Microbial ecology of the infant nasopharynx: Impact of the PCV-7 vaccination

Martin Antonio PhD

MRC Laboratories, The Gambia

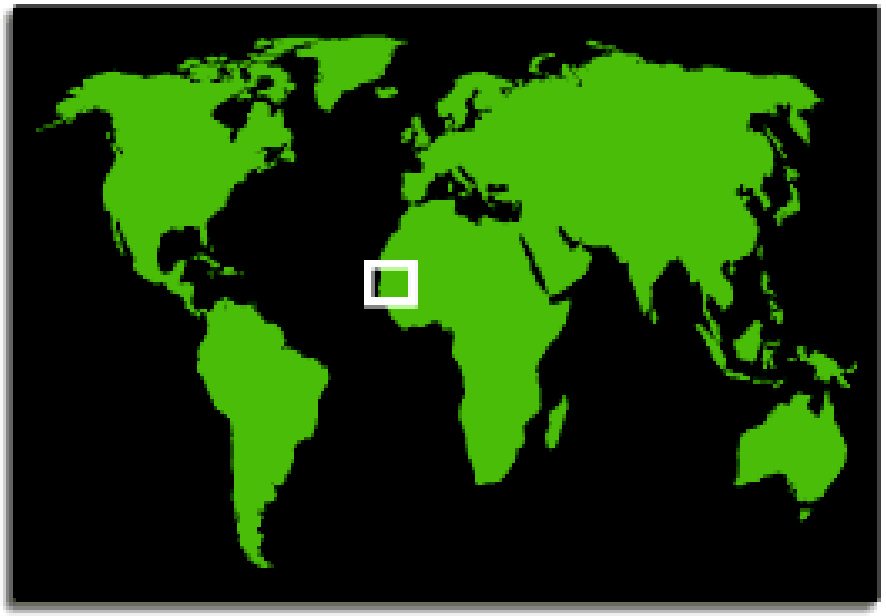
Presentation at

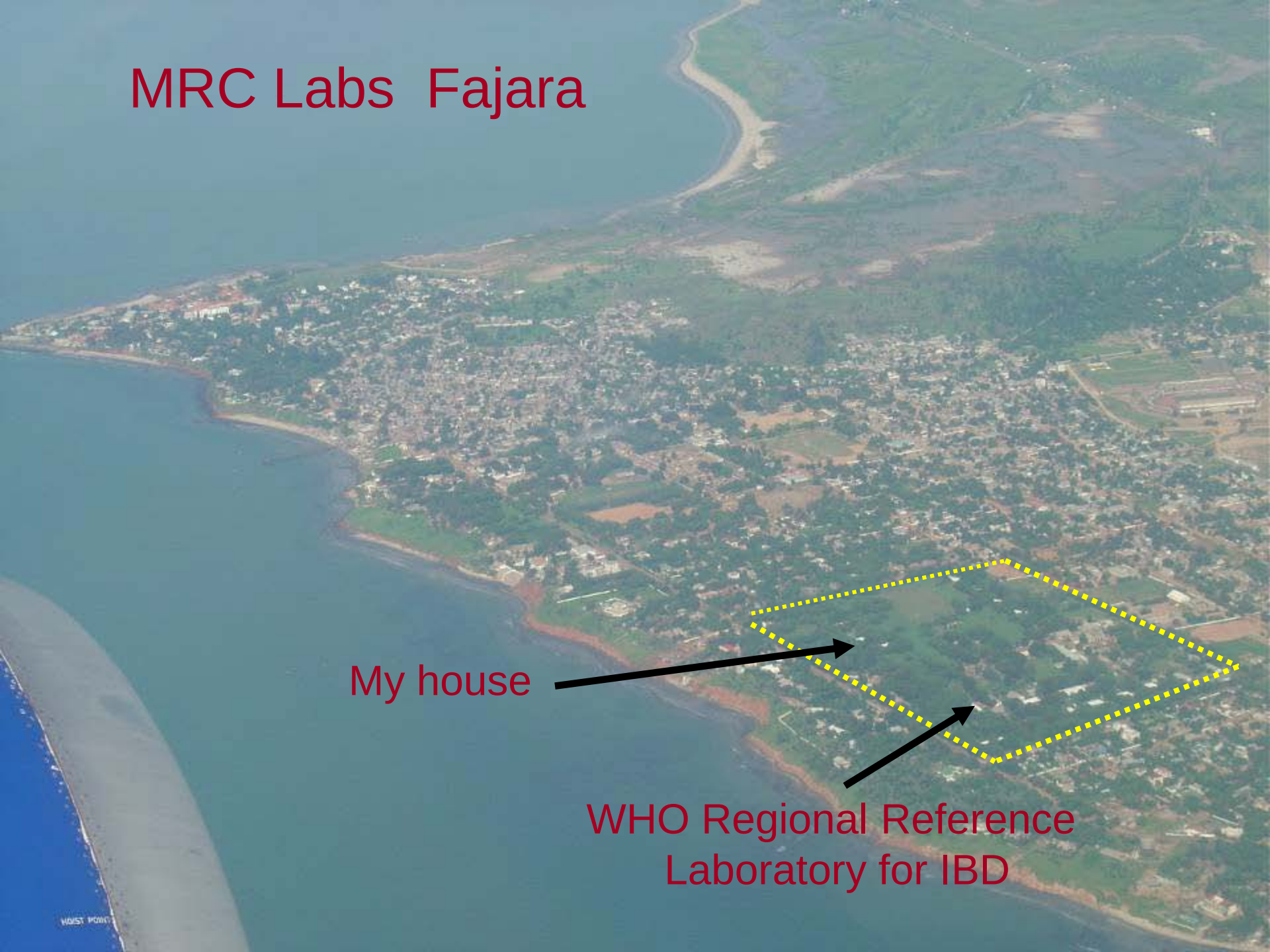
International Human Microbiome Congress

Vancouver, Canada

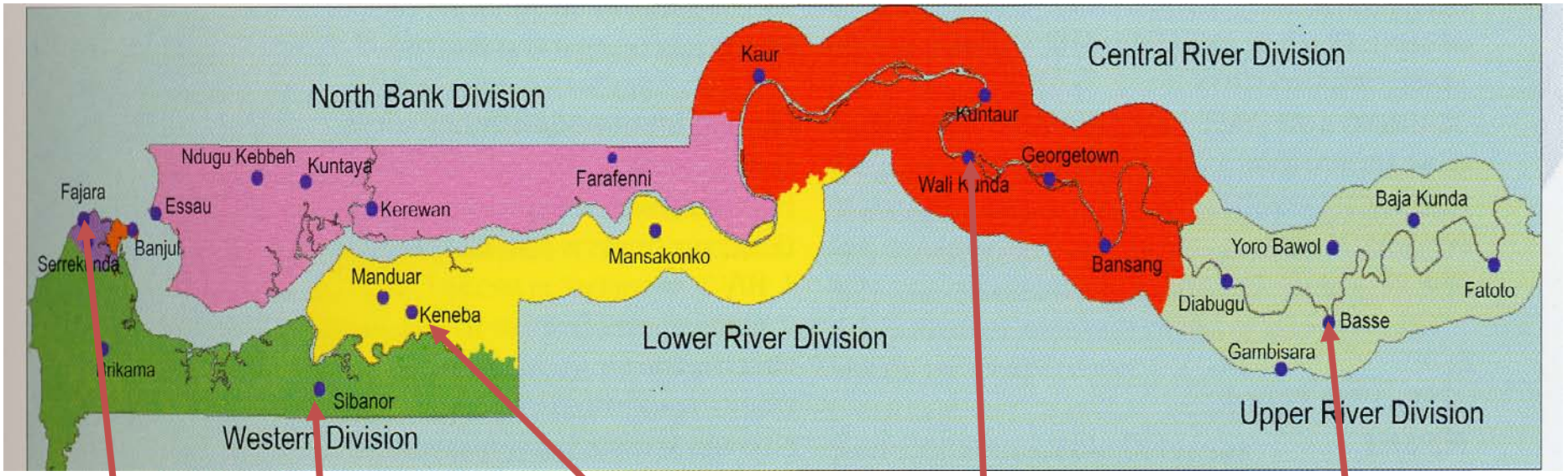
March 9-11, 2011

The Gambia




MRC Labs Fajara
My house
WHO Regional Reference
Laboratory for IBD

MRC Labs and Field Sites



Fajara Study site Keneba Wali Kunda Basse

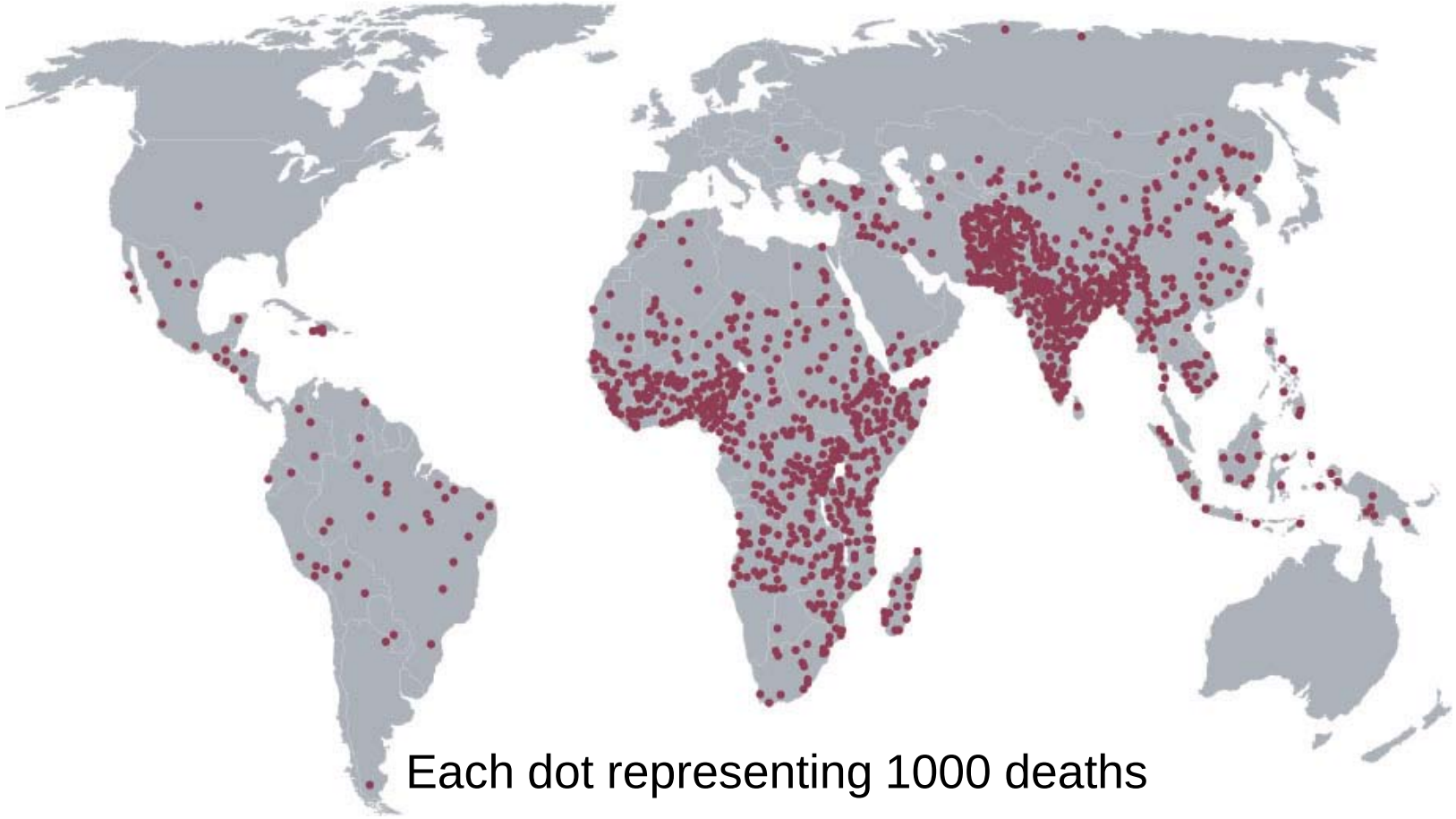
Facilities for research in a rural setting with a high disease burden


BJL 3839 A


MRC
85
THE GAMBIA

Nearly 70% of child pneumonia deaths occur in Africa & South Asia

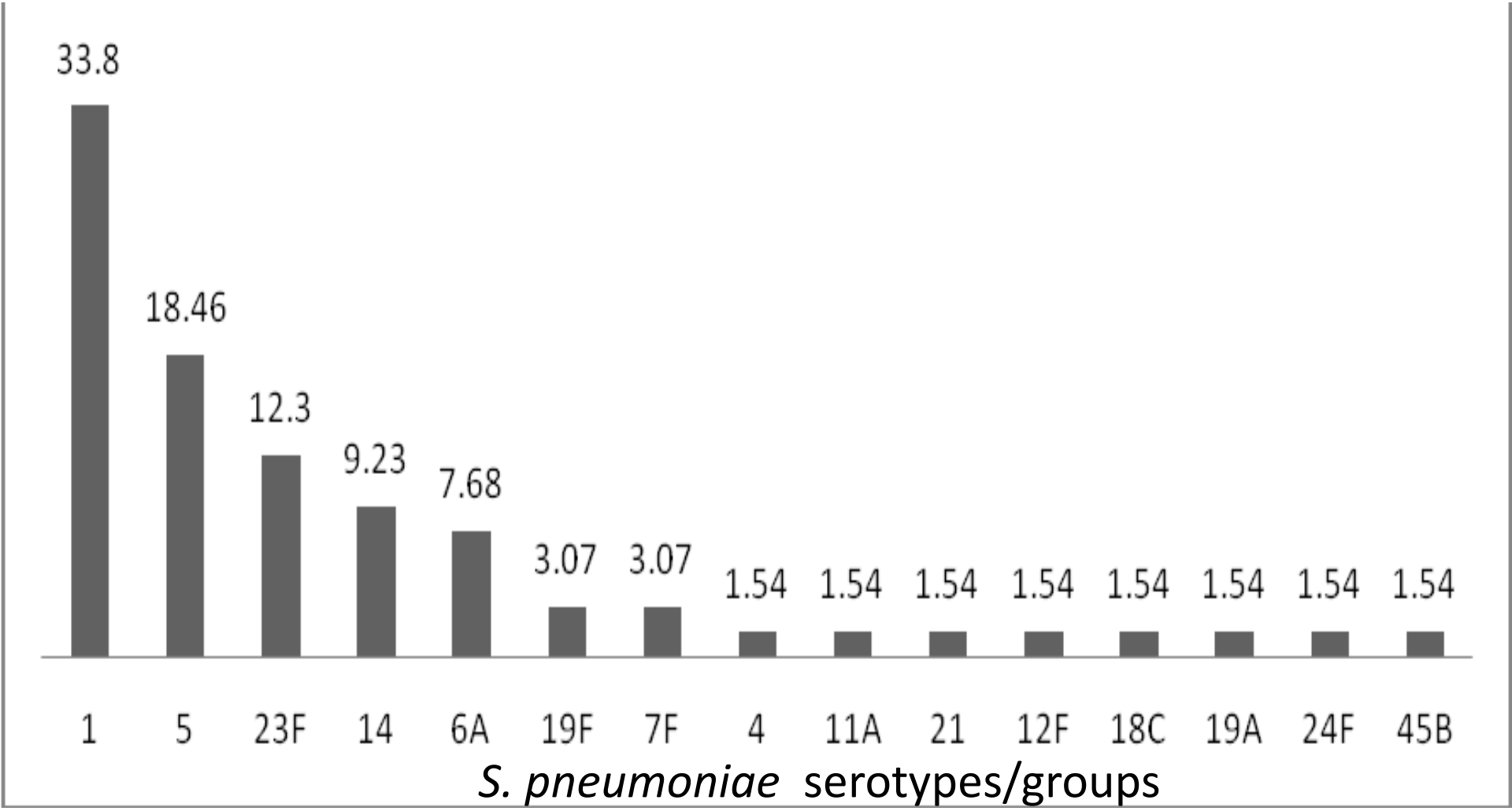
Pneumococcus is the leading cause of child pneumonia deaths (~40%)



Each dot representing 1000 deaths

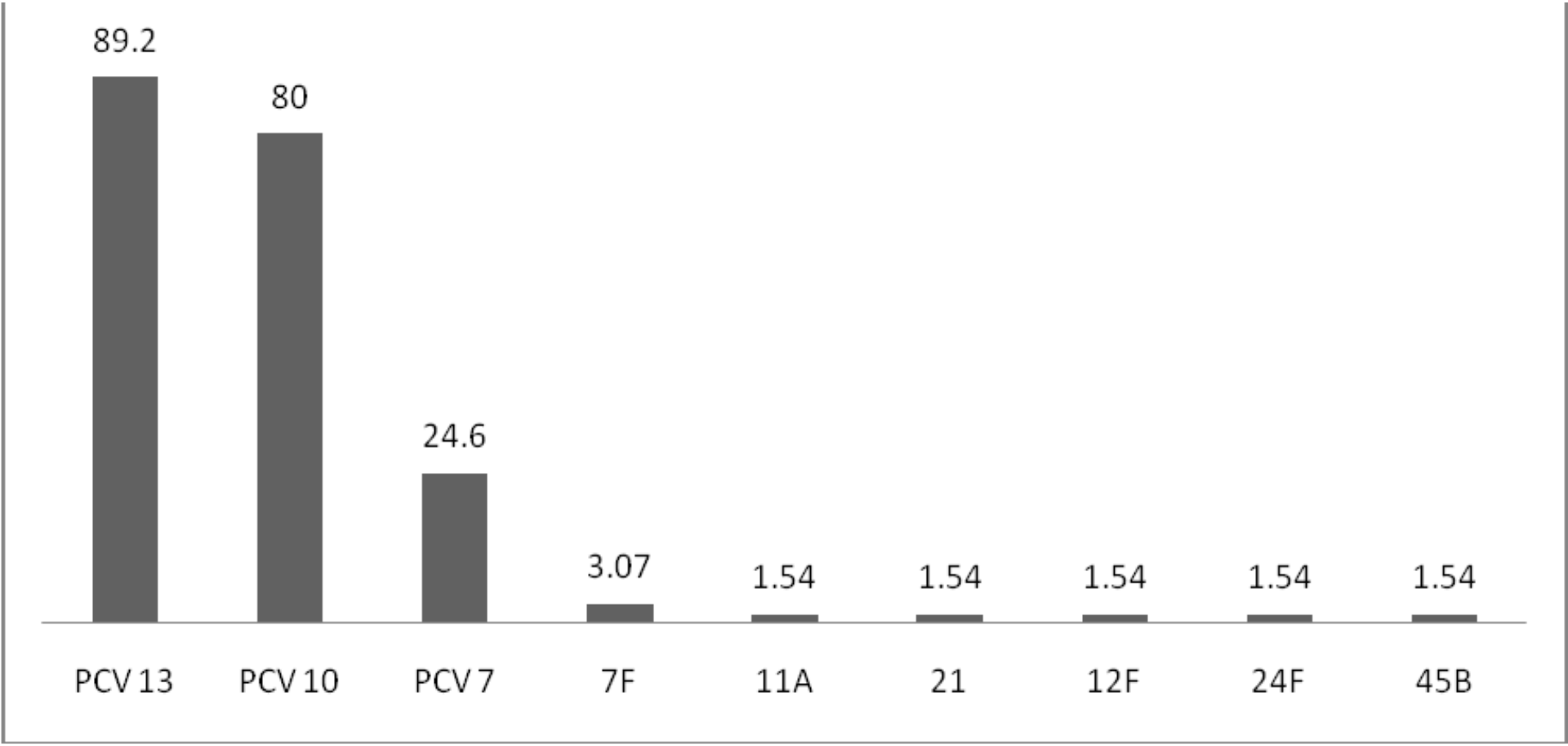
(Williams BG et al Lancet 2002)

Pneumococcal serotype (%) distribution from pneumoWAR sites (2010)



Source: Dr Martin Antonio, pneumoWAR

PCV13, PCV10 & PCV 7 coverage (%) in West Africa (2010)



S. pneumoniae serotypes/groups

Source: Dr Martin Antonio, pneumoWAR

Streptococcus pneumoniae

- The available licensed pneumococcal conjugate vaccines (Prevenar®) contain either 7 or 13 of the 94 pneumococcal serotypes

Concerns:

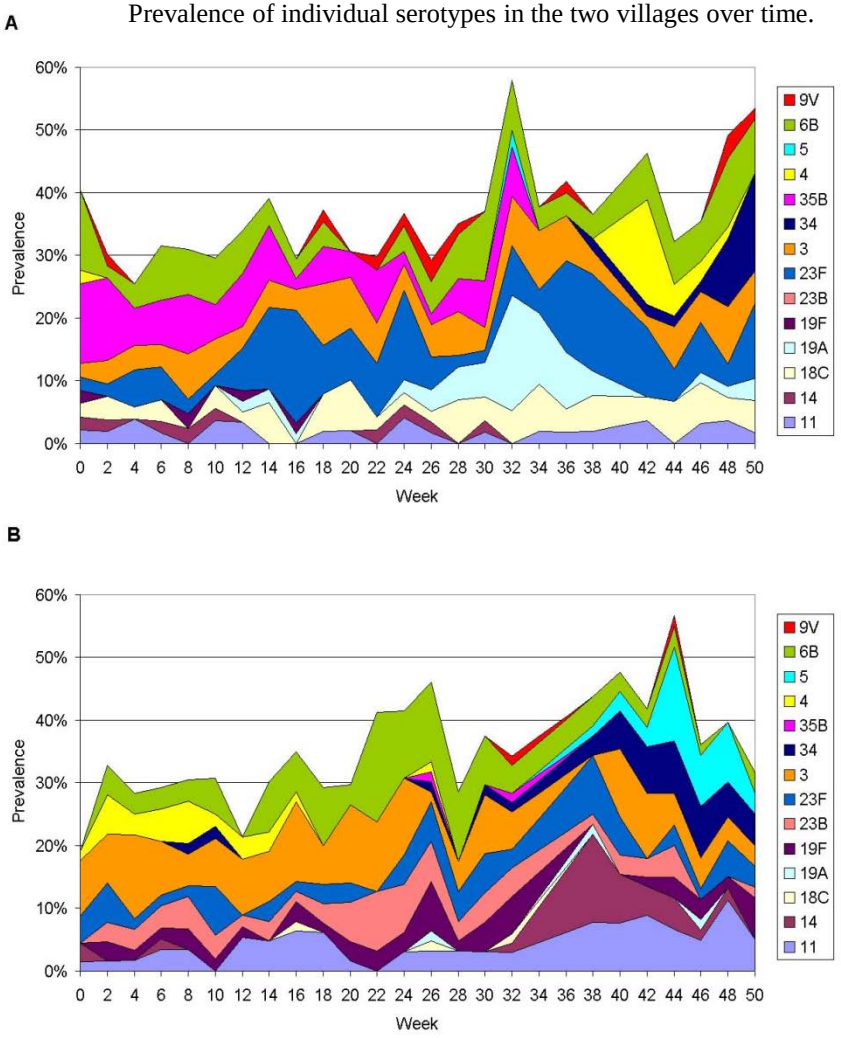
- Replacement colonisation by non-vaccine serotypes and a significant increase in IPD caused by these bacteria

- An occurrence of species replacement could be of public health concern

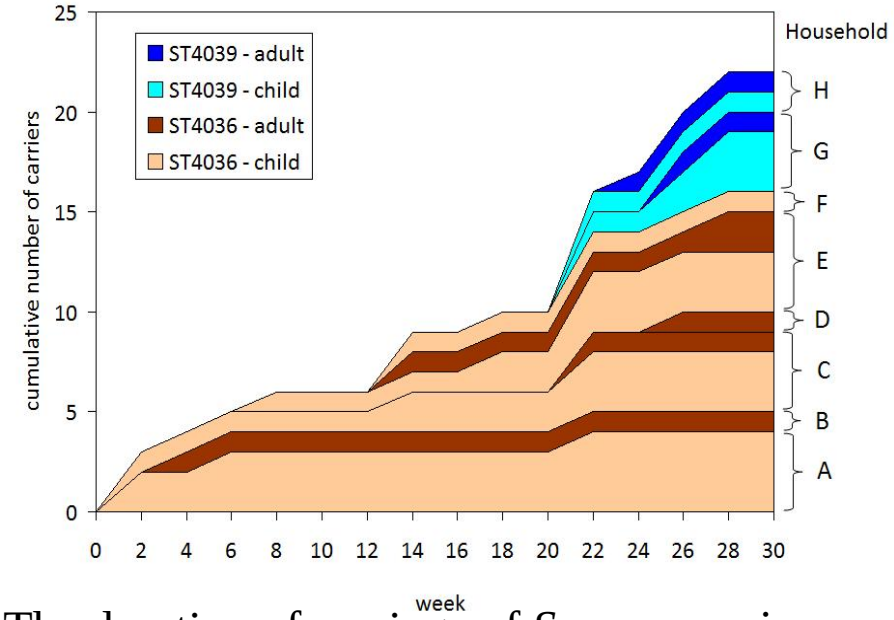
- The ecological events that occur after elimination of vaccine serotypes are unclear

Transmission of *Streptococcus pneumoniae* in rural Gambian villages: a longitudinal study

Prevalence of individual serotypes in the two villages over time.



Cumulative number of individuals in village B who had been carriers of ST4036 or ST4039, on MLST, up to and including the stated week in each of 8 households (A to H).

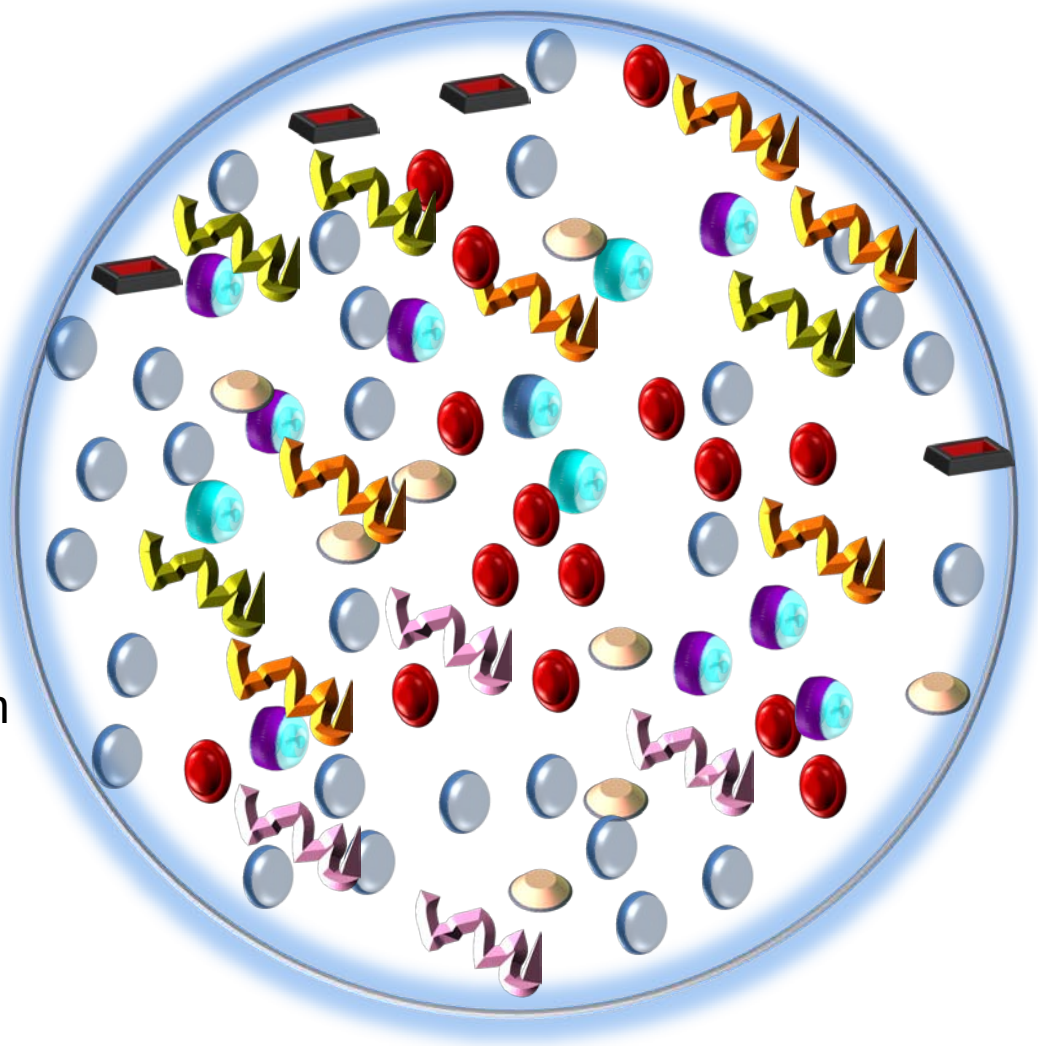


1. The duration of carriage of *S. pneumoniae* varies significantly by serotype.
2. Intra-household transmission is more important
3. than community transmission.
4. Serotype and sequence type specific analyses suggest children tend to bring *S. pneumoniae* into the household with subsequent spread among other children and adult members.

Nasopharyngeal Microbial Ecology and PCV-7

AIMS

- Investigate the impact of vaccination with a polysaccharide conjugate vaccine of limited valency on the nasopharyngeal microbiome
- Characterization of the development and composition of the nasopharyngeal microbiome in rural Gambian infants in the first twelve months of life



Brenda Kwambana
Graduate student

Studying the Nasopharyngeal Microbiota

- Sterile Calcium alginate swabs with aluminium shafts used (WHO recommended)
- Nasopharyngeal swabs are collected by sterile technique and stored in STGG
- NPS swabs are stored at -70 degrees Celsius

Trained field nurse prepares study infant for nasopharyngeal (NP) swabbing

The nurse collects the Nurse collects the NP swab by carefully inserting the swab in the nasopharynx, waiting 5 seconds and then rotating it 360 degrees before careful removal

Mother consoles baby while the trained field worker collects metadata including antibiotic use, respiratory tract infections, ear infections and travel and dietary information.

Environmental Factors that may influence the infant nasopharyngeal microbiota

MRC Medical Research Council

Overcrowding and exposure to many children

Malnutrition

MRC (UK) The Gambia

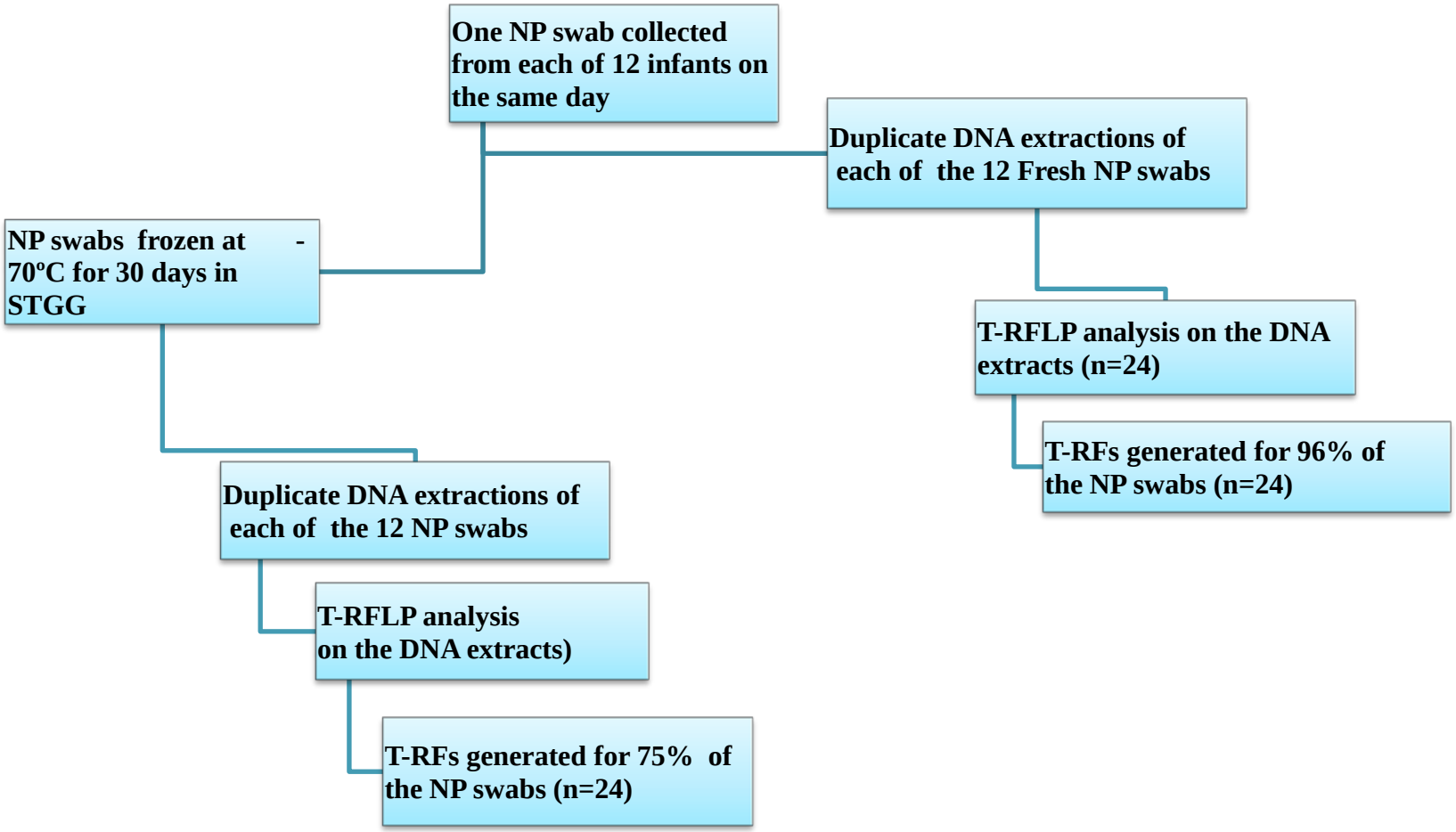
Diet

Proximity to domestic animals and livestock

Seasonal effects

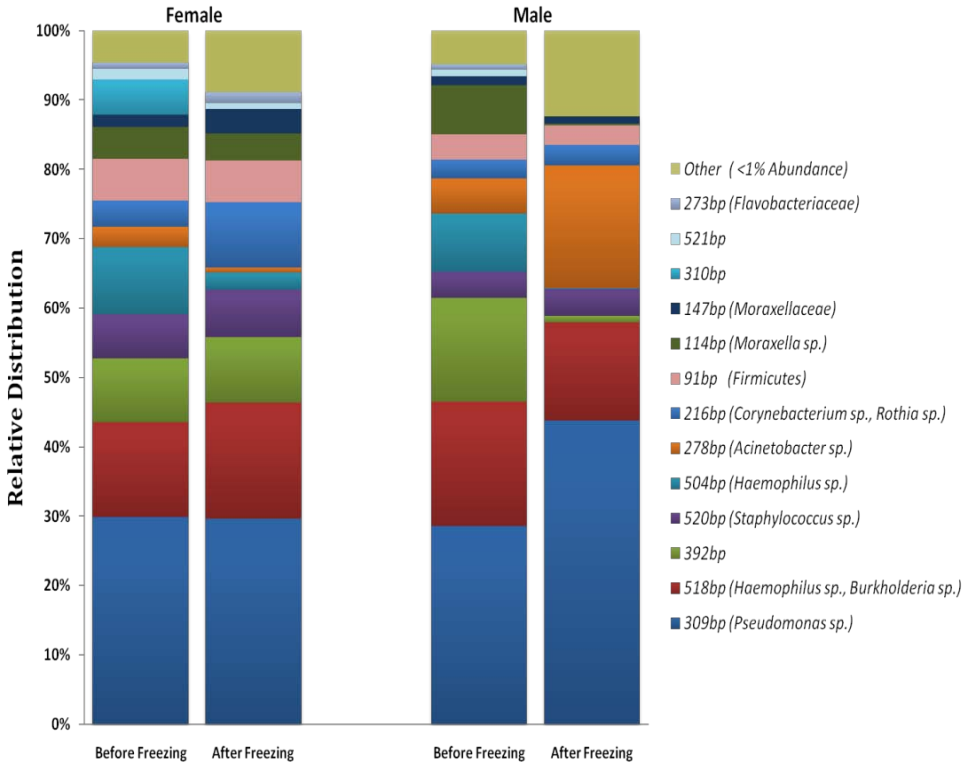
Breastfeeding

Effect of frozen-storage (-70°C) on the detection of bacterial taxa

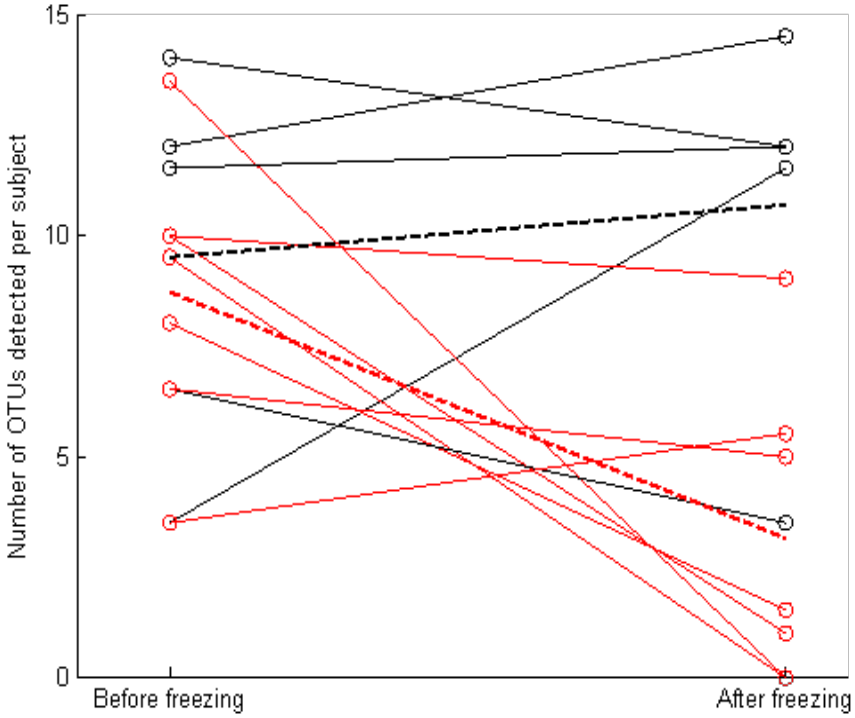


Kwambana et al. BMC Clin Pathol. 2011 Jan 24;11:2.

Differential effect of frozen-storage



Relative distribution of the bacterial OTUs detected before and after frozen storage of NP swabs at -70^{o}C amongst male and female infants

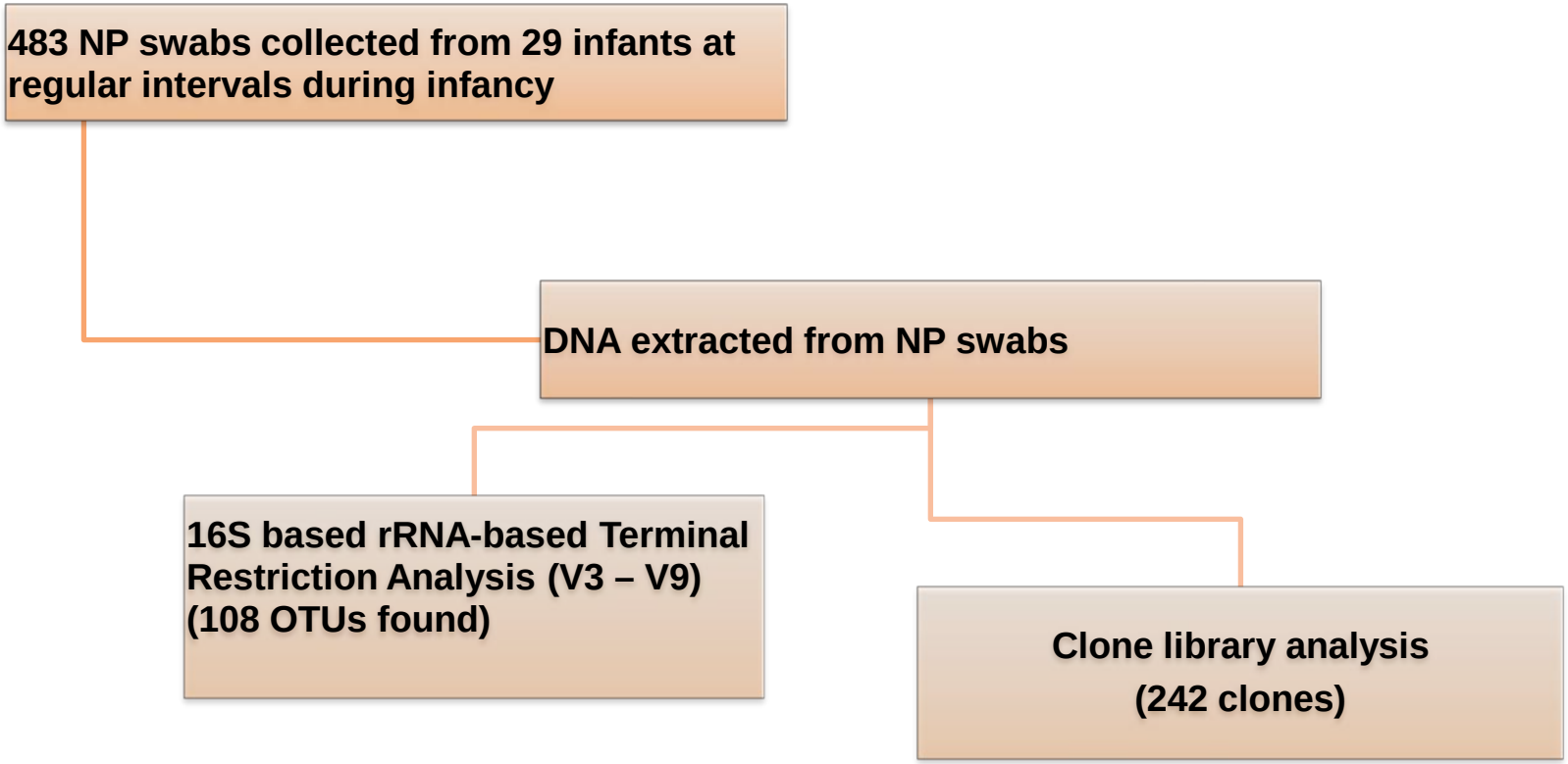


Bacterial OTU richness before and after freezing dichotomized by gender. Red lines represent females, and dotted lines show the mean change.

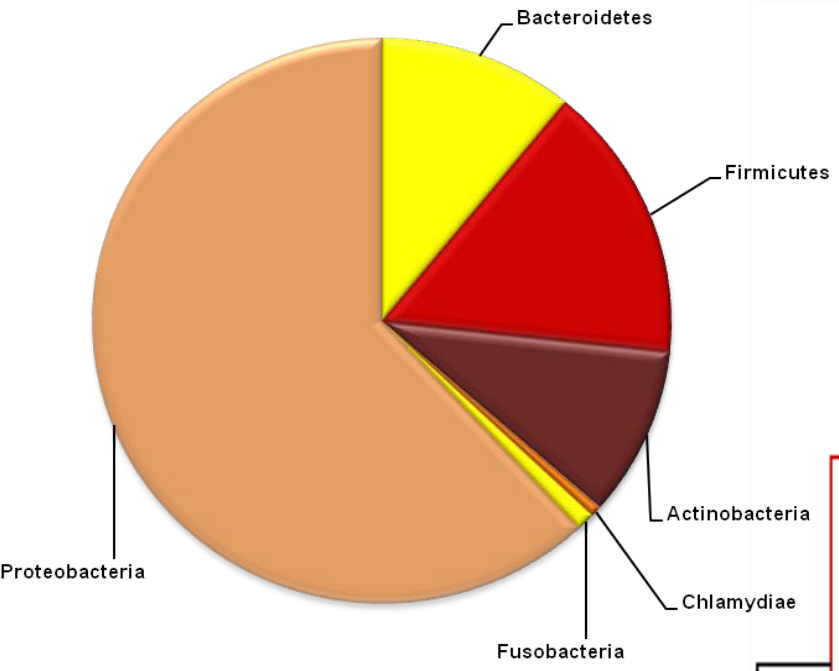
The difference in composition pre and post freezing was significant for female (p = 0.0014) but not for male infants (p=0.56).

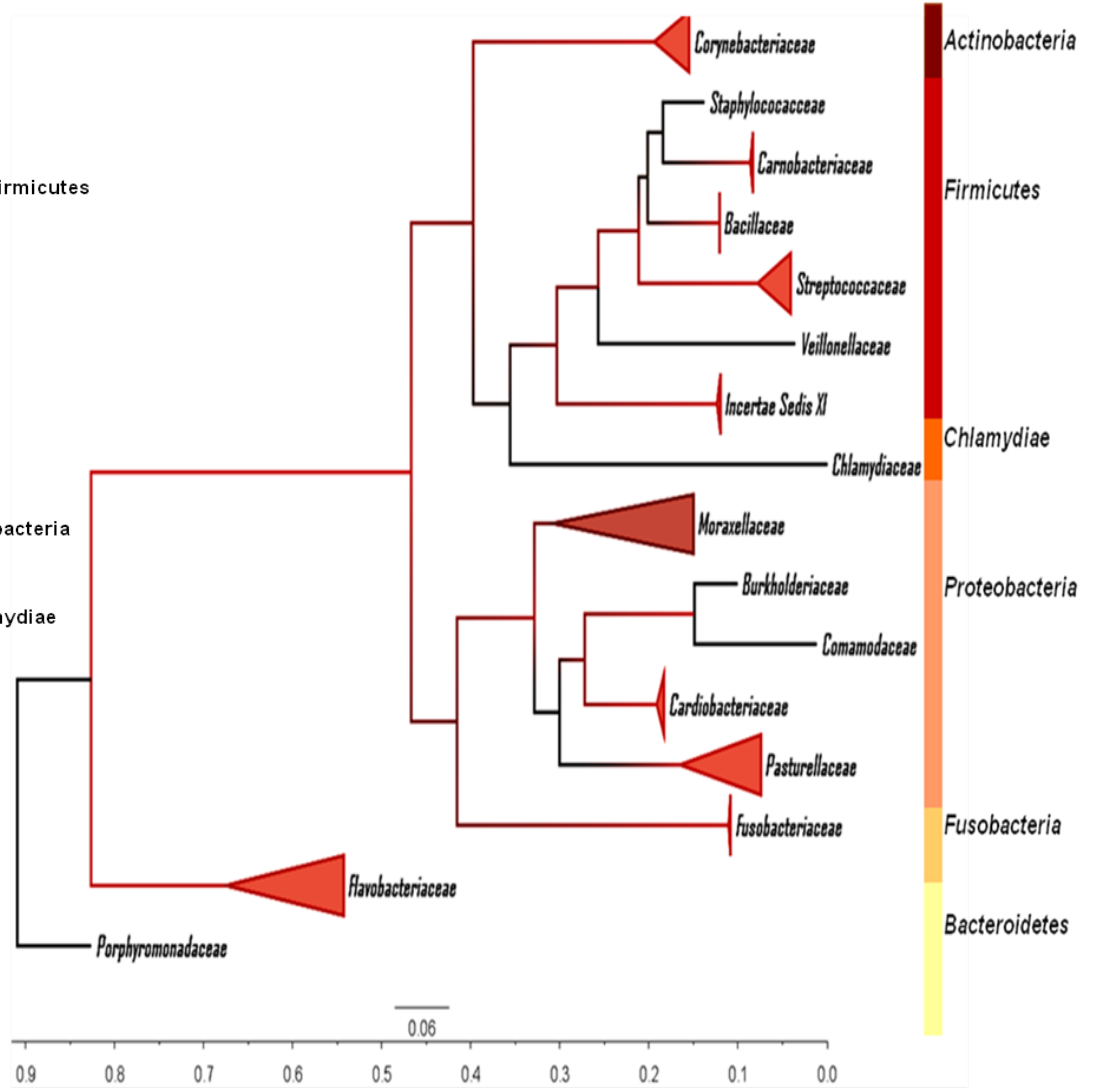
Kwambana et al. BMC Clin Pathol. 2011 Jan 24;11:2.

T-RFLP Comparative community analysis

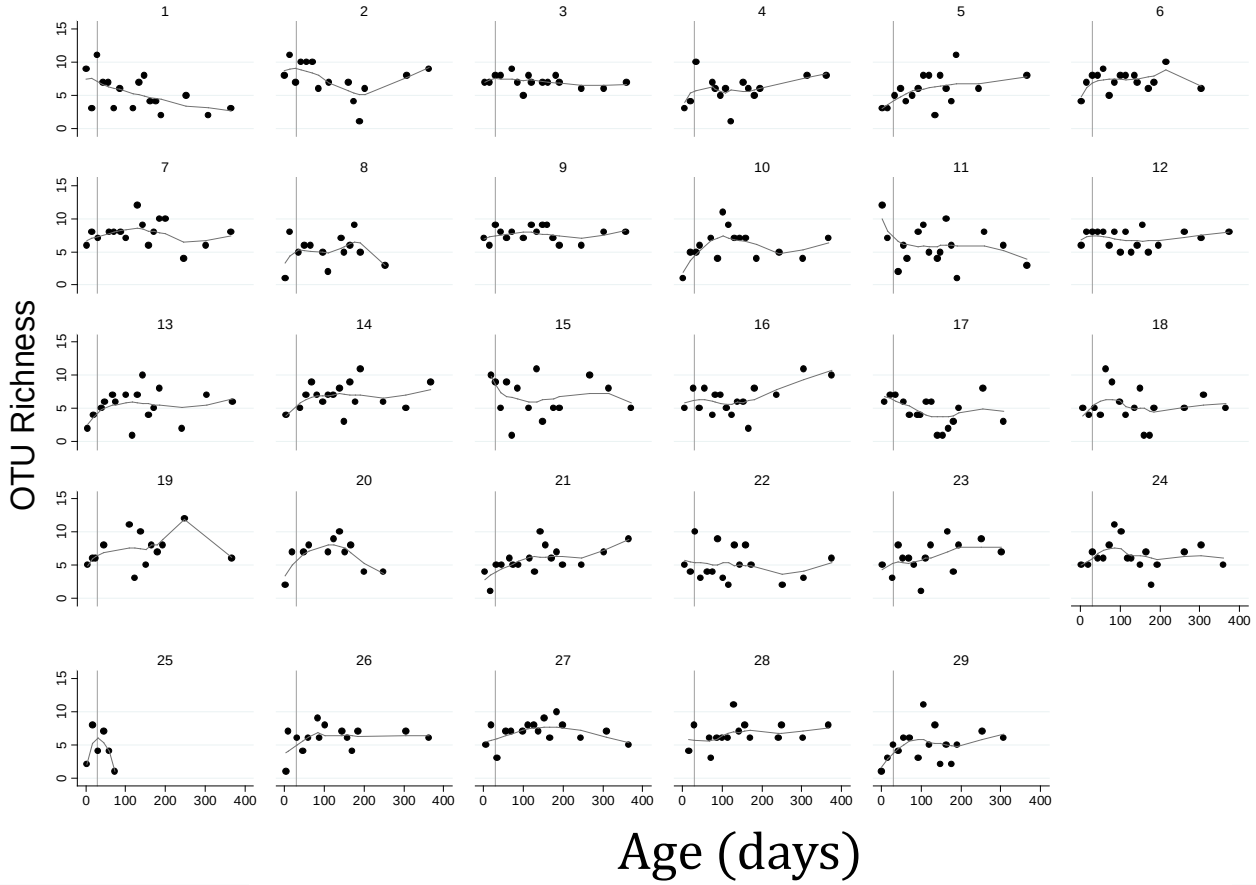


The infant nasopharyngeal microbiome (T-RFLP Analysis)



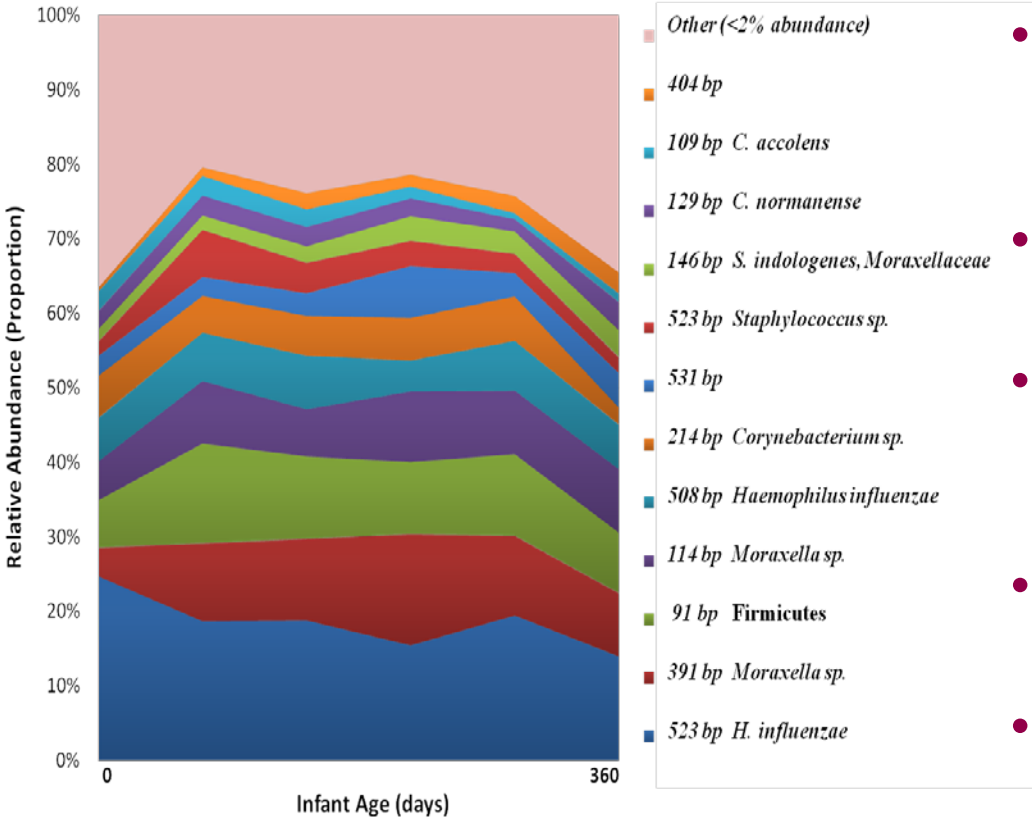


OTU Richness during infancy



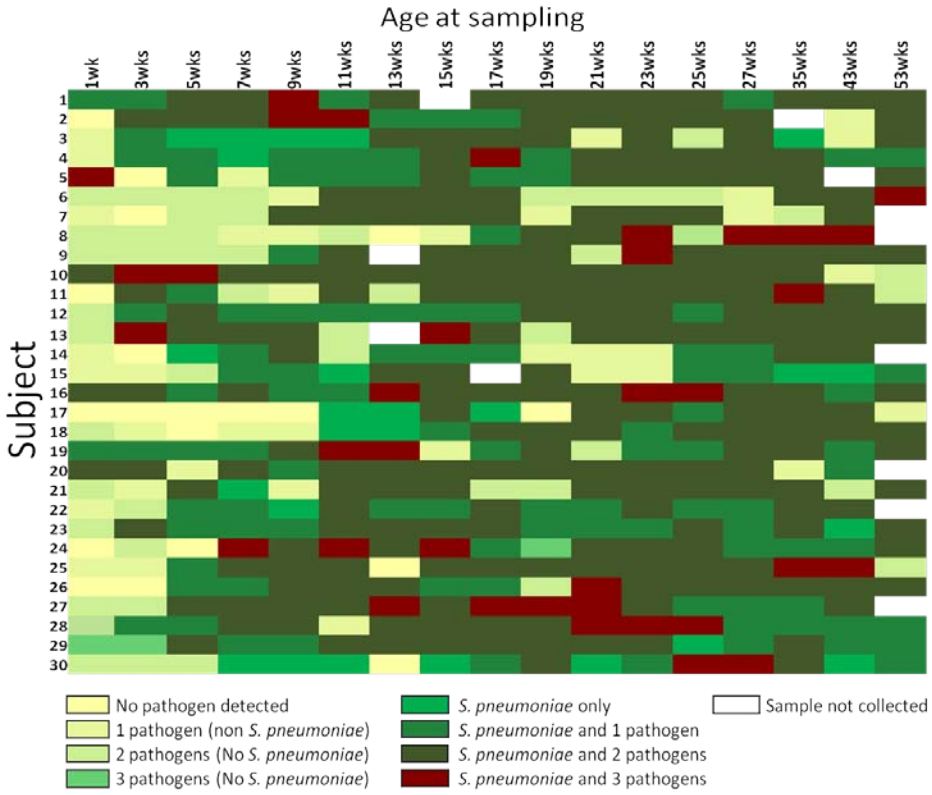
There was a significant difference in the number of OTUs detected between infants older and younger than one month of age (p=0.016) where older infants had on average 0.73 (95% CI: 0.13-1.32) more OTUs detected after allowing for gender.

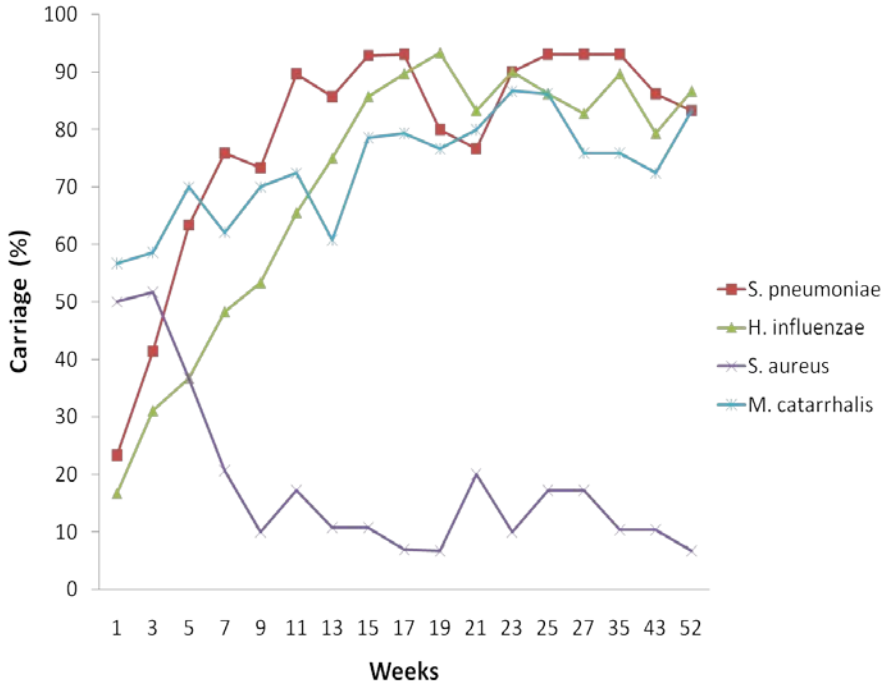
T-RFLP useful for community analysis but there are major limitations of T-RFLP



- From T-RFLP analysis it emerges that a few OTUs form the backbone of the microbiome
- Several low abundance OTUS are transient colonizers
- It is difficult to identify all the OTUs, even with parallel clone library analysis
- A single OTU may represent a wide spectrum of organisms
- T-RFLP-based analysis may misrepresent taxonomic diversity and richness

Co-detection of bacterial pathogens with pneumococci





Species specific PCR-based detection of three respiratory pathogens

Factors influencing co-carriage of respiratory pathogens with *S. pneumoniae*

				Unadjusted			Adjusted		
Risk Factors		**NP swabs**	***Co-occurrence* Prevalence (%)**	**OR**	**p Value**	**95% CI**	**OR**	**p Value**	**95% CI**
Age (weeks)	***≤ 5***	84	32 (38%)						
	≤ 12	92	61 (66%)	4.26	<0.01	2.08, 8.73	2.15	<0.01	0.96, 4.79
	≤ 20	112	93 (83%)	12.94		5.96, 28.11	7.72		3.21, 18.56
	≤ 28	115	99 (86%)	18.12		8.07, 40.72	8.37		3.31, 21.18
	> 28	95	79 (83)	13.26		5.90, 29.80	3.51		0.89, 13.83
Ethnic Group	***Mandinka***	182	13 (78%)						0.22, 1.63
	Jola	232	174 (75%)	0.86	0.07	0.41,1.84	0.60	0.06	0.61, 2.49
	Fula	34	24 (70%)	0.63		0.16,2.50	0.39		0.02, 0 .55
	Other	50	23 (46%)	0.21		0.07,0.65	0.11		
Sex (male)	**Male**	313	220 (70%)	1.51	0.13	0.69,3.31	1.17	0.77	0.42, 3.27
	Female	185	144 (78%)						
Antibiotic Course	***Not Administered***	410	319 (78%)	0.35	0.01	0.16,0.75	0.27	<0.01	0.12,0.63
	Administered	39	23 (59%)						
	NA	49	-						
Type of feeding	***Exclusive***	312	225 (72%	2.66	<0.01	1.47,4.79	2.36	0.13	0.77, 7.21
	Mixed	138	118 (85%)						
	NA	48	-						
Weight Kg				1.41	<0.01	1.19,1.67	1.01	0.52	0.98,1.03

*Logistic regression modelling was used, both unadjusted and adjusted presented. Adjusted model included age, ethnic group, sex, antibiotic course, type of feeding and weight. The baseline category is shown in bold italics for each factor. NA means that the metadata for the samples was missing. Red indicates significant data.

102 Infants Recruited with Parental Informed Consent

SNM STUDY DESIGN

MRC Medical Research Council

GROUP I
33 Infants Recruited

CONTROL
Non-Vaccinated Infants
9 Non-Vaccinated Villages

GROUP II
30 Infants Recruited

DIRECT IMPACT OF VACCINATION
Vaccinated Infants
9 Non-Vaccinated Villages

Group III
39 Infants Recruited

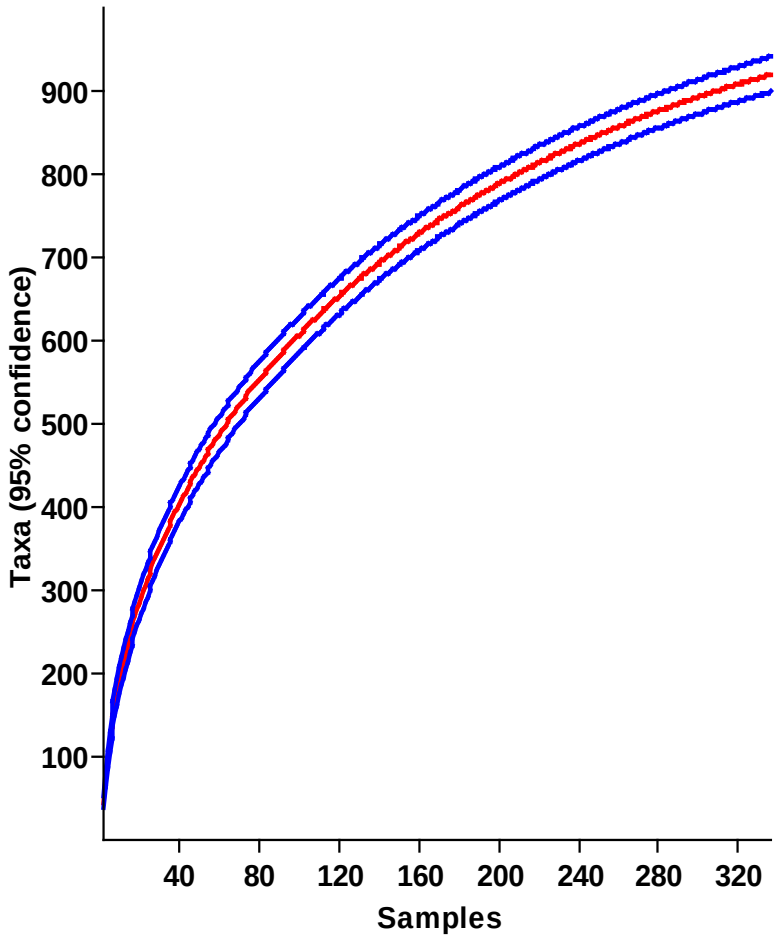
DIRECT & INDIRECT IMPACT OF VACCINATION
Vaccinated infants
9 Vaccinated villages

Herd Effect Group

	Group I	Group II	Group III
SAMPLE COLLECTION SCHEDULE			
NPS Sample A	<7 days after birth	<7 days after birth	<7 days after birth
NPS Samples B to N (First 6 months)	Bi-weekly (14 times)	Bi-weekly (14 times)	Bi-weekly (14 times)
NPS Samples O to Q (Last 6months)	Bi-monthly (3 times)	Bi-monthly (3 times)	Bi-monthly (3 times)
VACCINATION SCHEDULE			
PCV7 Dose 1	NONE	8 Weeks (sample E)	8 Weeks (sample E)
PCV7 Dose 2	NONE	12 Weeks (sample G)	12 Weeks (sample G)
PCV7 Dose 3	NONE	16 Weeks (sample I)	16 Weeks (sample I)

454-pyrosequencing of the nasopharyngeal microbiome

- For the V3-V5 region, 7 - 203 (Avg. 43) unique genera detected per sample
- Microbes represent at least 31 phyla
- For both regions, the number of unique genera present in a single sample ranged from 19 to 311 (median 65).
- The number of unique genera present in a single week (any subject) ranged from 292 to 498
- Each subject had between 2 and 10 bacteria that were present at every time point.



Effect of vaccination on bacterial loads: Individual analysis

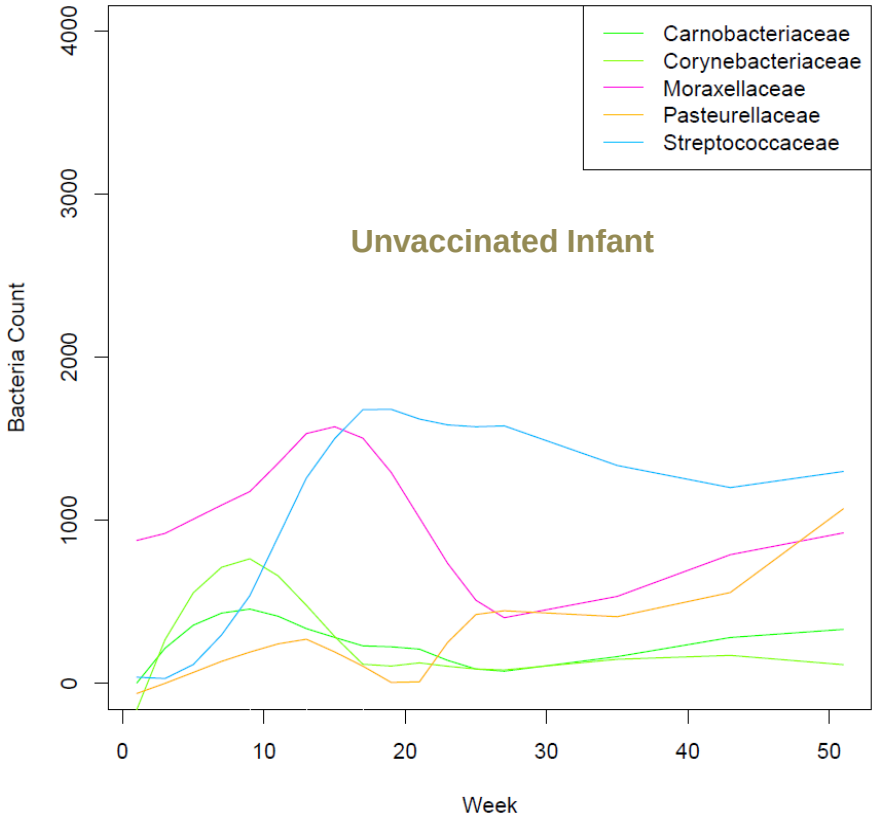


Figure 8a. Bacteria loads for the 5 most prevalent Families found in the nasopharynx of a PCV-7 naïve infant in the first year of life based on 454-pyrosequencing

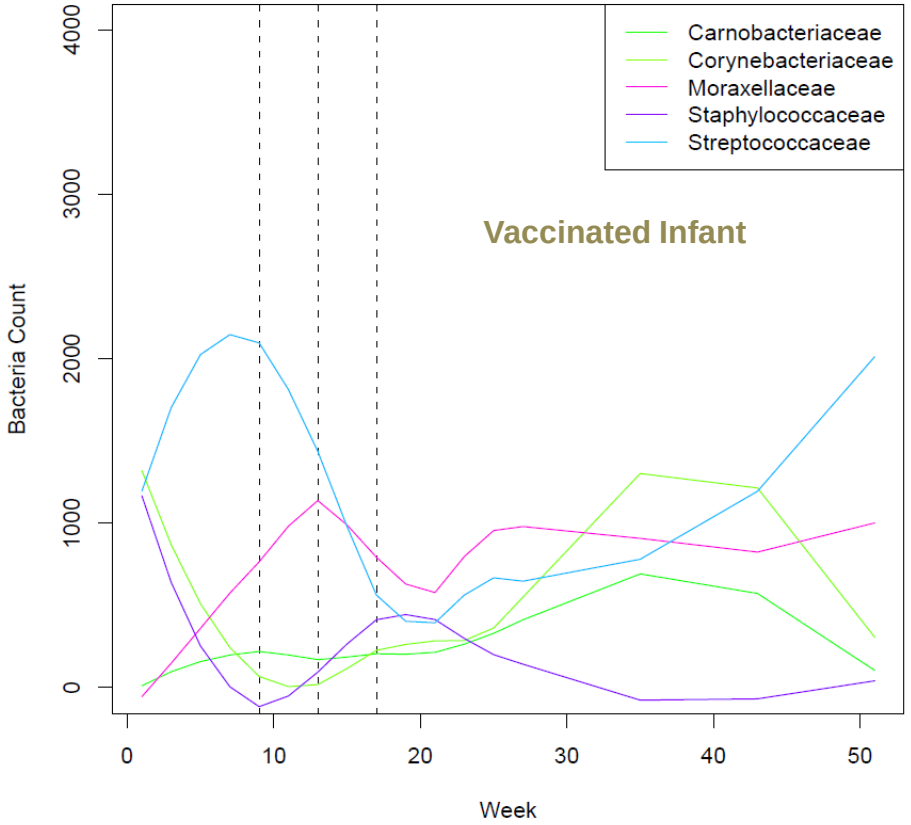
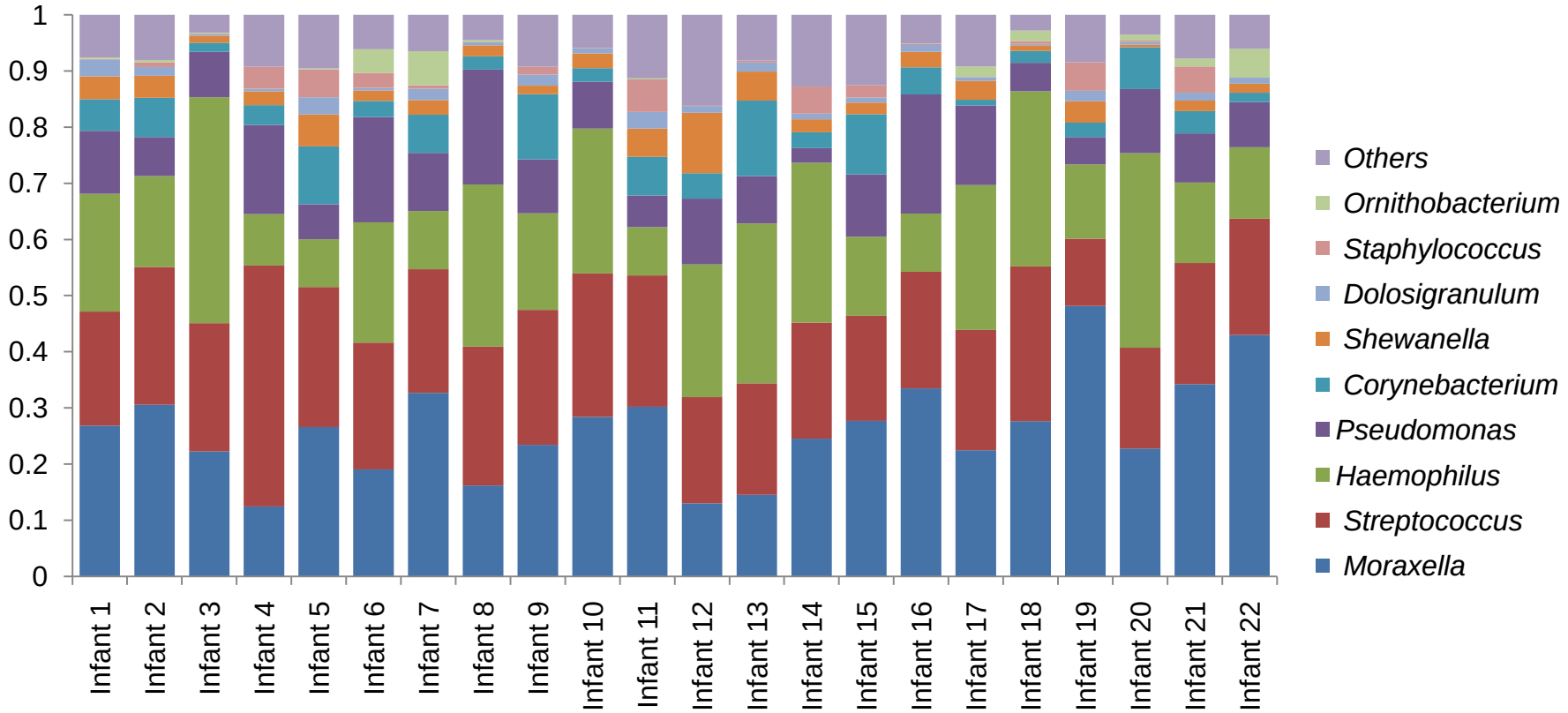


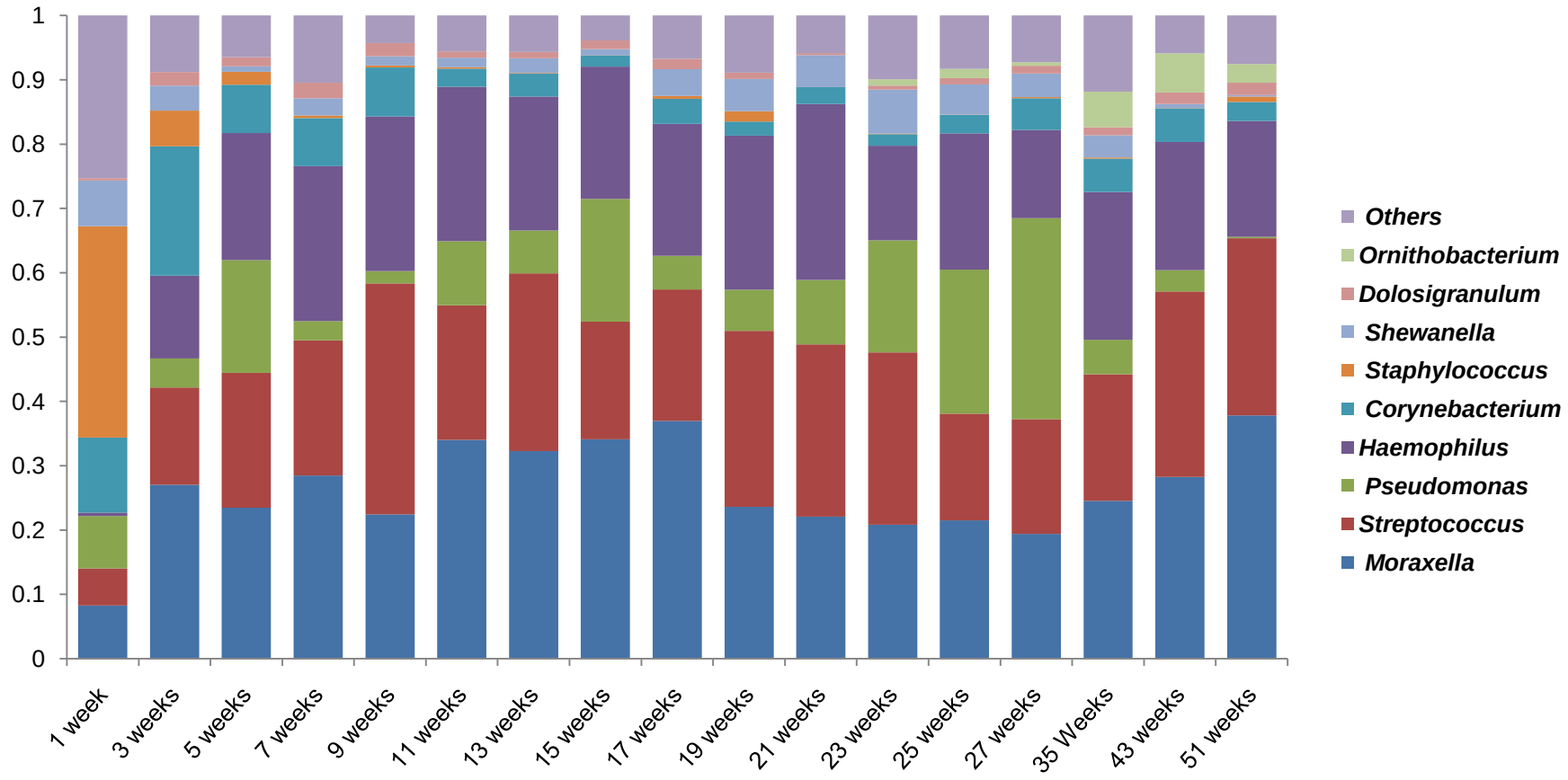
Figure 8.b Bacteria loads for the 5 most prevalent Families found in the nasopharynx of a PCV-7 vaccinated infant in the first year of life based on 454-pyrosequencing. Dotted lines represent vaccination points

Nasopharyngeal microbiome across individuals



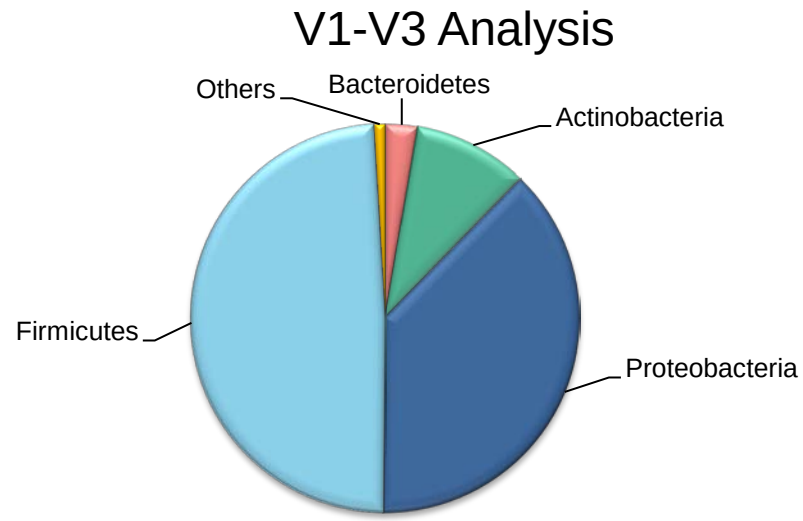
Streptococcus, *Moraxella*, *Haemophilus*, *Corynebacterium* and *Shewenella* make up at least 80% of the microbiome amongst the infants

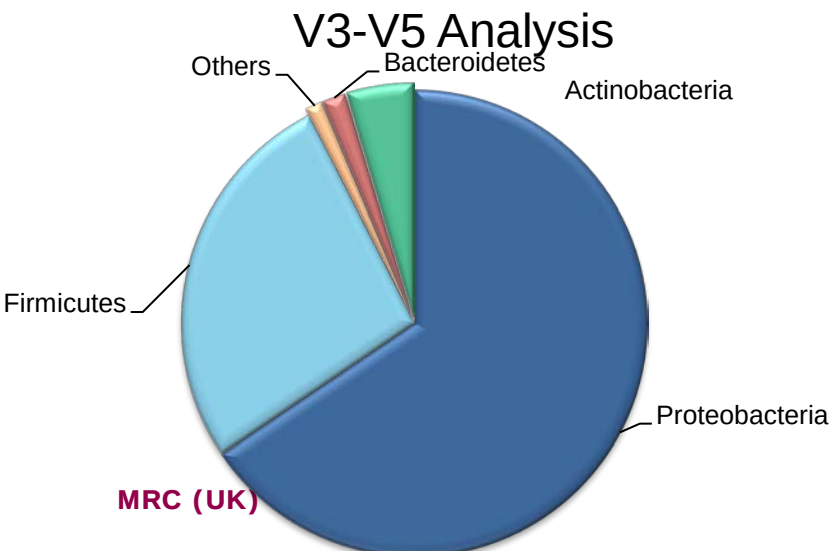
Nasopharyngeal microbiome across time

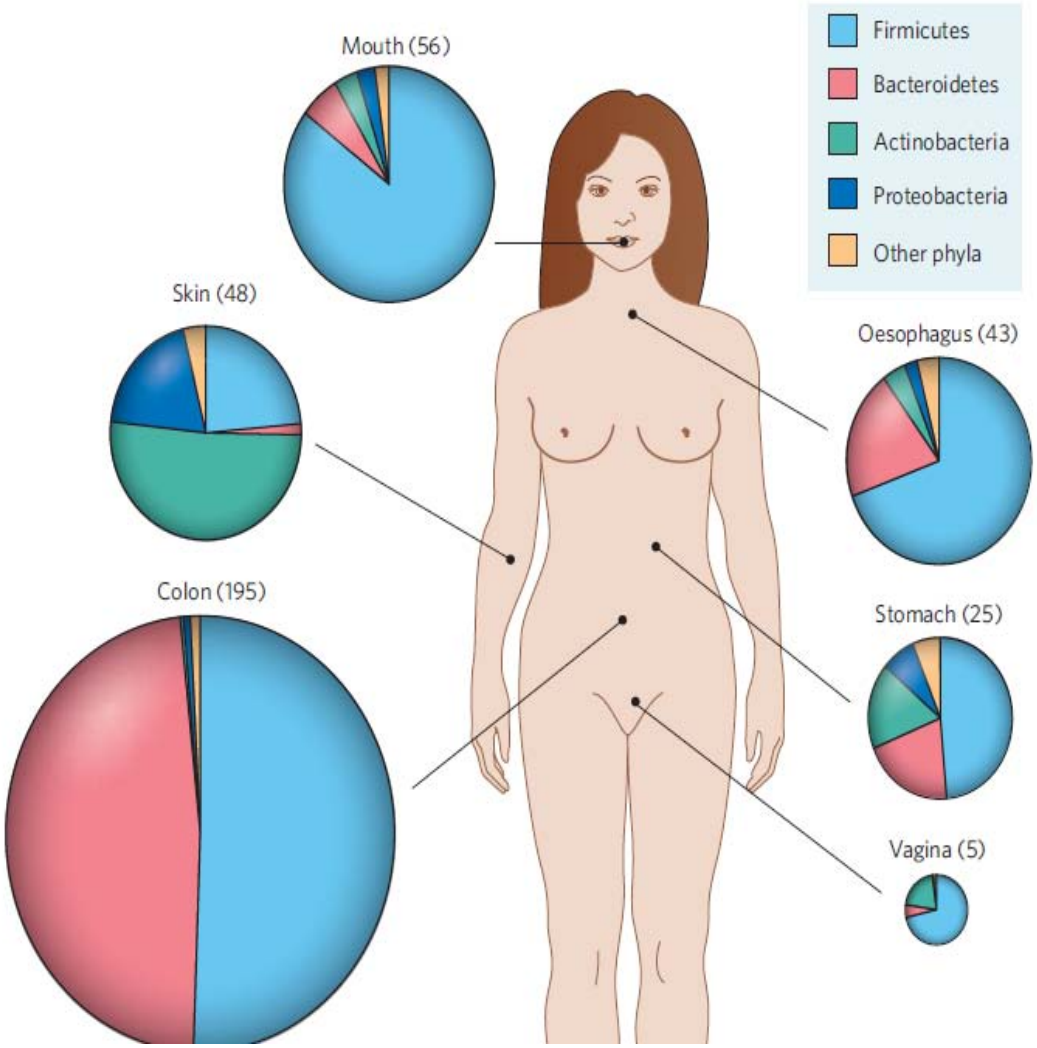


Major shift in relative distribution occurs in the between 1 and 3 weeks of birth

Composition of the infant nasopharyngeal microbiome



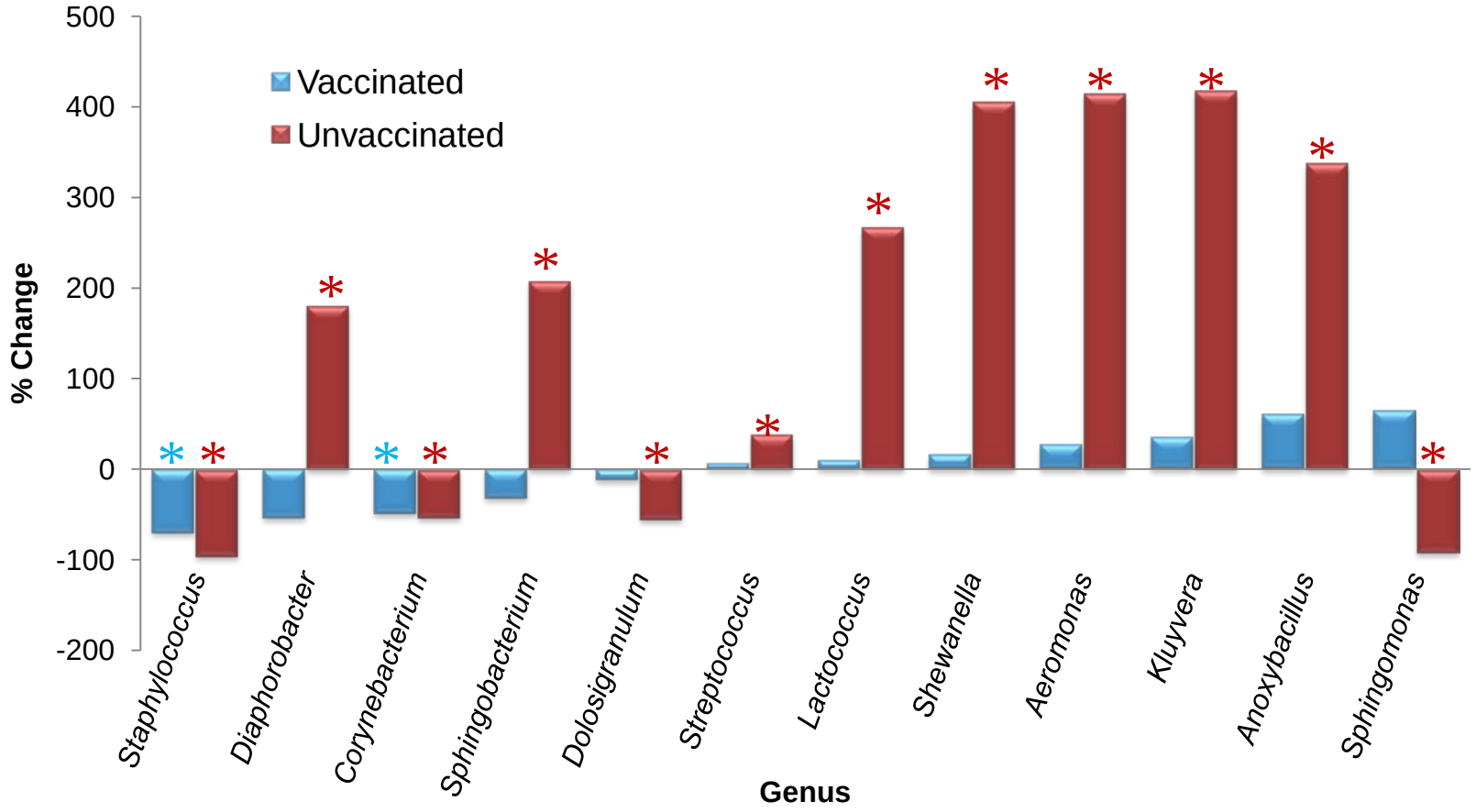




Dethlefsen et al. 2007

MRC (UK)

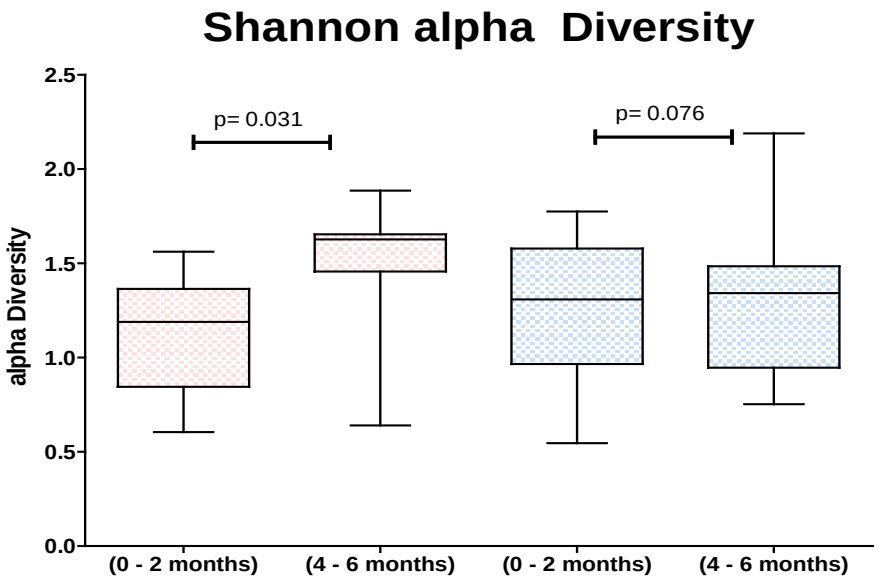
Development of the microbiome in vaccinated and unvaccinated infants

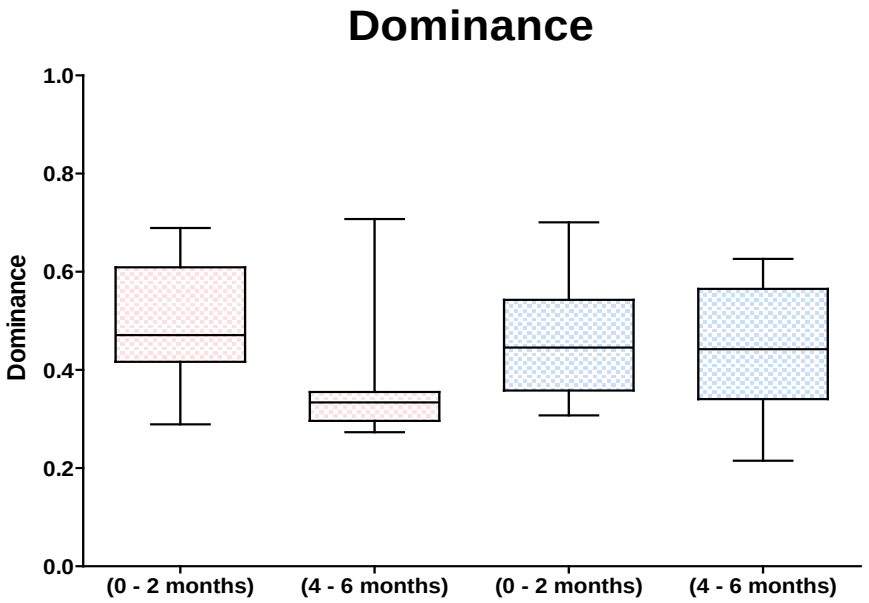


* Significant changes in vaccinated children

* Significant change in unvaccinated children

Effects of vaccination on microbial ecology





Unvaccinated Vaccinated

Unvaccinated Vaccinated

Diversity increases significantly in unvaccinated infants but not vaccinated infants – this is interesting!

Summary

- The infant nasopharyngeal microbiome is dynamic
  - High rates of acquisition and loss
- Acquisition of bacteria, including pathogenic microbes occurs rapidly after birth
- The infant nasopharyngeal microbiome is diverse but,
  - A few taxonomic groups make up the bulk of the microbiome including pathogens *S. pneumoniae, H. influenzae* and *M. catarrhalis* which display high carriage
  - There are numerous low abundance transient taxonomic groups
- There is preliminary evidence of a vaccine non-effect on other taxa though there may be large variations between individual subjects

Villagers came out to Welcome George

Acknowledgements

Many thanks to the infants participating in the study, their mothers and village leaders

MRC Hosts First **Genomics Symposium** in The Gambia

Martin Antonio's report continues on page 2

Classical and emerging infectious diseases still represent the single most important threat to human health on a global scale and infectious diseases are seldom out of the news. However, advances in genetics and genomic technologies promise to provide new approaches to understanding and combating these diseases.


Antonio Lab