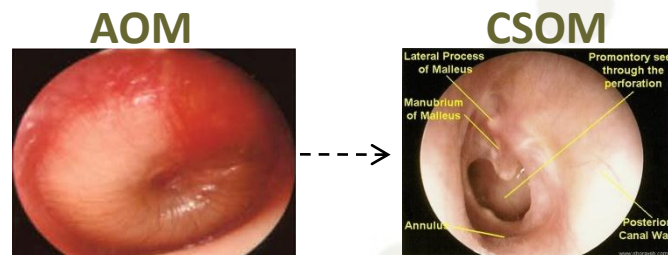


The importance of considering BACTERIAL LOAD in OTITIS MEDIA research

Heidi Smith-Vaughan, Robyn Marsh, Michael Binks, Mirjam Kaestli,
Peter S Morris, Amanda J Leach

OM in Indigenous children

- OM is endemic in children in remote Indigenous communities
- High NP carriage rates of OM pathogens (~80%)
- Prevalence of perforation ~20% (<2.5yo)
- Many will progress to CSOM



In Northern Territory Indigenous children

Ear discharge³

- Nasopharyngeal microbes
- Pseudomonas aeruginosa
- Staphylococcus aureus
- Alloiococcus otitidis⁴
- Proteus
- Fungi (Aspergillus)
- Yeast

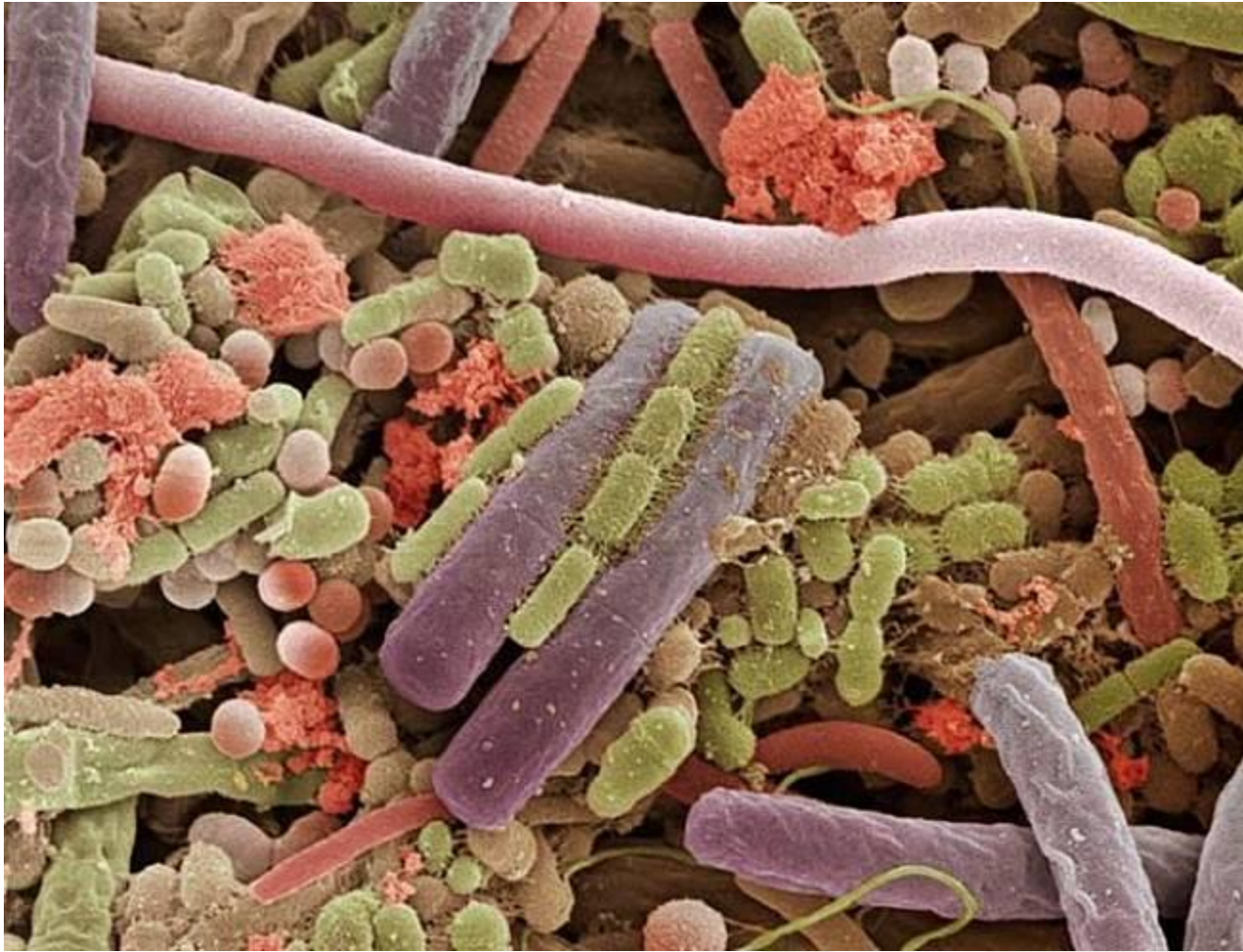


Nasopharynx¹

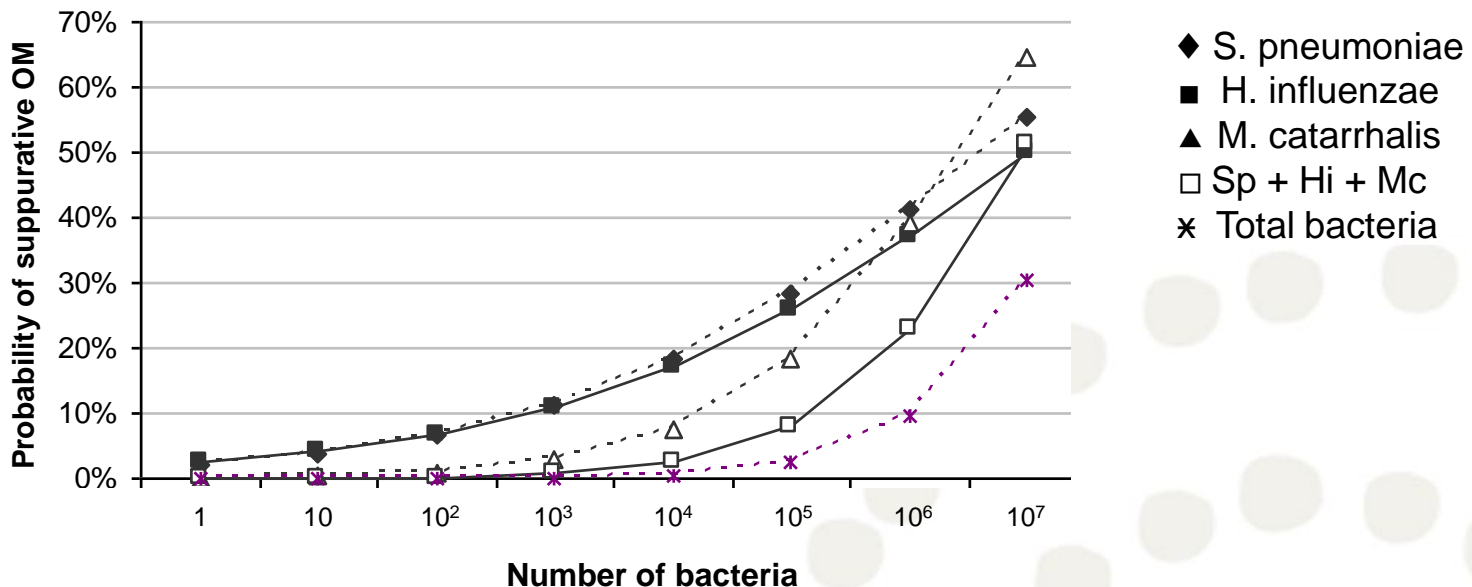
- Haemophilus influenzae
- Streptococcus pneumoniae
- Moraxella catarrhalis
- Staphylococcus aureus
- Respiratory viruses²

1. Leach et al. 1994. PIDJ: 13(11).
2. Binks et al. 2011. BMC Infect Dis.11.161.
3. Leach et al. 2008. PIDJ; 27(8).
4. Marsh et al. 2012. Submitted.

Bacterial load – why it's important



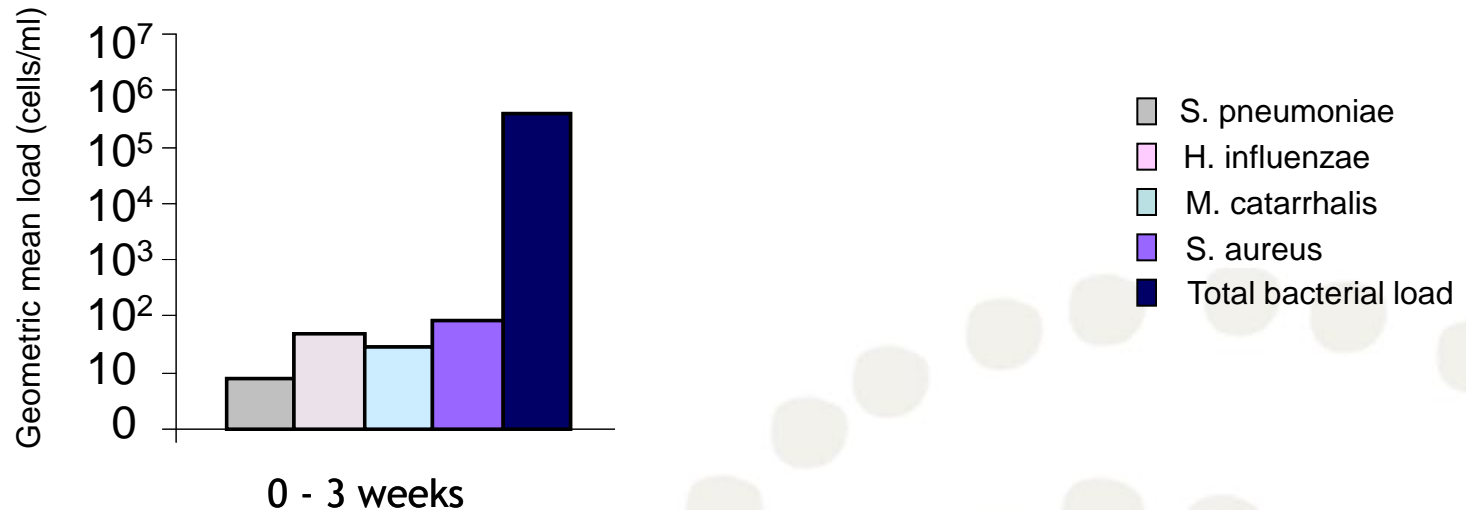
As bacterial load in the NP increases, so does the probability of acute otitis media



Aboriginal and non-Aboriginal children (18-36mo)

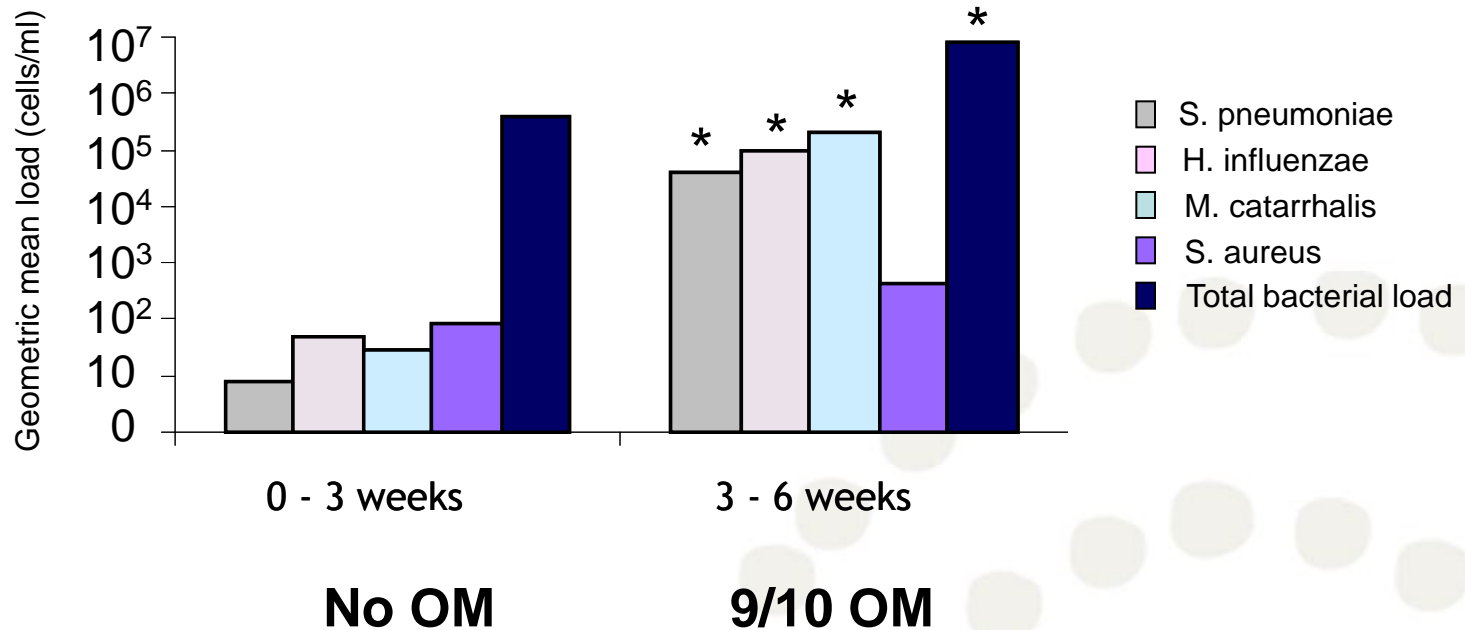
For Indigenous children, interventions need to be early in life

NASOPHARYNGEAL SWABS



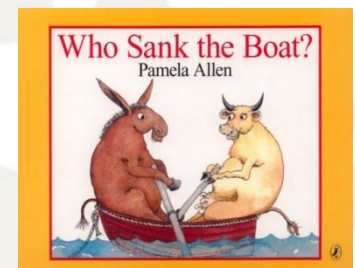
10 Children with no OM 0-3 weeks of age

NASOPHARYNGEAL SWABS



1. *S. pneumoniae* load in NP associated with pneumonia¹
2. *S. pneumoniae* load in serum associated with pneumonia severity²
3. Bacterial load positively associated with inflammation³

1. Vu et al. *Pediatr Infect Dis* 2010. 30(1): 11-8.
2. Werno et al. *J Med Microbiol*. 2012. Epub ahead of print.
3. Hill et al. 2000. *Am J Med* 109: 288-295.



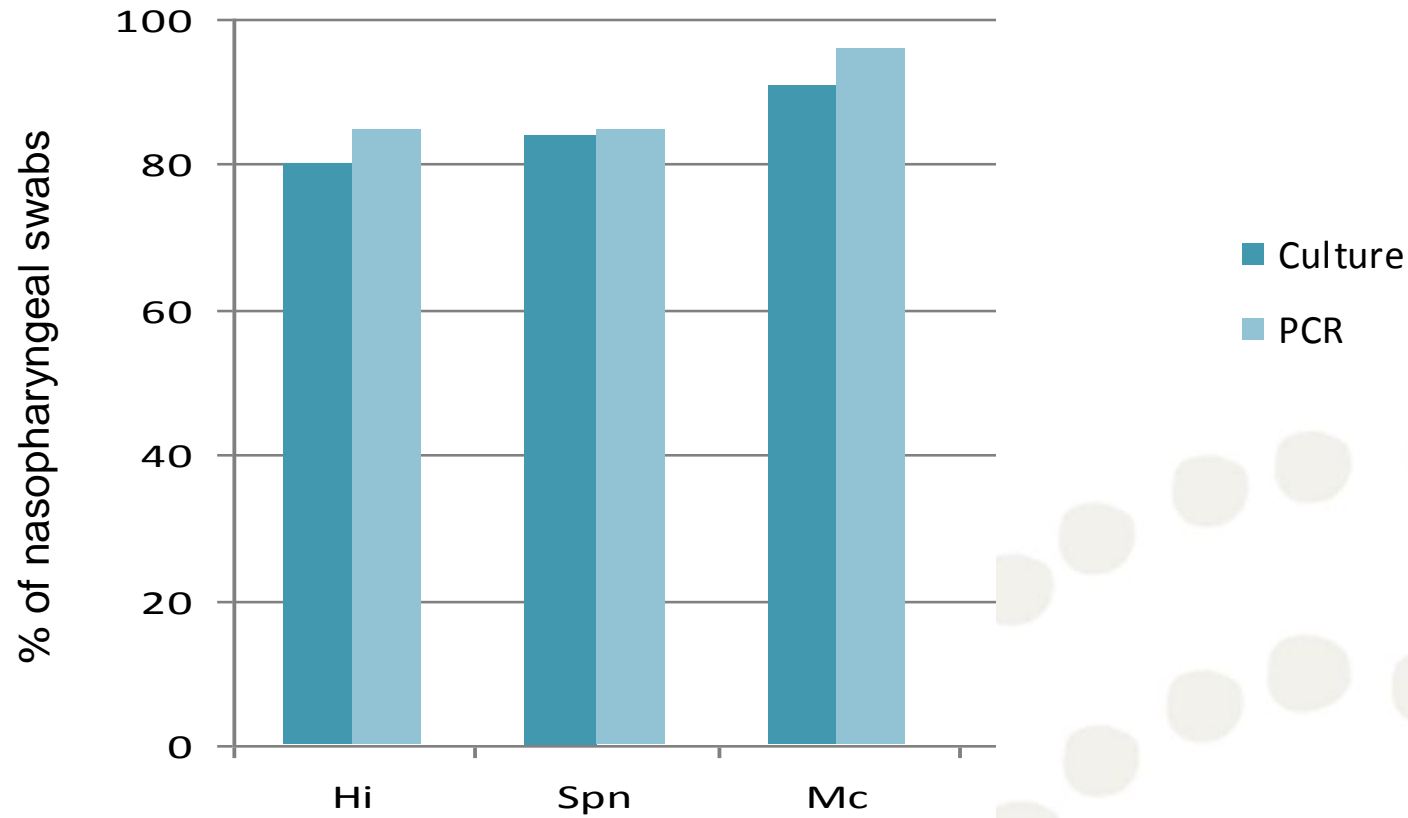
To explore bacterial load in children with acute otitis media with perforation (AOMwiP)



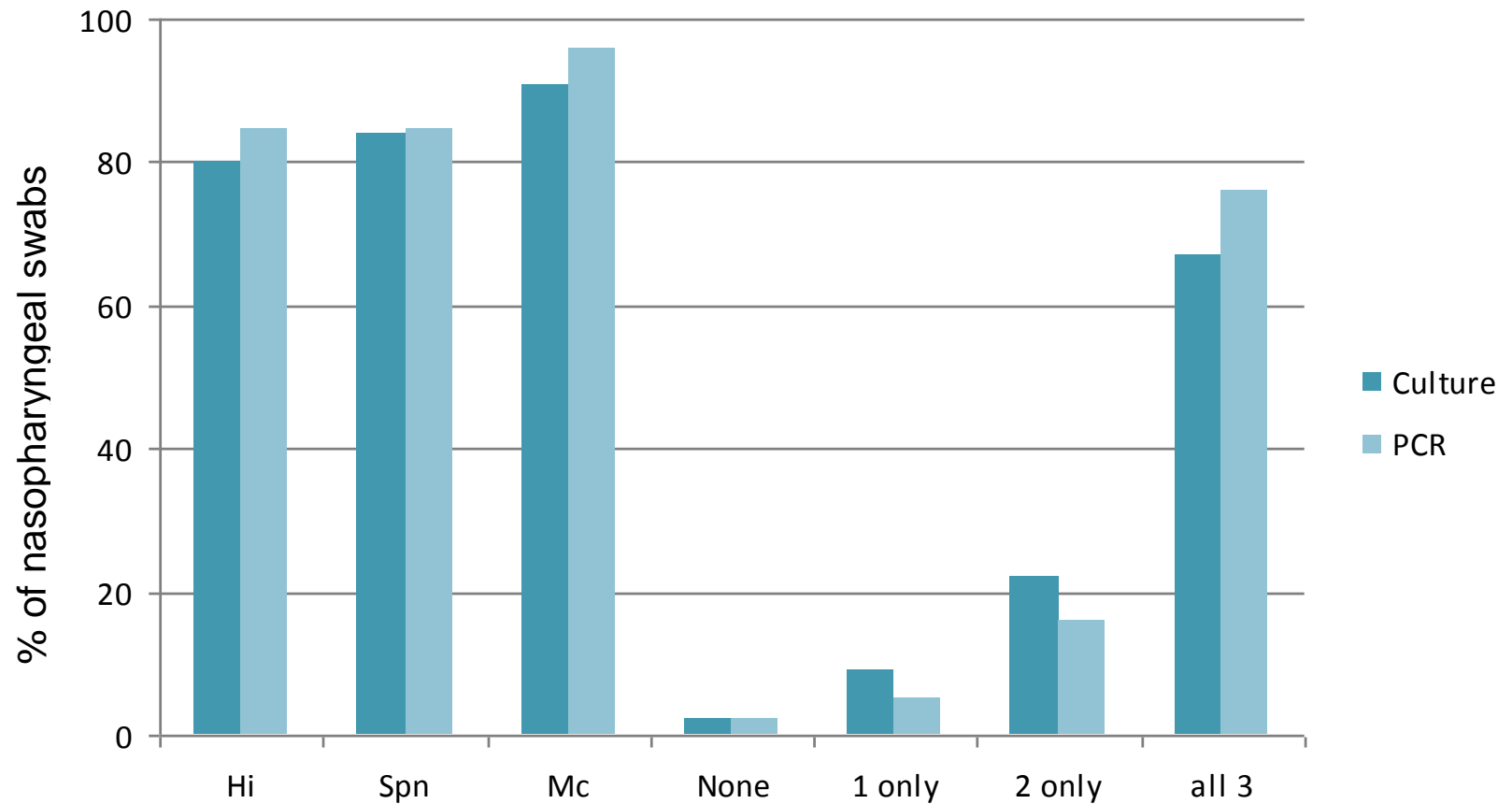
- 55 NT Indigenous children aged 4 – 55 months
- AOMwip <2% pars tensa and <6 weeks' duration
- no ABs in previous week

- paired NP and ED swabs
- Culture optimised for Spn and Hi
- qPCR to estimate bacterial load

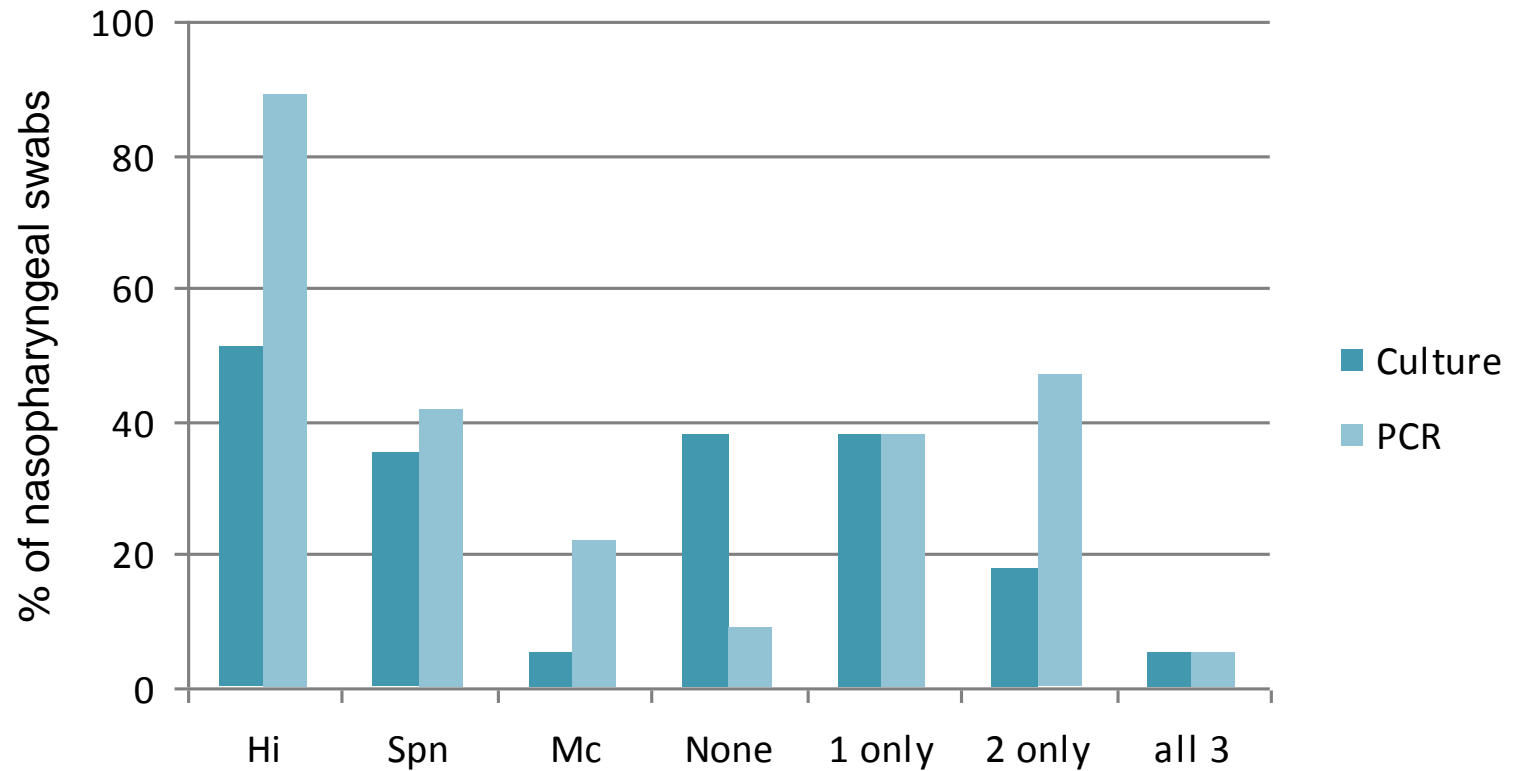
NASOPHARYNX



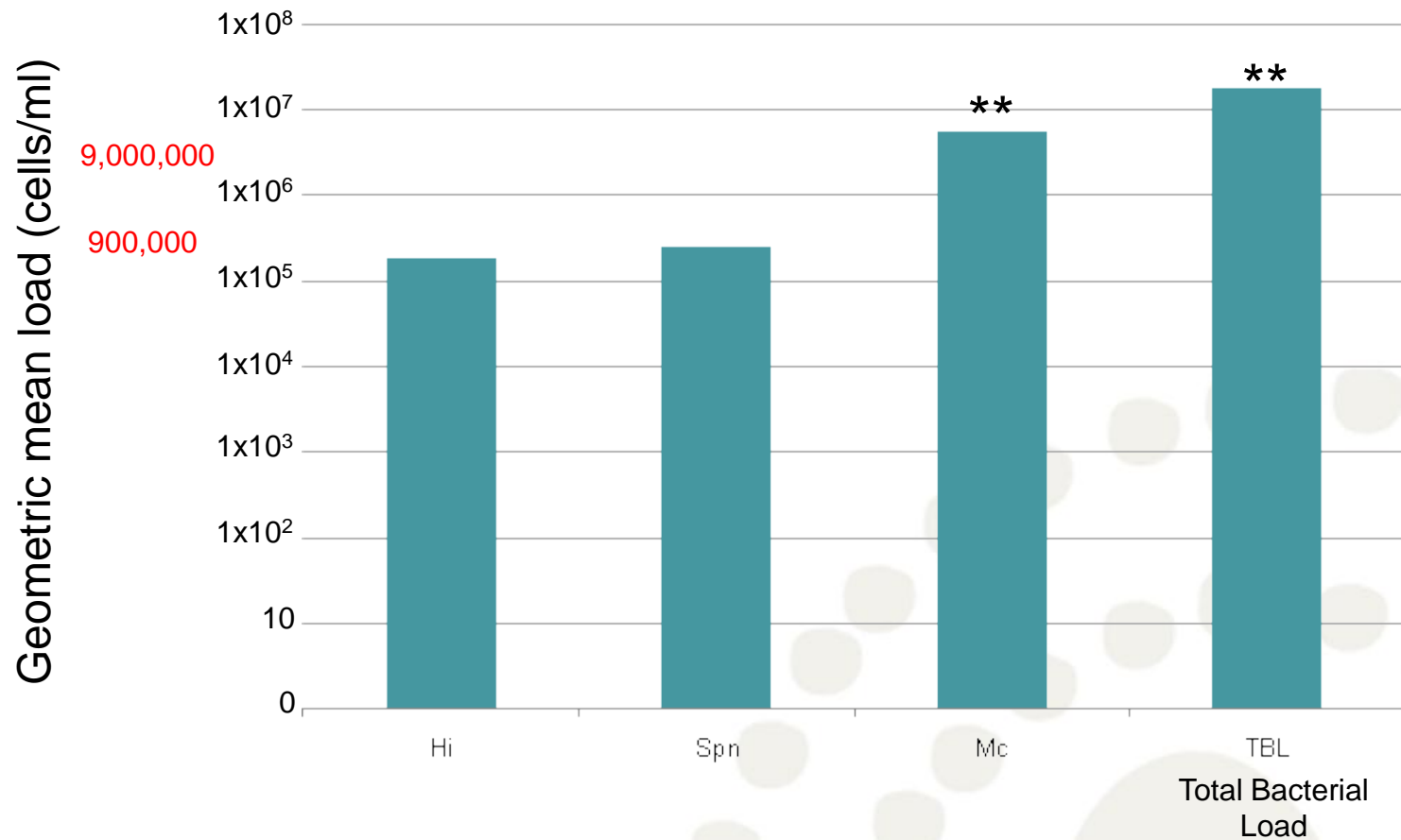
NASOPHARYNX



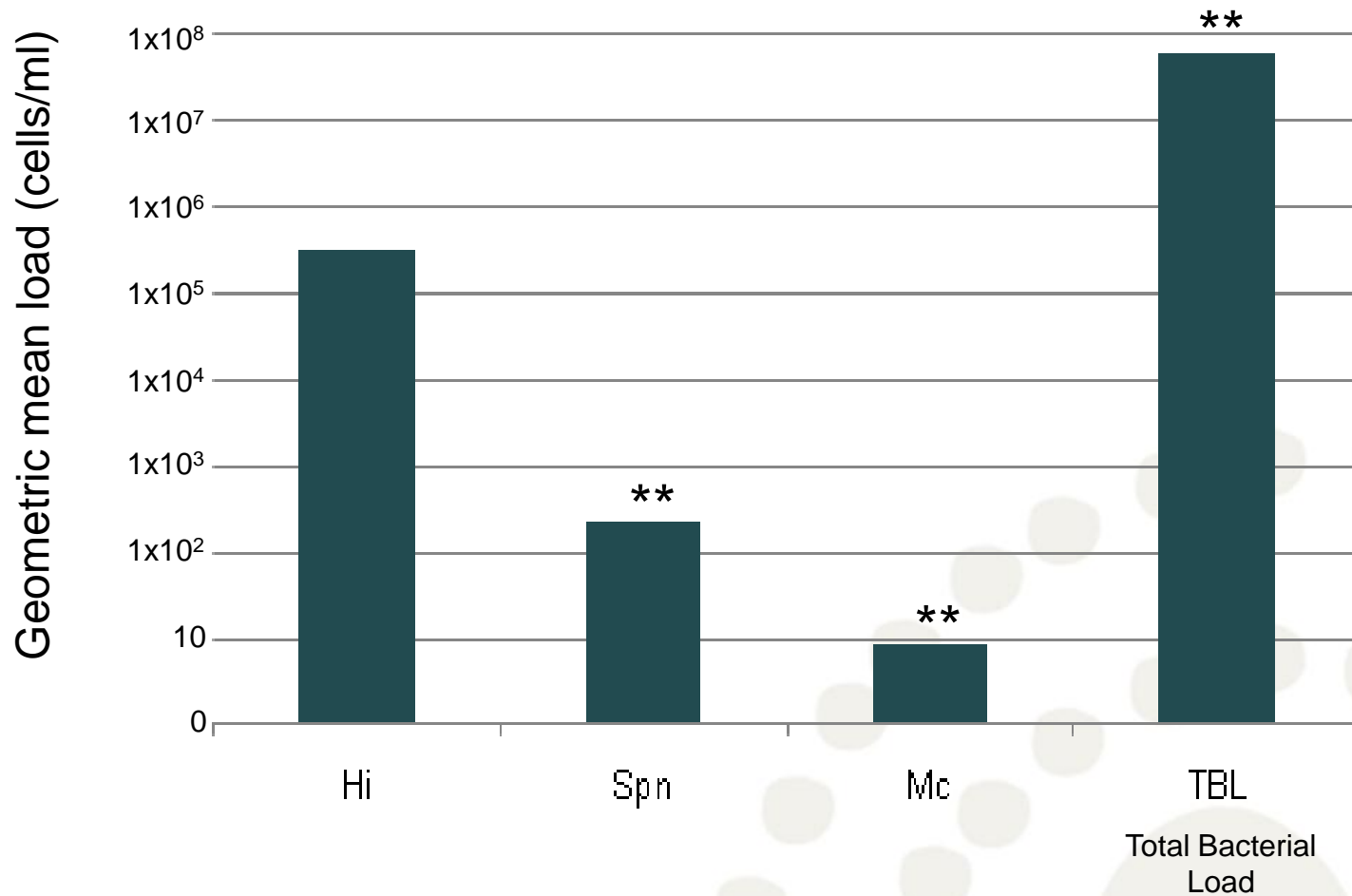
EAR DISCHARGE



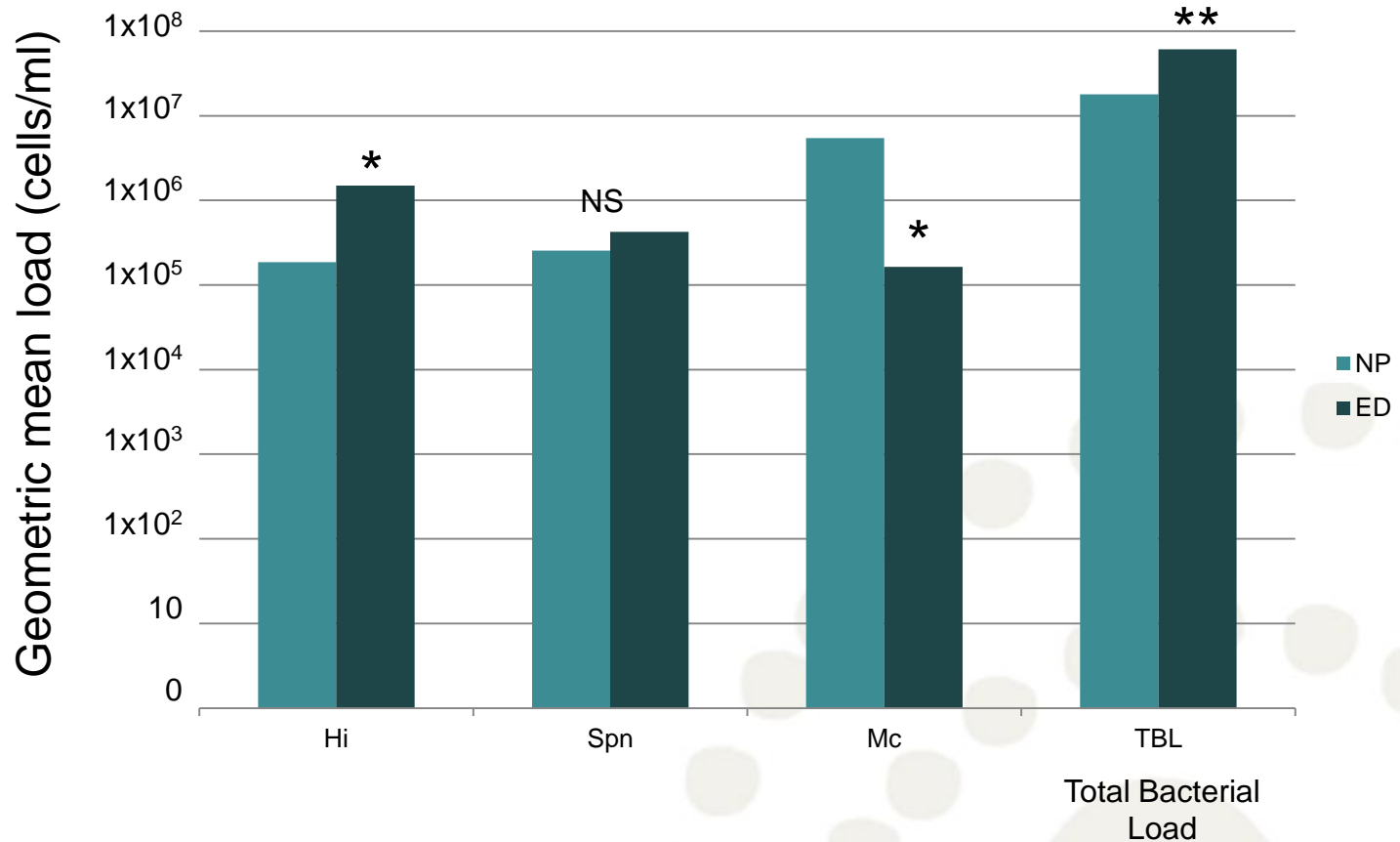
PCR ON NASOPHARYNGEAL SWABS



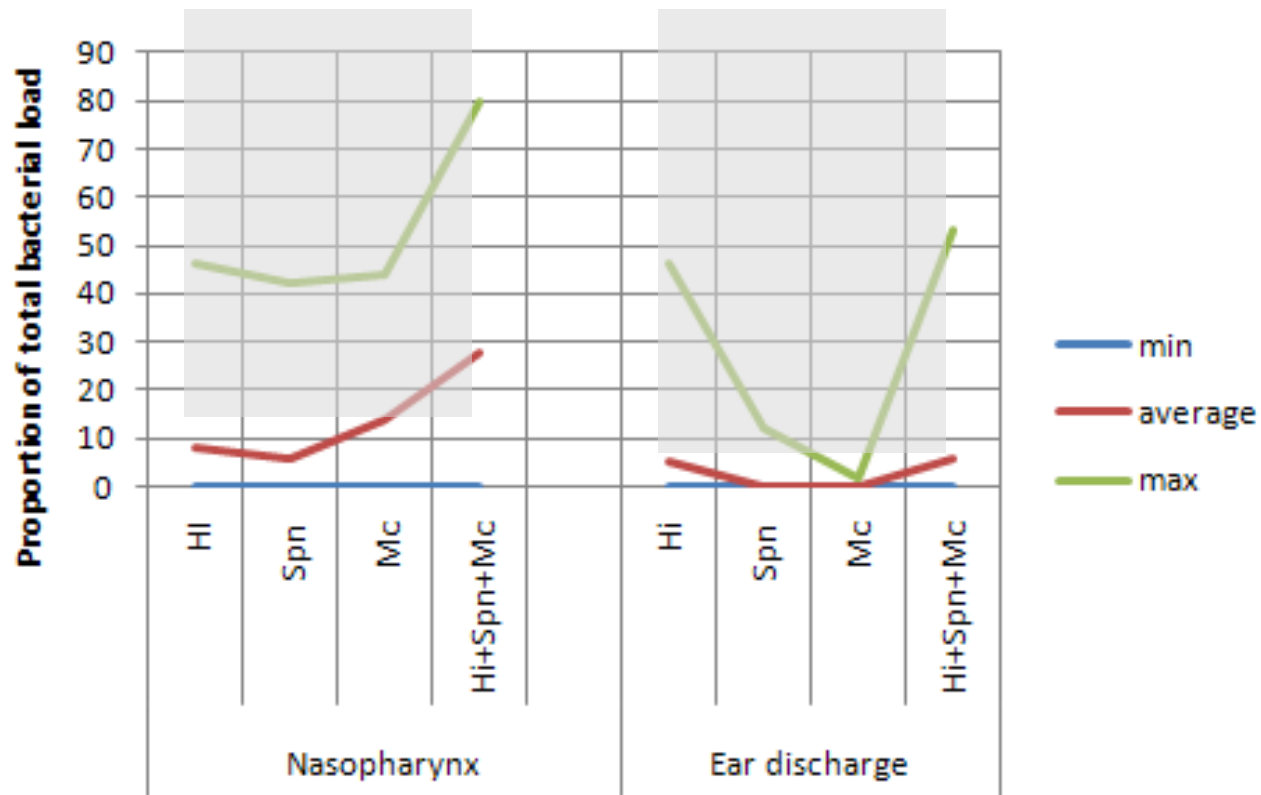
PCR ON EAR DISCHARGE SWABS



(pos swabs only)



Proportion of the total bacterial load



- OM has a complex pathogenesis
...and bacterial loads can help us understand the potential importance of various pathogens in OM
- *H. Influenzae* is a more dominant pathogen in ear discharge from children with AOMwip than Spn or Mc
- ...but there are more than the usual suspects (Robyn Marsh presentation)

Bustin *et al.* *BMC Molecular Biology* 2010, **11**:74
<http://www.biomedcentral.com/1471-2199/11/74>



BMC
Molecular Biology

EDITORIAL

Open Access

MIQE précis: Practical implementation of minimum standard guidelines for fluorescence-based quantitative real-time PCR experiments

Stephen A Bustin^{1*}, Jean-François Beaulieu², Jim Huggett³, Rolf Jaggi⁴, Frederick SB Kibenge⁵, Pål A Olsvik⁶, Louis C Penning⁷, Stefan Toegel⁸



miqe
a blueprint for successful qPCR assay design
<http://www.miqe.info/>



We would like to thank the families who participated in these studies and for their continued support of our research.

We are grateful to the NHMRC (Australia) and Channel 7 Children's Research Foundation for research support.

Menzies Ear Health Research Team and Child Health Laboratory Team for clinical specimens, clinical data, and laboratory support.

The OMOZ2012 Organisers!



- DNA extraction consistency
 - decide on a method (incl. enzymatic vs bead-beating pre-treatments) and stick to it.
- Store and prepare standards in TE (stability)
- Apply strict criteria for accepting results
 - R^2 must be $>$ or $= 0.99$
 - Efficiency must be $>$ or $= 0.80$
 - Replicates are $<$ or $= 0.5$ Cq different
 - Negative control.
 - If probe-free PCR, check the melt curve
- Internal control
- LOD - lowest standard concentration at which specific amplification was detected in at least 95% of replicates
- LOQ – lowest standard concentration at which replicates consistently within 0.5Cq
- POSBLOQ – median value between LOQ and LOQ

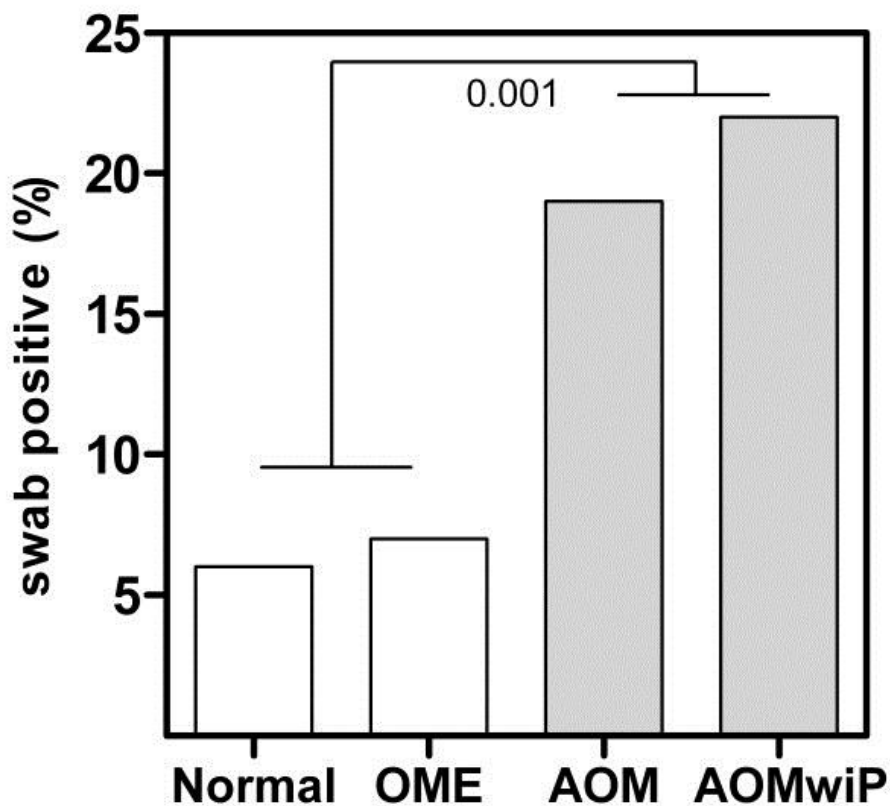
In Northern Territory Indigenous children (to 2 years of age)



Nasopharyngeal viruses

- Human rhinovirus (38%)
- Human polyomavirus (14%)
- *Human adenovirus* (13%)*
- Human bocavirus (8%)
- Human coronavirus (4%)

Human Adenovirus and AOM



The clinical staff!!!

Critical for meaningful results

- Good specimens
- No contamination (eg. of tubes or swabs)
 - Frozen (LN2 shipper or dry ice) soon after collection and not thawed

Plotting the individual specimens

