (0.59, 1.32)	0.55	1.18 (1.03, 1.35)	1.15 (1.00, 1.33)	1.03 (0.85, 1.25)	0.78	1.06 (0.87, 1.28)	0.58
Pn 3[2]	1.37 (1.15, 1.64)	1.52 (1.21, 1.90)	0.90 (0.68, 1.20)	0.48	0.91 (0.68, 1.21)	0.51	0.57 (0.50, 0.65)	0.50 (0.44, 0.57)	1.14 (0.95, 1.37)	0.15	1.17 (0.98, 1.39)	0.09
Pn 4[2]	0.53 (0.41, 0.69)	0.50 (0.40, 0.63)	1.06 (0.75, 1.51)	0.73	1.09 (0.77, 1.54)	0.61	0.22 (0.20, 0.25)	0.21 (0.19, 0.23)	1.05 (0.90, 1.22)	0.52	1.07 (0.92, 1.24)	0.39
Pn 5[2]	2.91 (2.17, 3.89)	3.46 (2.71, 4.43)	0.84 (0.57, 1.23)	0.36	0.92 (0.63, 1.33)	0.65	0.96 (0.83, 1.12)	0.88 (0.76, 1.02)	1.09 (0.89, 1.34)	0.41	1.14 (0.93, 1.39)	0.22
Pn 6A[2]	4.82 (3.66, 6.34)	4.81 (3.81, 6.08)	1.00 (0.70, 1.44)	1.00	1.01 (0.72, 1.42)	0.97	1.08 (0.93, 1.25)	0.93 (0.78, 1.10)	1.16 (0.93, 1.45)	0.19	**1.24 (1.01, 1.53)**	**0.04**
Pn 6B[2]	1.64 (1.04, 2.59)	2.27 (1.46, 3.54)	0.72 (0.38, 1.36)	0.31	0.72 (0.38, 1.34)	0.29	0.56 (0.47, 0.68)	0.47 (0.39, 0.57)	1.20 (0.93, 1.56)	0.17	1.25 (0.97, 1.61)	0.08
Pn 7F[2]	5.72 (4.51, 7.27)	6.62 (5.31, 8.26)	0.86 (0.63, 1.20)	0.38	0.85 (0.62, 1.15)	0.29	1.93 (1.70, 2.18)	1.83 (1.61, 2.07)	1.06 (0.89, 1.26)	0.55	1.09 (0.93, 1.30)	0.29
Pn 9V[2]	2.40 (1.83, 3.16)	2.52 (2.01, 3.16)	0.95 (0.67, 1.36)	0.79	0.99 (0.71, 1.37)	0.94	0.63 (0.55, 0.72)	0.57 (0.49, 0.65)	1.12 (0.92, 1.36)	0.26	1.16 (0.96, 1.40)	0.12
Pn 14[2]	2.54 (1.82, 3.56)	2.81 (1.86, 4.26)	0.90 (0.54, 1.53)	0.70	0.88 (0.52, 1.51)	0.65	1.09 (0.93, 1.29)	1.00 (0.85, 1.18)	1.09 (0.87, 1.37)	0.46	1.13 (0.90, 1.43)	0.28
Pn 18C[2]	2.68 (1.96, 3.65)	3.27 (2.59, 4.13)	0.82 (0.55, 1.21)	0.31	0.83 (0.56, 1.22)	0.35	0.69 (0.60, 0.78)	0.62 (0.54, 0.71)	1.11 (0.92, 1.34)	0.28	1.16 (0.97, 1.40)	0.10
Pn 19A[2]	1.77 (1.34, 2.34)	1.67 (1.25, 2.22)	1.06 (0.71, 1.58)	0.77	1.14 (0.76, 1.69)	0.52	0.48 (0.38, 0.60)	0.45 (0.37, 0.54)	1.06 (0.79, 1.42)	0.70	1.10 (0.83, 1.47)	0.51
Pn 19F[2]	10.20 (7.71, 13.50)	13.22 (10.40, 16.79)	0.77 (0.53, 1.11)	0.17	0.80 (0.56, 1.15)	0.22	3.21 (2.72, 3.79)	3.00 (2.52, 3.58)	1.07 (0.84, 1.36)	0.58	1.11 (0.87, 1.40)	0.40
Pn 23F[2]	2.27 (1.64, 3.15)	2.41 (1.69, 3.44)	0.94 (0.58, 1.52)	0.81	0.96 (0.60, 1.52)	0.85	0.63 (0.52, 0.77)	0.50 (0.41, 0.61)	1.26 (0.96, 1.66)	0.10	1.27 (0.97, 1.68)	0.08

	Antibodies to 12-month vaccines measured 13 months of age					
	Female (n=73) **GMC (95% CI)**	**Male** (n=71) **GMC (95% CI)**	**Unadjusted GMR (95% CI)**	**p-value**	**Adjusted* GMR (95% CI)**	**p-value**
Measles[1]	**3.66 (3.05-4.40)**	**2.49 (1.83-3.41)**	**1.47 (1.03, 2.10)**	**0.03**	**1.45 (1.01, 2.09)**	**0.04**
Mumps[1]	58.93 (43.31, 80.20)	57.40 (42.07, 78.32)	1.03 (0.67, 1.58)	0.90	0.94 (0.65, 1.35)	0.73
Rubella[1]	60.78 (44.83, 82.40)	45.04 (31.74, 63.92)	1.35 (0.85, 2.14)	0.20	1.25 (0.86, 1.81)	0.25
MenC[2]	19.47 (16.47, 23.00)	15.89 (12.84, 19.66)	1.23 (0.94, 1.60)	0.14	1.27 (0.97, 1.65)	0.08
Hib[2]	18.21 (13.08, 25.35)	13.01 (8.74, 19.38)	1.40 (0.84, 2.33)	0.20	1.53 (0.93, 2.53)	0.10
Tetanus[1]	0.83 (0.64, 1.08)	1.19 (0.88, 1.60)	0.70 (0.47, 1.04)	0.07	0.73 (0.49, 1.08)	0.12

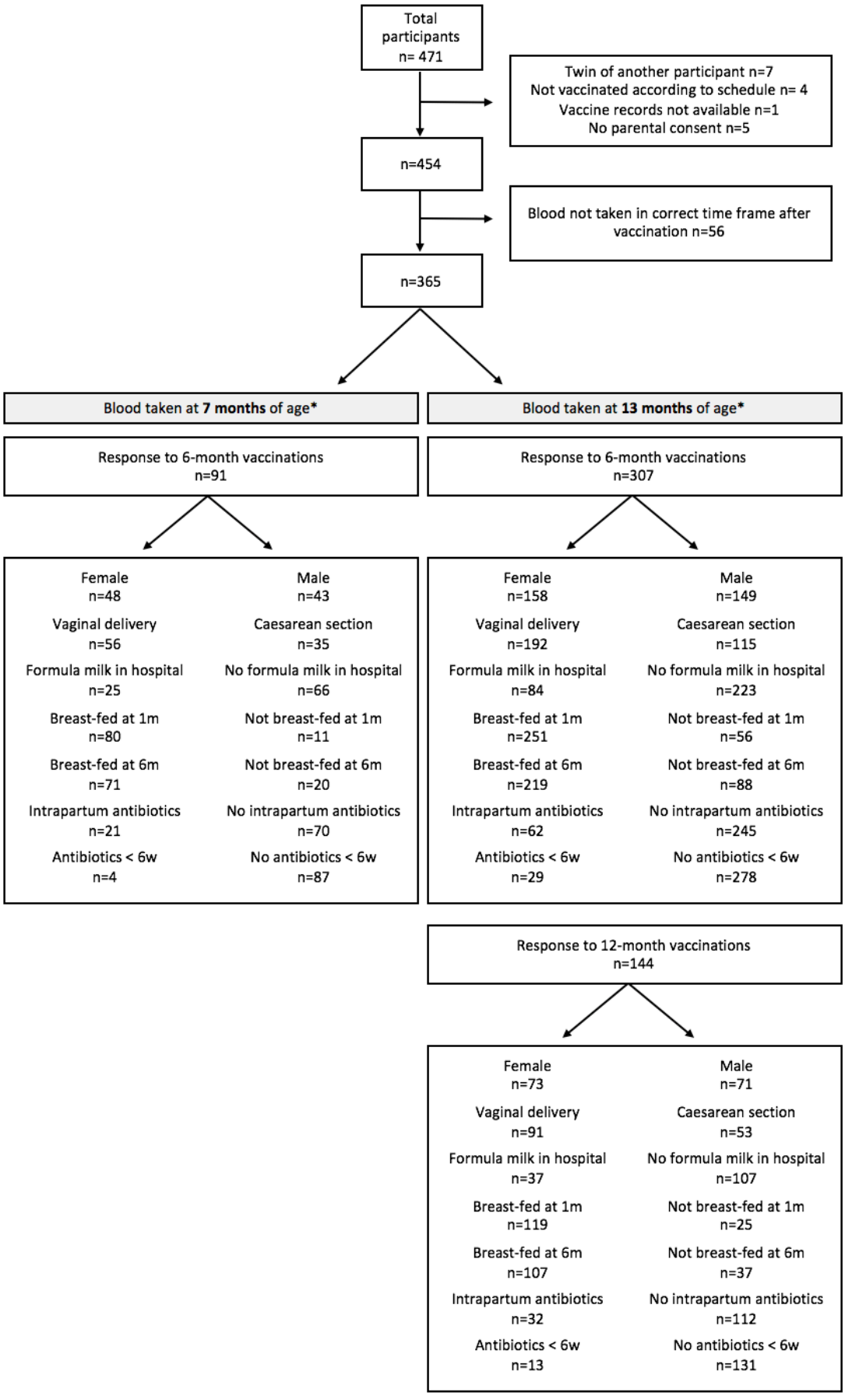
[1] IU/ml [2] µg/ml [3] includes only participants who have not had Hib-MenC

BCG = Bacille Calmette-Guérin, CI = confidence interval, GMC = geometric mean antibody concentration, GMR = geometric mean antibody ratio, FHA = filamentous haemagglutinin, Hib = *H. influenzae* type b, MMR = measles-mumps-rubella, MenC = meningococcus C, Pn = pneumococcus serotype, PRN = pertactin, PT = pertussis toxin

*adjusted for maternal dTpa (diphtheria-tetanus-aceulluar pertussis) and trivalent influenza vaccine during pregnancy, gestational age, BCG vaccination status, age at first DTPa-HepB-IPV-Hib / PCV13 vaccination, age at sampling and time between vaccination and sampling using multiple linear regression

**adjusted for maternal dTpa (diphtheria-tetanus-aceulluar pertussis) and trivalent influenza vaccine during pregnancy, gestational age, BCG and MMR vaccination status, age at first DTPa-HepB-IPV-Hib / PCV13 vaccination, age at sampling and time between vaccination and sampling using multiple linear regression

Figure 1 Selection of participants



* 33 participants had their blood analysed at both 7 and 13 months of age

Figure 2 Seroprotection rates and geometric mean antibody concentrations (GMCs) in female and male infants