Zambia SAMIPS cohort study

Gill positivity estimates ratio

SAMIPS Cohort - Zambia

- **Study Design & Cohort**
  - Southern Africa Mother Infant Pertussis Study (SAMIPS) longitudinal birth cohort in Zambia
  - 1981 Mother-infant pairs enrolled at ≈ 1 week of age
  - Followed every 2–3 weeks through 14 weeks post-birth
  - NP sampling at every visit - including baseline, scheduled and unscheduled (resp complaints) visits
  - Infants received scheduled vaccines at 6, 10, and 14 weeks with the pentavalent vaccine
- **Pertussis Case Definitions**
  - The CDC case definition for a laboratory-confirmed pertussis infection can be met in **either** of two ways:
    1. Culture-positive Bordetella pertussis with any duration of cough, or
    2. PCR-positive B. pertussis plus ≥ 2 weeks of cough together with at least one classic symptom: whooping, paroxysmal cough, apnea, or post-tussive vomiting.
  - A screening definition was developed - an infant presenting with any of the signs or symptoms on our screening form with a positive PCR result.
  - Positive cases were further classified as severe/nonsevere pertussis using the modified perziosi scale

https://doi.org/10.1093/cid/ciw526

Gill et al, 2016

What is a pertussis positive PCR Test?

The 2016 paper used the following diagnostic algorithm to define PCR positive cases.

- PCR-positive when the IS481 Ct < 35 or
- IS481 Ct fell between 35–40 together with a PTxS1 signal

Supplementary Table 1a. Diagnostic algorithm for interpreting PCR results

Primer reactions				Interpretation
IS481	PTxS1	PIS1001*	HIS1001*	
Ct<35	(+) or (-)	NA	NA	*B. pertussis*
35<=CT<40	(+)	NA	NA	*B. pertussis*
35<=Ct<40	(-)	NA	NA	Indeterminate
(+ at any CT value <40)	(-)	(-)	(+)	*B. holmesii*
(-)	(+)	(+)	(-)	*B. parapertussis*
(-)	(-)	(-)	(-)	Non-Bordetella respiratory disease

IS481

- highly sensitive target
- most common insertion sequence in B. pertussis
- multiple copies per genome

PTxS1

- highly specific target
- coding for pertussis toxin
- exists as a single or double copy

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Gill et al, 2016 contd.

RESULTS

- 10 positive pertussis cases were identified
- Only 1 infant qualified as "severe pertussis" who was unimmunized and 3 weeks old
- 100% pertussis positive cases were symptomatic
- 0% with severe pertussis in vaccinated while 14% of unvaccinated cases with typical/severe pertussis

Pertussis +		**N[a] =10**	**Typical/Severe (paroxysmal cough >=14d)**	**Moderate/ Nonsevere**	**Sx (any)**
Vaccinated		3	0	3 (100%)	3 (100%)
Age (m)	3 - <4	3	0	3 (100%)	3 (100%)
Unvaccinated[1] (includes not effectively vaccinated)		7	1 (14%)	6 (86%)	7 (100%)
Age (m)	0- <1	2	1 (50%)	1 (50%)	2 (100%)
	1-<2	2	0	2 (100%)	2 (100%)
	2-<3	3	0	3 (100%)	3 (100%)

[1] includes vaccinated children with less than 21 days from last immunization to diagnosis

[a] Denominator for Row%

Table 6. Incidence of Infant Pertussis by Prior Number of Whole-Cell Pertussis Vaccinations

Immunization Status[a]	No. of Infants	Person-time, months	Incidence Rate per 1000 Person-months[b]
All pertussis			
No immunization	4	1882	2.1
1 DTP dose	4	1458	2.7
2 DTP doses	2	914	2.2
Nonsevere pertussis			
No immunization	3	1882	1.6
1 DTP dose	4	1459	2.7
2 DTP doses	2	915	2.2
Severe pertussis			
No immunization	1	1882	0.5
1 DTP dose	0	1463	0
2 DTP doses	0	921	0

Abbreviation: DTP, diphtheria-tetanus-pertussis vaccine.

[a] The final nasopharyngeal swab was obtained at the same visit at which DTP dose 3 was given. Therefore, we have no incidence data after DTP dose 3.

[b] In all cases, incidence was calculated allowing for 2 weeks for the infants to respond to the

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Gill et al, 2021

A detecting assay is any IS481 qPCR test that produces a measurable fluorescence signal before the assay reaches its maximum number of cycles. In this study every sample was run for 45 PCR cycles;
In 2016 paper,

- Threshold Ct=35
- Pertussis -ive cases: Ct >35

In 2021 analysis,

- Detecting assay: a Ct value ≤ 45 (target DNA detected)
- Non-detecting assay: Ct = 45 (no signal after the full run)

The authors wanted to justify that lab diagnosed testing where only Ct<35 are considered positive for pertussis might not be representative of actual pertussis circulation in the population.

EFI = Evidence of Infection

For every subject

- IS481 PCR Cycle Threshold values for all assays (Ct) arranged from the highest to the lowest
- For every assay, proportion of assays that were as strong or stronger calculated and graphed = RCD (reverse cumulative distribution)
- Geometric mean of the RCD calculated
- 1 – Geometric Mean = EFI

EFI = 0 means all assays were non-detecting NO EVIDENCE
EFI = 1 means repeatedly strong Ct values.

To categorize infection strength, they set a threshold based on the number of detecting assays:
Strong EFI: participants with ≥ 3 detecting assays,
Weak EFI: those with > 0 but < 3 detecting assays
None EFI: those with no detecting assays

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Gill et al, 2021

Among infants with strong molecular evidence of infection, the percent asymptomatic or minimally symptomatic was **59% among unvaccinated** and **79% among vaccinated**

(no difference by #doses: 1d: 5/6 = 83.3%, 2d: 20/26 = 76.9%, 3d: 101/128 = 78.9%)

Only one infant presented with classic symptoms of whooping and paroxysmal cough (unvaccinated, 26 days of age).

Unvaccinated

	EFI Category				
		+ Pertussis			
Symptoms	**Neg**	**Weak**	**Strong**	**Total+**	**Sum**
None	18	4	2	6	24
Minimal [1]	33	21	8		62
Moderate/Severe [2]	16	10	7	17	33
Typical (parox.)				1/52 (1.9%)	
Sum	67	35	17	52	119
% Asx				**11.5%**	
% Asx or mild			10/17= **58.8**		
% mod/sev Sx	**23.9%**	**28.6%**	7/17= **41.2%** **(21.6%–64.0%)**	17/52= **32.7%**	

Vaccinated

	EFI Category				
		+ Pertussis			
Symptoms	**Neg**	**Weak**	**Strong**	**Total+**	**Sum**
None	428	89	39	128	556
Minimal [1]	279	107	87		473
Moderate/Severe [2]	92	46	34	80	172
Typical (parox.)				0/402 (0%)	
Sum	799	242	160	402	1201
% Asx				**31.8%**	
% Asx or mild			126/160= **78.8**		
% mod/sev Sx	**11.5%**	**19.0%**	34/160= **21.2%** **(15.6%–28.2%)**	80/402= **19.9%**	

[1] Minimal symptoms include coryza and/or uncomplicated cough.

[2] Moderate to severe symptoms include all other pertussis symptoms in the Modified Preziosi Scale.

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