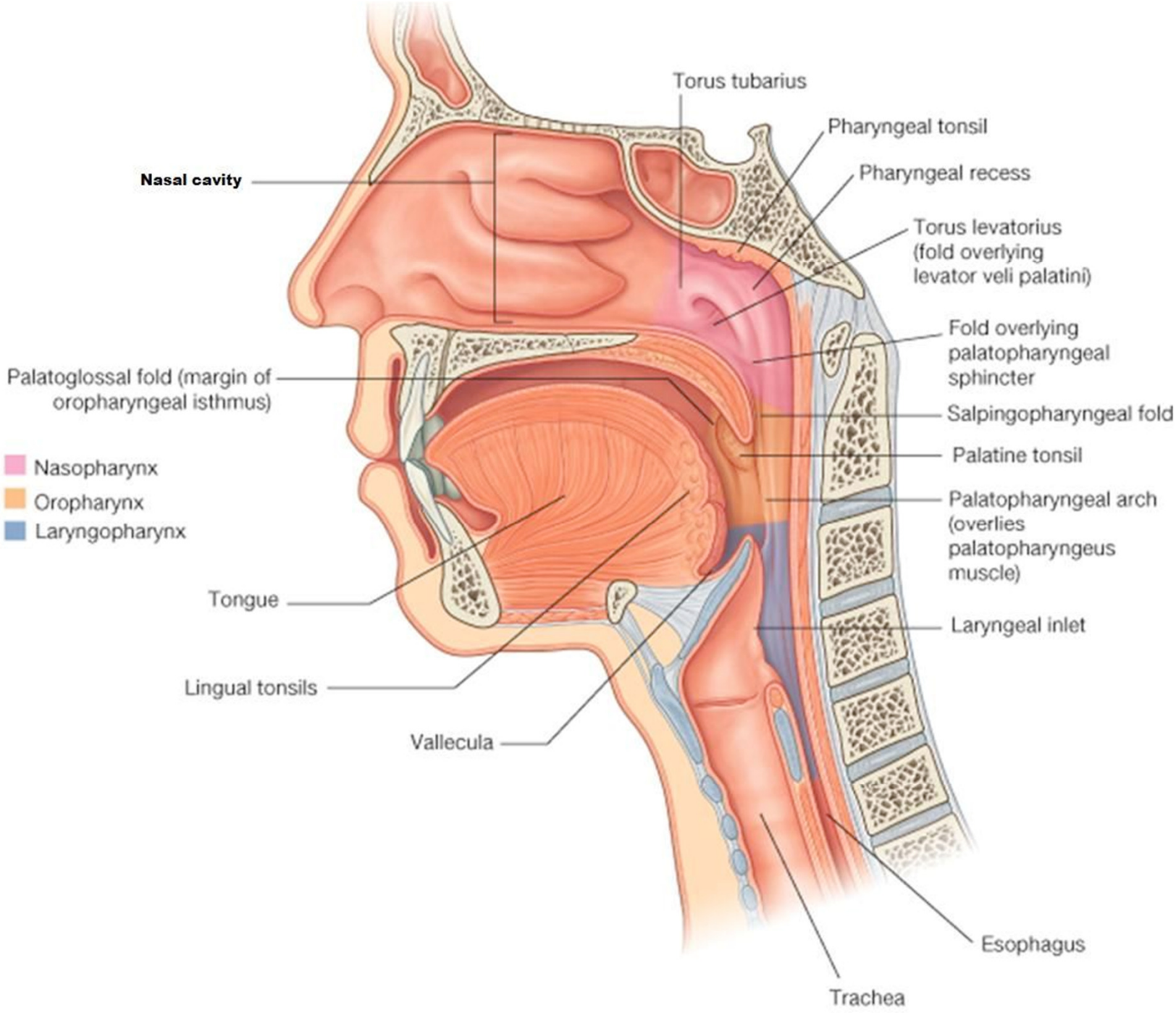
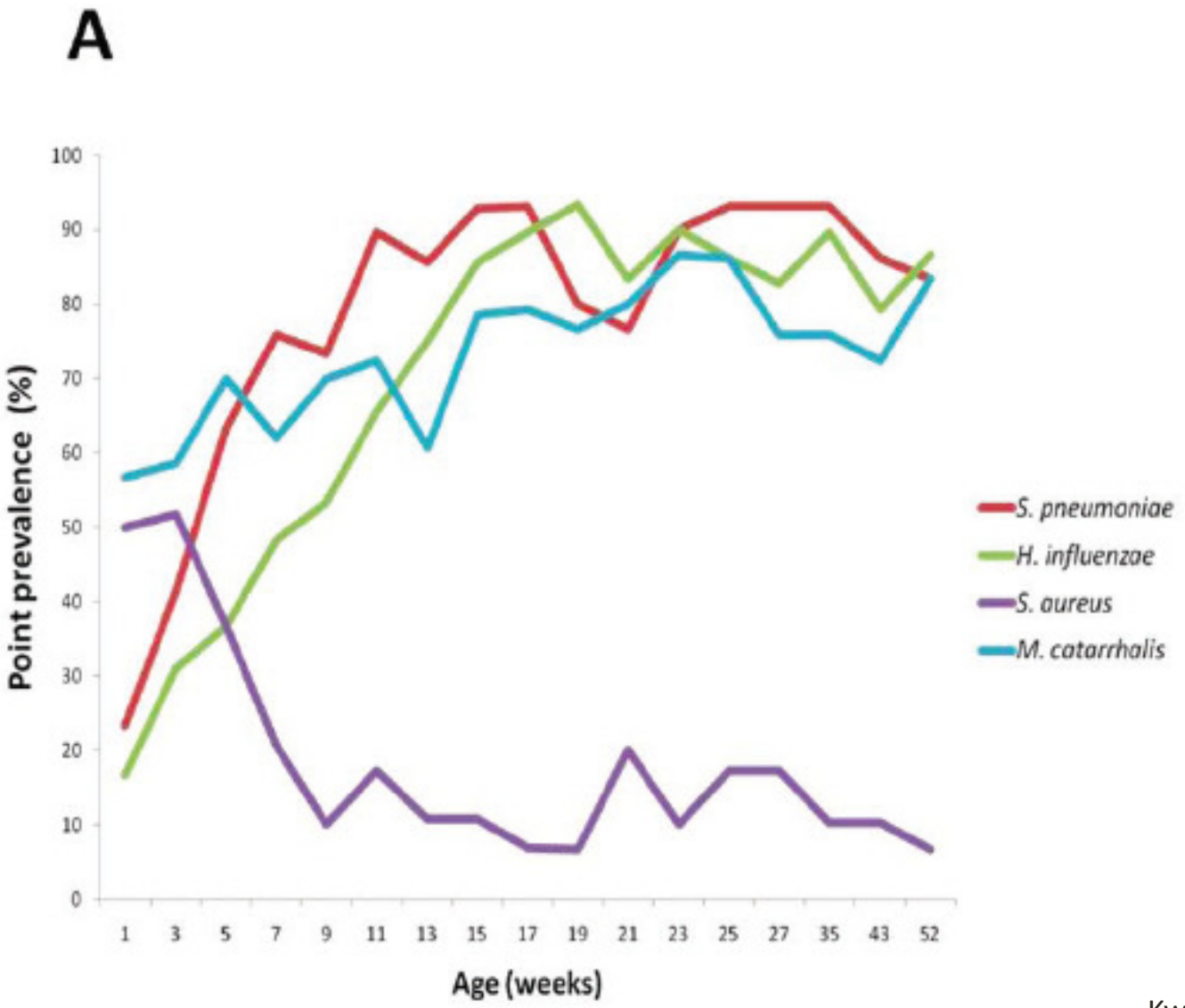
# The nasopharyngeal microbiome and respiratory disease in African children

Mark Nicol
Division of Medical Microbiology, Department of Clinical Laboratory Sciences and
Institute for Infectious Diseases and Molecular Medicine,
University of Cape Town and National Health Laboratory Service

Acknowledgements:
Heather Zar – co-PI, UCT
Landon Myer, UCT
Nicky Mulder, UCT
Bill Nierman - JCVI


Torus tubarius
Pharyngeal tonsil
Nasal cavity
Pharyngeal recess
Torus levatorius
(fold overlying
levator veli palatini)
Fold overlying
palatopharyngeal
sphincter
Palatoglossal fold (margin of
oropharyngeal isthmus)
Salpingopharyngeal fold
Palatine tonsil
Nasopharynx
Oropharynx
Laryngopharynx
Palatopharyngeal arch
(overlies
palatopharyngeus
muscle)
Tongue
Laryngeal inlet
Lingual tonsils
Vallecula
Esophagus
Trachea

# Important bacterial constituents of the NP flora




Kwambana et al. BMC Infect Dis 2011

# Invasive pneumococcal disease

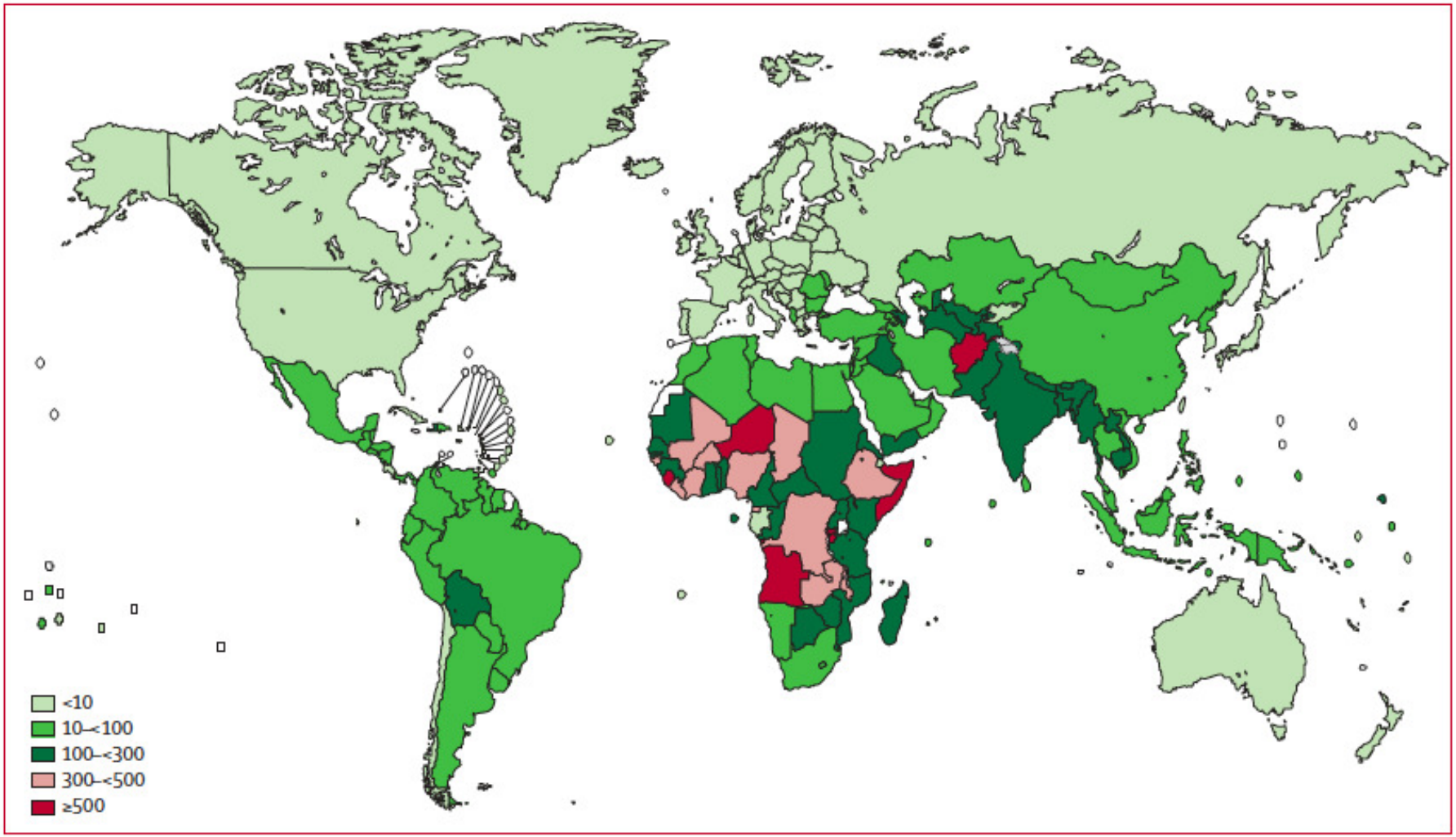


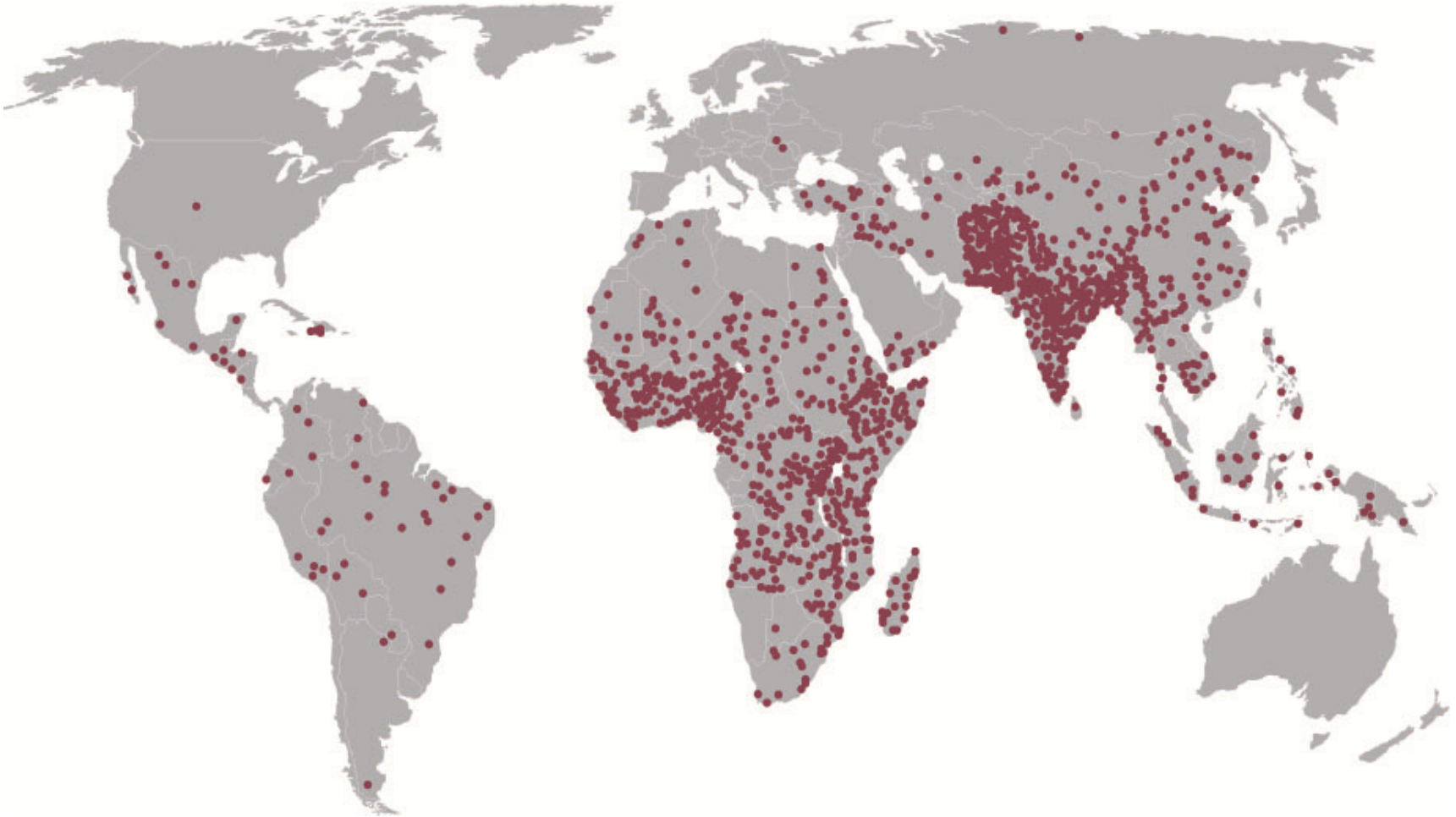

***Figure 2:*** **Pneumococcal mortality rate**

Pneumococcal deaths in children aged 1–59 months per 100 000 children younger than 5 years (HIV-negative pneumococcal deaths only). The boundaries shown and the designations used on this map do not imply the expression of any opinion by WHO concerning the legal status of any country, territory, city, or area, or of its authorities, or concerning the delimitation of its frontiers or boundaries. Dotted lines on maps represent approximate border lines for which there may not yet be full agreement.

O' Brien et al. Lancet 2009

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

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



Each dot represents 1000 deaths (Williams BG et al Lancet 2002)

Slide courtesy Martin Antonio

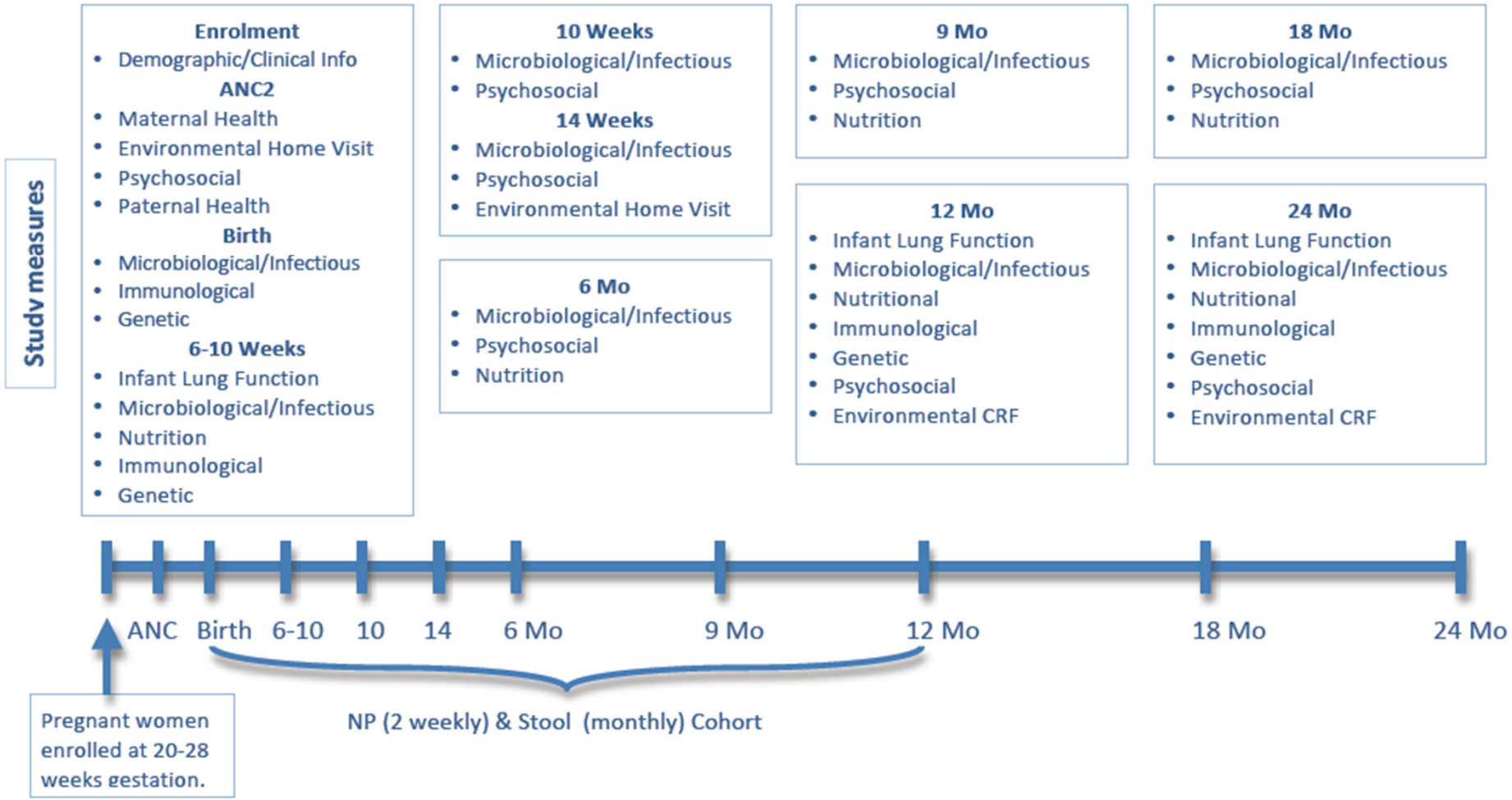
# Drakenstein Child Lung Health Study

- A birth cohort of 500 mother-infant pairs followed for 2 (5) years
- Longitudinal data on incident pneumonia and its aetiology
- Investigate antecedent risk factors for pneumonia and for pneumonia progression

# Key points in our strategic approach

- Drakenstein sub-district –peri-urban, high prevalence of risk factors, births at a single hospital
- Birth Cohort – temporality, repeated measures of risk factors
- Outcomes: pneumonia and wheezing illness
- Very detailed phenotyping (including repeated lung function testing) and extensive biological and environmental sample collection
- Diverse risk factors in 7 areas – microbiological, immunological, environmental, maternal, nutritional, genetic & psychosocial

# Drakenstein Study Overview



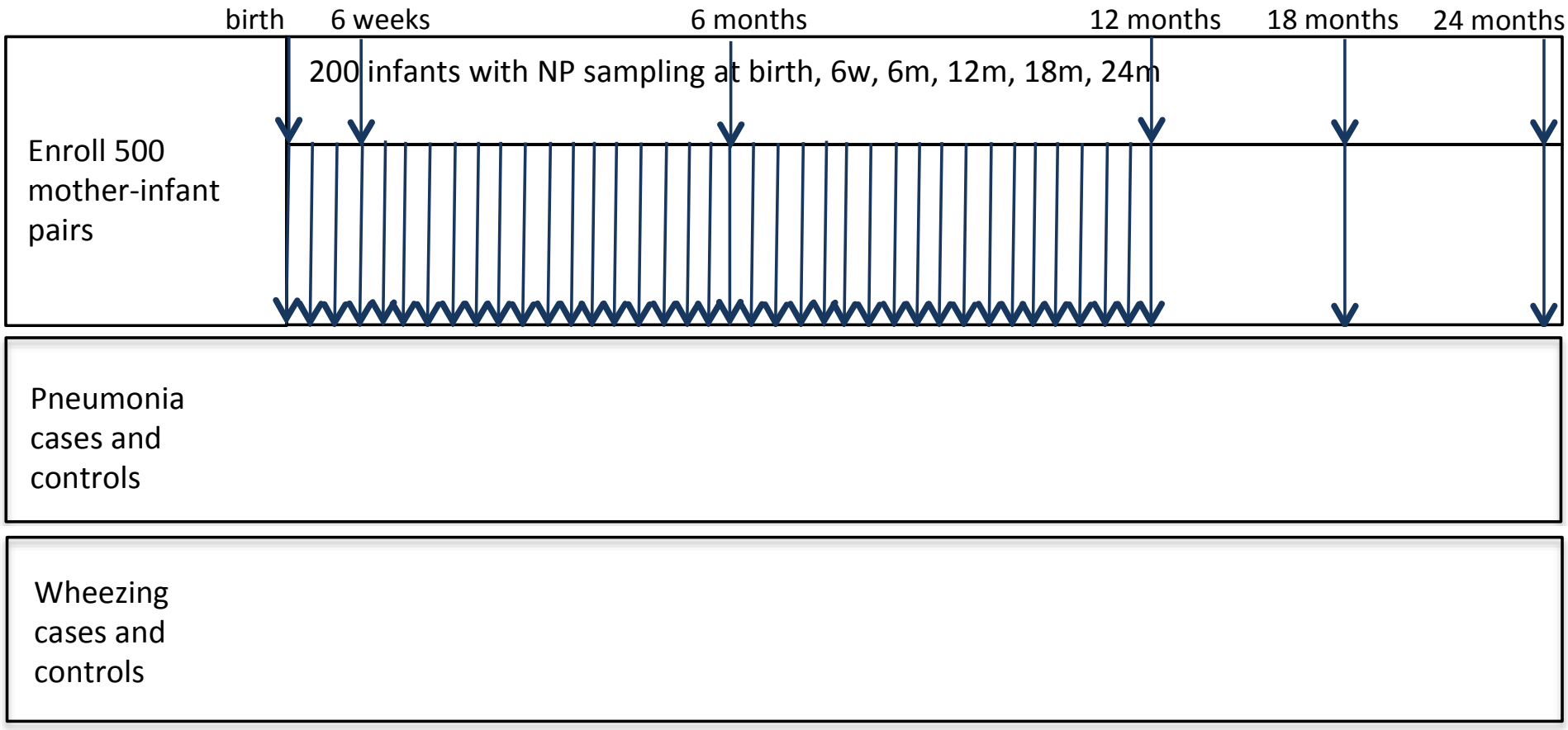
# Key H3Africa Project Aims

- Describe, in detail, patterns of nasopharyngeal colonization in healthy infants from birth to two years (bacterial and viral)

- Determine the role of the nasopharyngeal microbiome in pneumonia and wheezing illness in African children by:
  - Identifying patterns of nasopharyngeal colonization that are associated with the development of pneumonia/wheezing.
  - Investigating interactions between the nasopharyngeal microbiome and other risk factors

- Development of capacity for full pipeline microbiome analysis

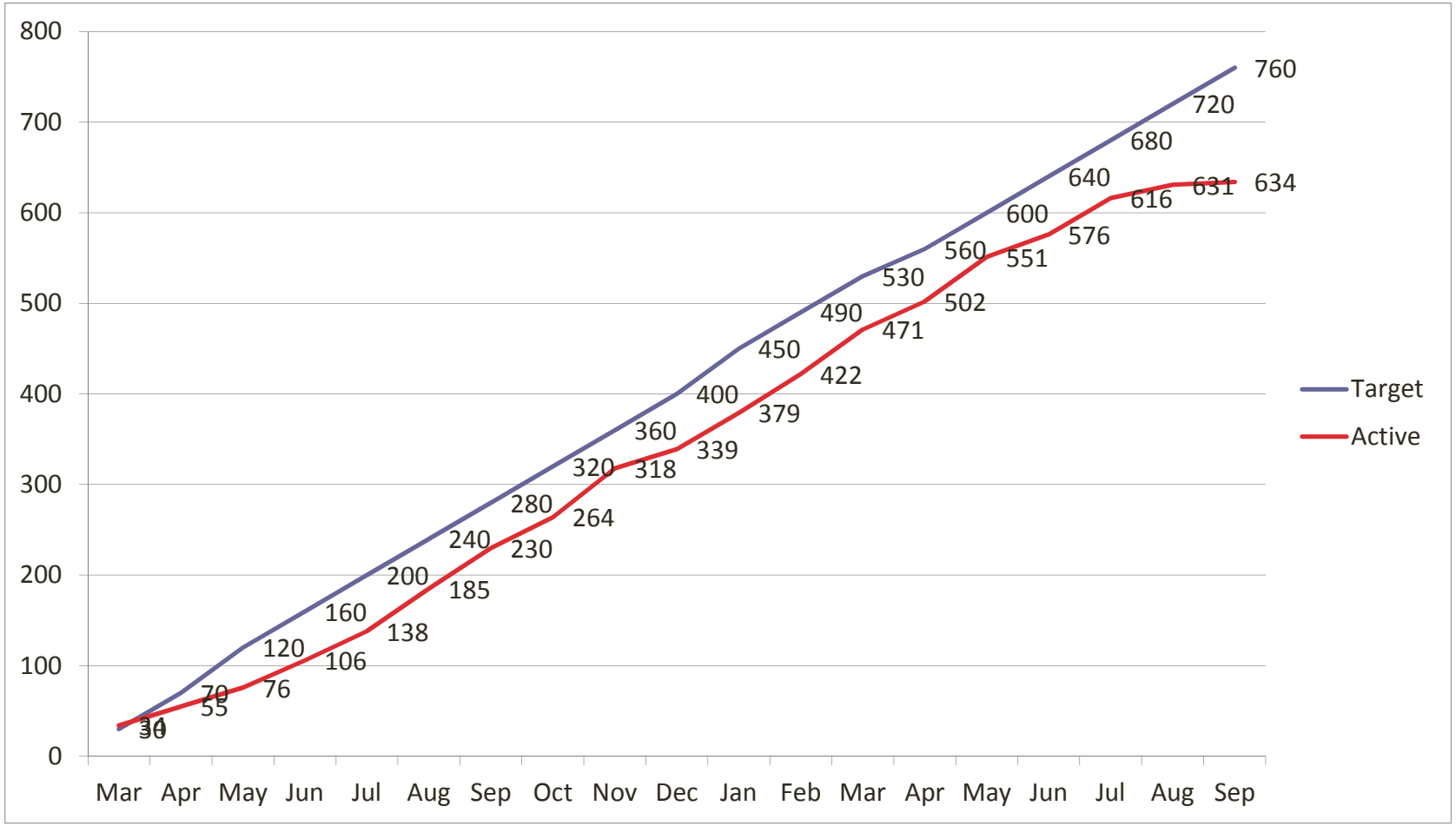
# Nested H3Africa studies: NP sampling



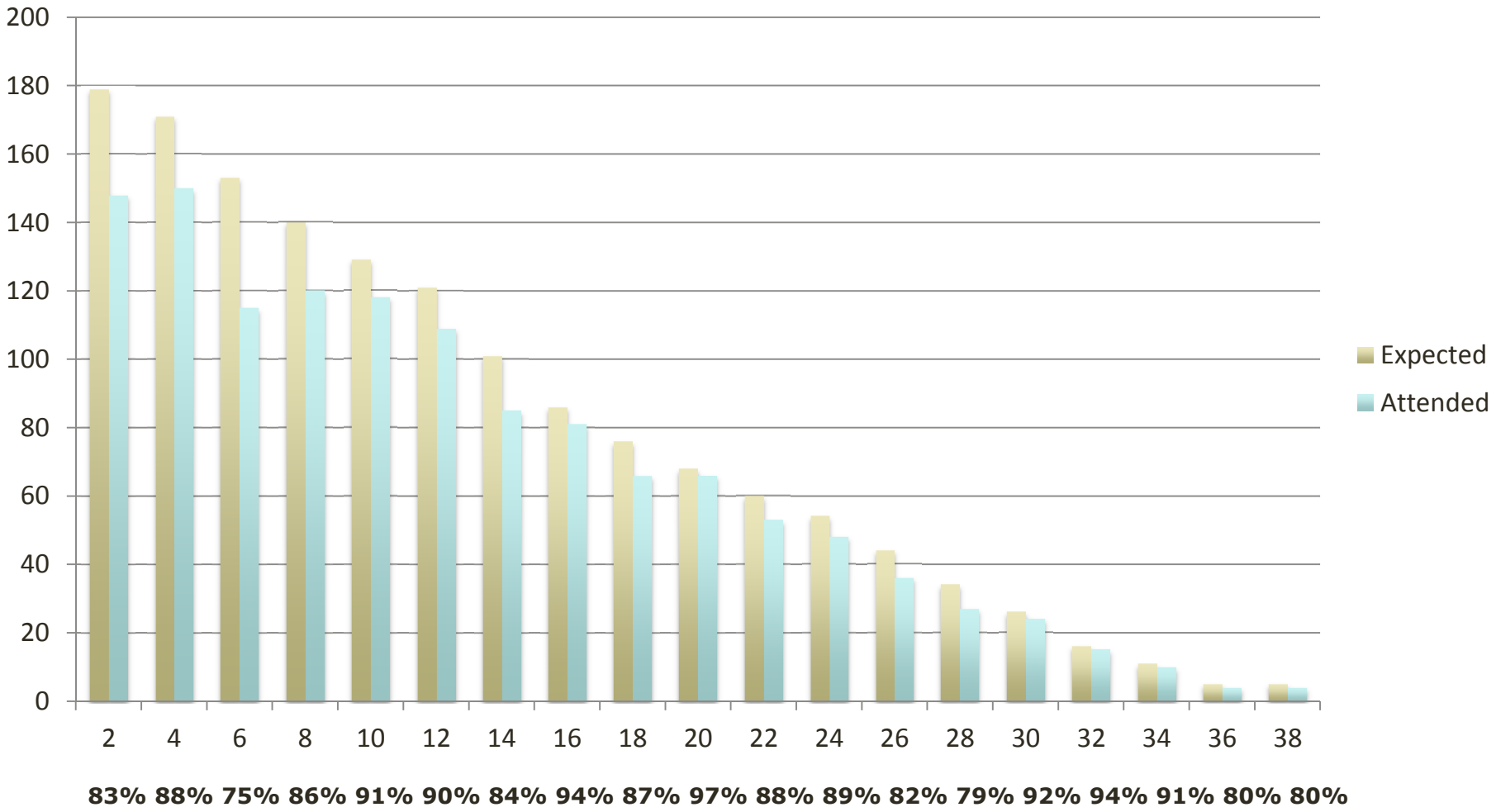
# Nested H3Africa studies: experimental approach

- Routine bacterial culture, with focus on respiratory pathogens
  - Sequetyping of pneumococci
  - Genotyping of *S. aureus*
- 33-plex real-time PCR assay (Fasttrack Diagnostics FTD33) for common bacterial and viral pathogens
  - Including quantitative assessment
- Microbiome analysis - sequencing 16S rRNA gene amplicons (MiSeq, Cape Town)
- Association analysis (incorporating time-series approaches) for pneumonia and wheezing outcomes

# Cumulative enrolments by month through 22 Sept 2013

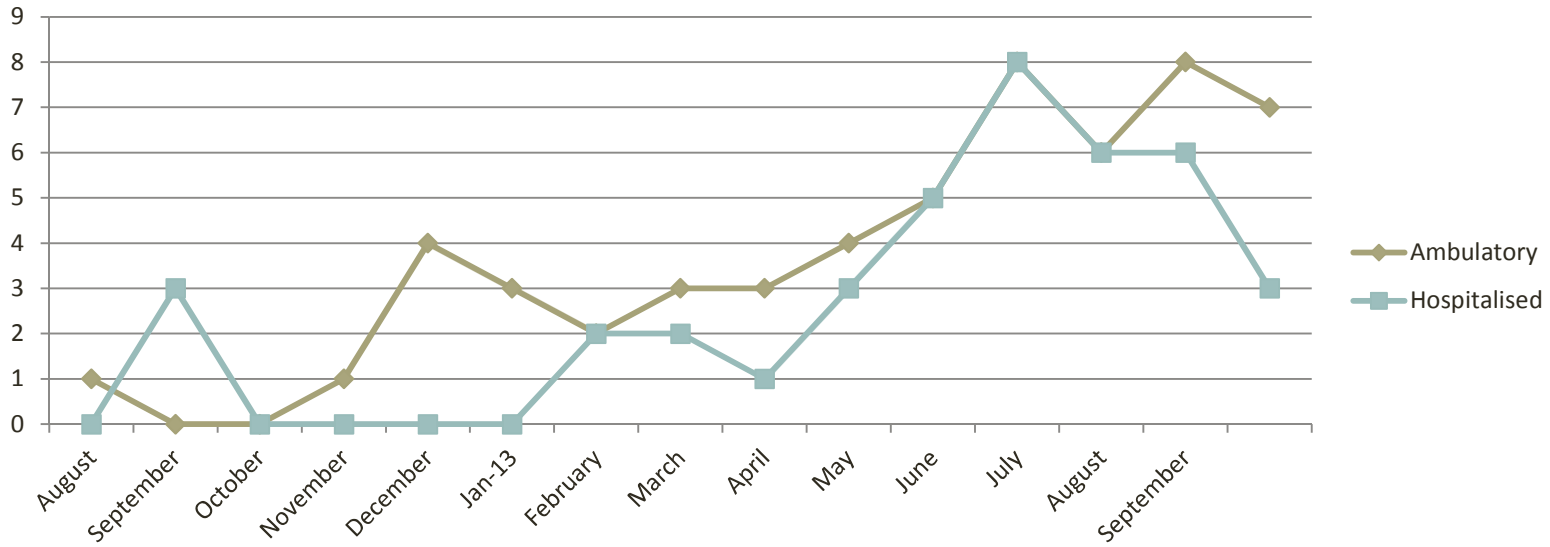


# 2-weekly NP Cohort: cohort retention

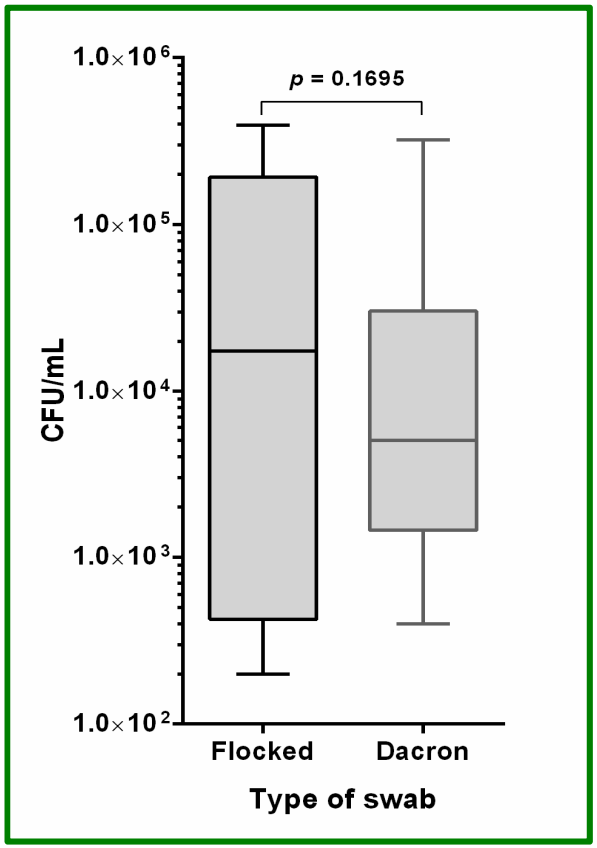


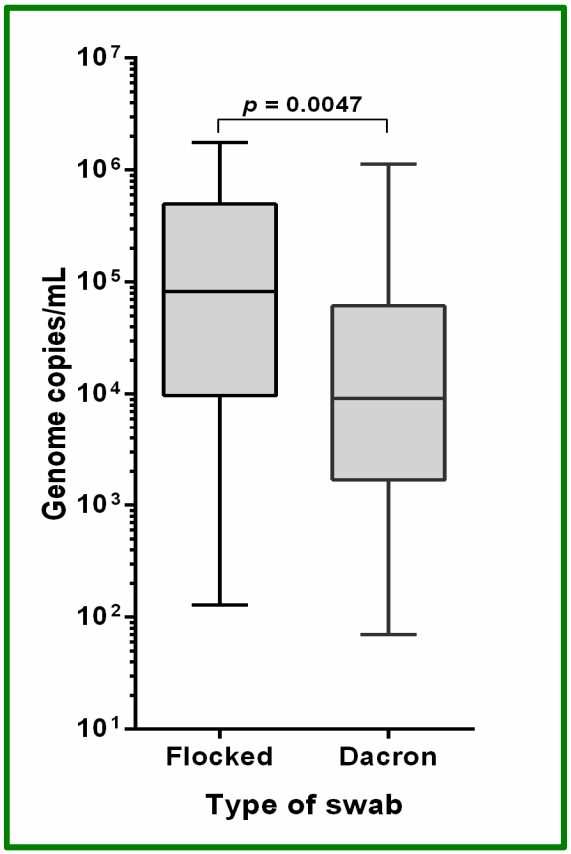
# Pneumonia Cases

- Total WHO-defined pneumonia cases: 118
  - <28 days: 26 cases (19 congenital)
  - >28 days: 92 cases (58 ambulatory, 34 hospitalised)



# Preparatory studies: Flocked NP swabs are superior to Dacron NP swabs

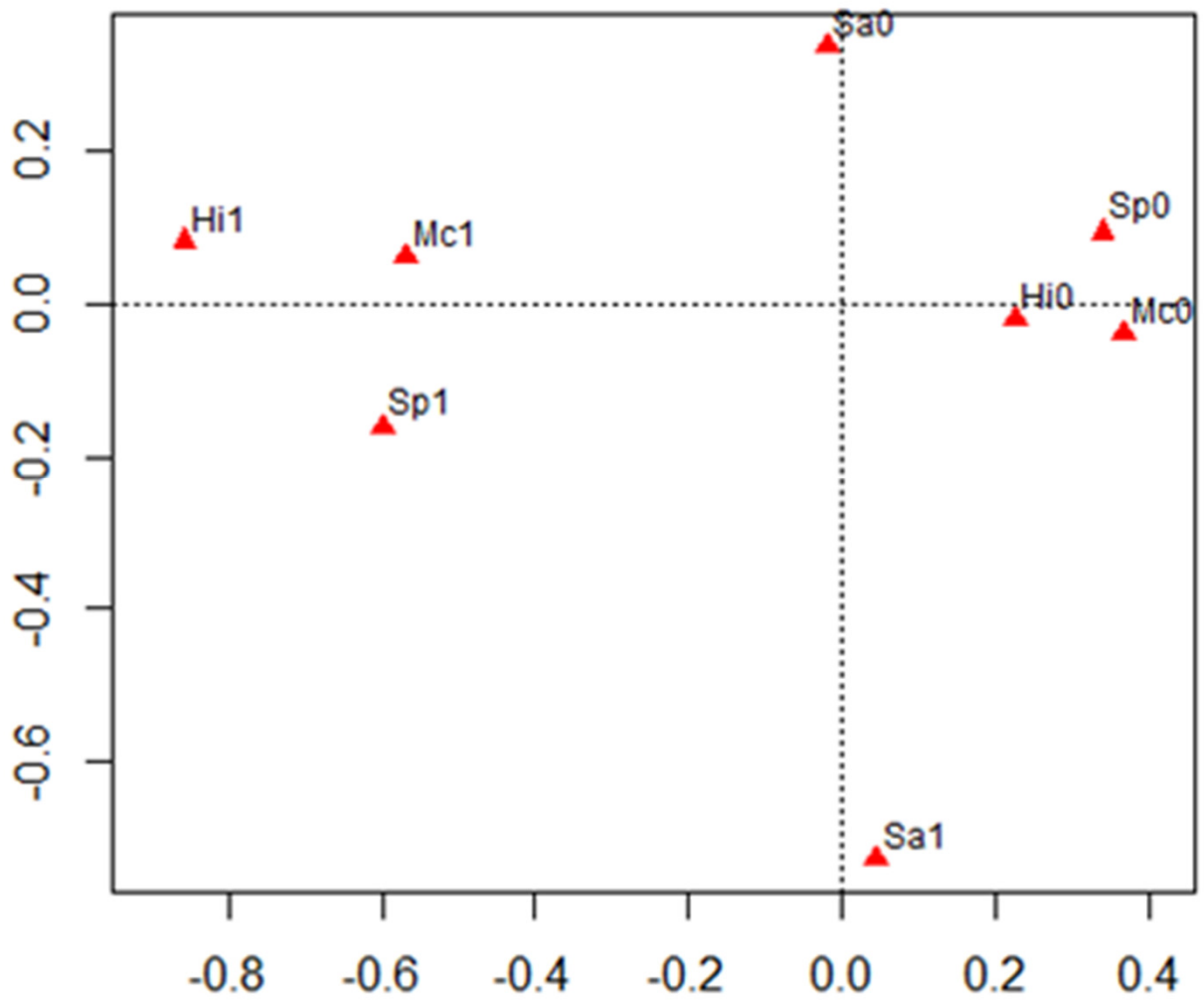



Flocked (3.0 x $10^5$copies/mL) vs. Dacron (9.3 x $10^4$ copies/mL)

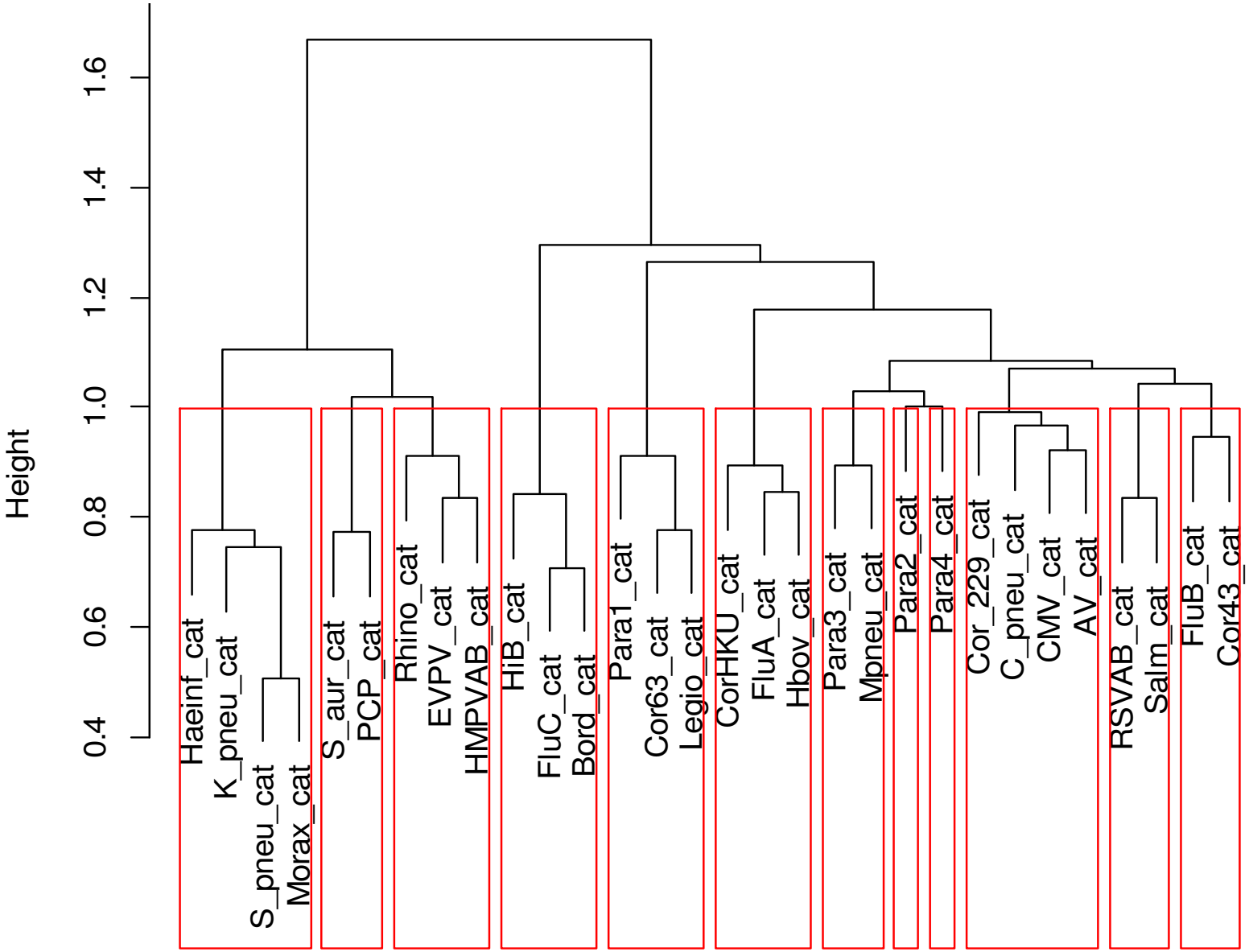
Dube et al. PLoS One In press

# Early data: NP bacterial co-colonization

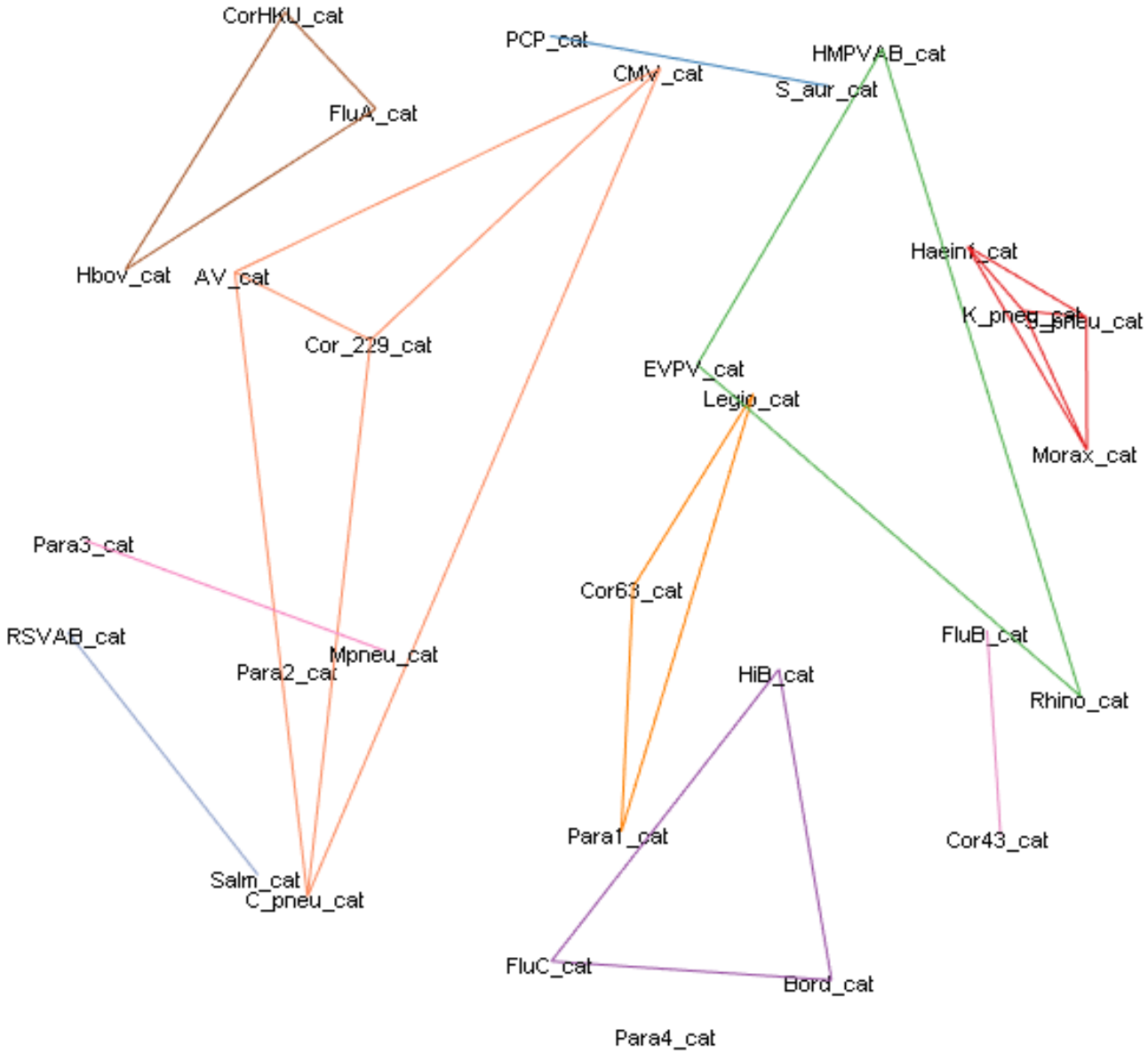



Multiple correspondence analysis: 321 NP samples from Drakenstein cohort

# Early data: NP co-colonization viruses/bacteria



# Early data: NP co-colonization viruses/bacteria



# Capacity development

- 1-week ‘wet’ workshop on 16S microbiome sequencing (with JCVI)
- Exchange programme with JCVI
- Postgraduate students
  - Current: MSc 3, PhD 3, postdoc 3

# Acknowledgements


- Clinical Group
  - Heather Zar
  - Landon Myer
  - Whitney Barnett
  - Emilee DaCosta
  - Leonie Coetzee
  - Dave le Roux
  - …and the rest of the team
- JCVI
  - Bill Nierman
- Laboratory Group
  - Mamadou Kaba
  - Widaad Zemanay
  - Samantha Africa
  - Elloise du Toit
  - Slee Mbhele
  - Felix Dube
  - Shantelle Klaassen
  - Menna Duyver
  - …and the rest of the team