

1 **Pneumococcal Conjugate Vaccine Dose-Ranging Studies in Humans: A**
2 **Systematic Review**

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24

25 Abstract

26 Background

27 *Streptococcus pneumoniae* is one of the most common bacterial pathogens of infants and young children.
28 Antibody responses against the pneumococcal polysaccharide capsule are the basis of vaccine-mediated
29 protection. We examined the relationship between the dose of polysaccharide in pneumococcal conjugate
30 vaccines (PCVs) and immunogenicity.

31 Methods

32 A systematic search of English publications that evaluated the immunogenicity of varying doses of
33 pneumococcal conjugate vaccines was performed in Medline and Embase (Ovid Sp) databases in August
34 2019. We included only articles that involved administration of pneumococcal conjugate vaccine in
35 humans and assessed the immunogenicity of more than one serotype-specific saccharide dose. Results
36 were synthesised descriptively due to the heterogeneity of product valency, product content and vaccine
37 schedule.

38 Results

39 We identified 1691 articles after de-duplication; 9 studies met our inclusion criteria; 2 in adults, 6 in
40 children and 1 in both. Doses of polysaccharide evaluated ranged from 0.44 mcg to 17.6 mcg. In infants,
41 all doses tested elicited IgG geometric mean concentrations (GMCs) above the established correlate of
42 protection (COP; 0.35 mcg/ml). A month after completion of the administered vaccine schedule, 95%
43 confidence intervals of only three out of all the doses evaluated had GMCs that crossed below the COP.
44 In the adult studies, all adults achieved GMCs that would be considered protective in children who have
45 received 3 standard vaccine doses.

46 Conclusion

47 For some products, the mean antibody concentrations induced against some pneumococcal serotypes
48 increased with increasing doses of the polysaccharide conjugate, but for other serotypes, there were no
49 clear dose-response relationships or the dose response curves were negative. Fractional doses of

50 polysaccharide which contain less than is included in currently distributed formulations may be useful in
51 the development of higher valency vaccines, or dose-sparing delivery for paediatric use.

52

53

54 **Key words**

55 Pneumococcal conjugate vaccines. Dose-range. Systematic review. Immunogenicity.

56

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59 Background

60 The polysaccharide capsule of *Streptococcus pneumoniae* is the principal target of the mature human
61 response to pneumococcal infection and the reason initial vaccine development focused on pneumococcal
62 polysaccharide vaccines [1]. However, polysaccharides are poor immunogens, especially in infants and
63 the elderly [1, 2]. Conjugation of serotype-specific capsule polysaccharides to a carrier protein improves
64 immunogenicity by stimulating T-cell dependent responses [3].

65
66 Early conjugate vaccine candidates differed in the dose of saccharide conjugated to the carrier protein, the
67 saccharide chain length, the carrier protein used, the ratio of carrier protein to saccharide, the conjugation
68 method, the adjuvant used and the vaccination schedule[4-27] (Table 1).

69
70 Some of the evidence that led to the vaccine formulations in use today has been summarised previously
71 [3]. In brief, polysaccharides were found to be more immunogenic than oligosaccharides [2, 28]. Proteins
72 used in other conjugate vaccines, like Tetanus Toxoid (TT) or *Neisseria meningitidis* outer membrane
73 protein (OMPC) generated lower immune responses when used in PCVs compared to other carrier
74 proteins such as Diphtheria Toxoid (DT) and Diphtheria Toxoid mutant (Dip. CRM197) [3]. PCVs using
75 TT, OMPC or protein D seemed to elicit a peaked response (immunogenicity increased with dose until a
76 threshold and then decreased thereafter) or no dose-dependent response. However, candidates using DT
77 or Dip. CRM197 as the carrier protein elicited a linear dose-response relationship and were less likely to
78 induce epitopic B-cell suppression (CIES) than vaccines using TT or OMPC carrier proteins.

79 Additionally, higher valency PCVs using Dip. CRM197 benefit from coadministration with other infant
80 vaccines using DT protein [3].

81
82 The need to keep the total saccharide and carrier protein doses low to avoid interference and/or hypo
83 responsiveness, while incorporating multiple serotypes into the vaccine, led to the development of
84 candidates with lower saccharide doses and lower carrier protein load than the Hib conjugate vaccines

85 previously developed[3]. Doses of saccharide in current conjugate vaccines were determined before the
86 correlate of protection was known. Immunogenicity was measured in fold-rises of IgG titres compared to
87 baseline. Relatively low concentrations of serotype-specific IgG (0.35 mcg/ml) in response to vaccine
88 have since been shown to correlate with protection against invasive pneumococcal disease in infants[29],
89 while protection against acquisition of carriage of pneumococci in the nasopharynx may require higher
90 concentrations (2-5 mcg/ml)[30].

91
92 As of March 2019, 75% of countries globally had introduced PCV. Since 2010, Gavi, the Vaccine
93 Alliance, has supported PCV introduction in 60 low and middle-income countries (LMICs) [31]. PCV
94 alone represents the largest proportion of the Gavi budget when compared to all other vaccines [32] and,
95 at approximately US\$10 per fully immunized child, the most expensive vaccine in the routine vaccination
96 schedule for many LMICs [33]. One approach to reducing the financial cost of PCV programmes is to use
97 a fractional dose at each vaccination but this is only possible if lower doses are sufficiently immunogenic
98 to indicate strong protection. We examined previous literature on the relationship between the dose of
99 polysaccharide in pneumococcal conjugate vaccines (PCVs) and immunogenicity in a systematic review.

100

101 **Methods**

102 **Search strategy**

103 The Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) guidelines were
104 followed [34]. Medline and Embase databases (Ovid SP) were searched in April 2018 and the search was
105 updated in August 2019. Search terms were built around 1) pneumococcal vaccination/ immunisation 2)
106 immunogenicity 3) dose/ dosage/ dose-response/ dose-ranging. The search had no restrictions based on
107 publication date. We included only English-language publications that involved administration of
108 pneumococcal conjugate vaccine in humans and assessed the immunogenicity of more than one serotype-
109 specific saccharide dose (Figure 1, Supplementary Table 1).

110 Screening of Articles

111 All articles retrieved from the two databases were exported into Endnote X8 (Clarivate Analytics, PA,
112 USA) and duplicates were automatically and manually removed.

113 The title and/or abstracts were screened by two reviewers (RKL and KEG) independently (Figure 1). Full
114 texts were screened by two of three reviewers (KEG, CH and RKL). Articles were excluded if they did
115 not assess more than 1 dose of polysaccharide conjugate and/or did not report serum IgG concentrations.

116

117 Data Extraction & Synthesis

118 Data from included articles were extracted into a template in Microsoft Excel 2013. Data on the study
119 population, setting, vaccine formulation, comparison arms/cohorts, schedule, outcome measure(s) and
120 timepoint of outcome measurement were noted alongside any analyses. The qualities of the included
121 studies were evaluated using the Cochrane GRADE system [35].

122 The studies were not combined in a meta-analysis because of the heterogeneity in the vaccine valency,
123 carrier protein, adjuvant, adjuvant dose, manufacturer and conjugation methods, the vaccination schedule
124 and the population of analysis (children, adults with or without prior vaccination). Instead, serotype-
125 specific dose response curves were estimated using data from studies with the same vaccination schedule
126 and immunogenicity endpoints.

127

128 We requested the corresponding authors to provide access to the raw data. Where data was not provided,

129 the proportion of infants and adults with IgG GMCs below the established correlate of protection (0.35
130 mcg/mL[36]) was estimated from the reported estimates of the geometric mean concentrations to each

131 dose and log-scale standard deviation by assuming a normal distribution. To evaluate whether the

132 assumption of normality was reasonable, the estimated proportions for one of the included studies which

133 provided raw data, Rupp *et al.*'s formulation B, were compared with the reported proportions. The

134 estimated proportions were found to be similar to those reported. Since Rupp *et al.* reported the proportion

135 of responders (rather than proportion non-responders), the proportion of non-responders for their study
136 was calculated as 1-proportion responders.

137

138 Results

139 The search identified 3791 articles; 1691 remained after de-duplication (Figure 1). A total of 360 full
140 texts were reviewed; 9 studies were included in the review [2, 28, 37-43] (Table 2). Of the nine, two
141 studies involved adult populations [41, 43], six involved paediatric populations [2, 28, 38-40, 42] and one
142 involved adult and paediatric populations [37].

143

144 Quality of included studies

145 All the included studies were individually randomised controlled trials. The included studies were graded
146 to have high to moderate quality of evidence (Supplementary table 2). The blinding procedures for four of
147 the nine studies [38-40, 42] were not reported. Only five of the nine included studies, [37-39, 41, 43],
148 mentioned the number of participants withdrawn or lost to follow up prior to the primary endpoint.

149

150 Immunogenicity in adult studies

151 Three studies involved adult populations [37, 41, 43] (Table 2). Lode *et al.* and Jackson *et al.* studied the
152 immunogenicity of PCV7(Prevnar ®, Wyeth Vaccines, NY) in healthy adults >70 years old with no
153 history of PPV [43] and in adults 70-79 years old with a previous history of PPV exposure [41, 44]
154 respectively. The vaccines were administered as a single dose with polysaccharide doses ranging from
155 0.44 to 8.8 mcg for serotypes 4, 9V, 14, 18C, 19F, 23F and 0.88 to 17.6 mcg for serotype 6B. Rupp *et al.*
156 evaluated the safety and immunogenicity of two formulations of PCV15 (Merck Sharp & Dohme Corp) in
157 healthy adults aged 18 to 49 years with no history of either PPV or PCV exposure. The vaccines were
158 administered as a single dose in each group at polysaccharide doses of 2 and 4 mcg. All PCV7 doses
159 evaluated by Lode *et al.* and Jackson *et al.* were also evaluated by Rupp *et al.*

160 A dose dependent increase in serum IgG GMCs which then plateaued was apparent for serotype 4 for all
161 three adult studies [37, 41, 43], serotype 6B for two out of three studies [41, 43] and for serotype 23F in
162 one of the three studies [41]. The overall IgG GMCs reported for Jackson *et al.* were lower than those
163 reported for Lode *et al.* for all serotypes. IgG GMCs for serotype 9V and 23F reduced at higher doses in
164 Lode *et al.* [43] and for both formulations in Rupp *et al.* [37], while those for serotype 19F, 18C, 9V and
165 23F for Lode *et al.* [43] increased with higher doses (Supplementary Figure 1).

166

167 Estimated proportions of adults with IgG GMCs below the infant correlate of protection were calculated
168 for the studies which reported IgG GMCs and the confidence intervals around these means, assuming a
169 normal distribution (Supplementary Figure 2). These proportions ranged between 0.1% (95% confidence
170 interval (CI): 0-17.0%) (Lode *et al.*, serotype 18C, dose: 4.4 mcg/mL) and 22.3% (95% CI: 12.4-36.8%)
171 (Jackson *et al.*, serotype 4, dose: 0.44 mcg/mL).

172 Immunogenicity in paediatric studies

173 A total of 7 studies involved paediatric populations ranging from 2 to 30 months of age [2, 28, 37-40, 42]
174 (Table 2). Daum *et al.* [28], Ahman *et al.* (1998 and 1999) [39, 40] and Zangwill *et al.* [42] evaluated
175 varying doses of experimental PCVs in 3-dose schedules at 2, 4 and 6 months of age. Steinhoff *et al.* [2]
176 evaluated the immunogenicity of varying doses of PCV2 after a single dose administered at 18-30
177 months. Anderson *et al.* [38] evaluated varying doses of an experimental PCV3 with two different carrier
178 proteins (Dip. CRM197 and Tetanus Toxoid) after two doses administered at 24 and 26 months. Rupp *et*
179 *al.* [37] evaluated varying doses of two PCV15 formulations (Merck Sharp & Dohme Corp) after
180 administration of a 4-dose schedule at 2, 4, 6 and 12-15 months of age. Concomitant vaccinations as per
181 national vaccination schedules were allowed for all the studies. As immunogenicity varies with age, the
182 two studies in toddlers [2, 38] were not included in the descriptive synthesis as toddlers are not the target
183 population for current routine immunization programmes. The common serotype evaluated by the toddler
184 studies [2, 38] was 23F. The proportion of toddlers with > 4-fold increase in IgG GMCs from baseline

185 after a single dose for serotype 23F in these two studies ranged between 20% (group that received 5.1
186 mcg of PCV)[38] and 94% (group that received 2 mcg of PCV)[2].

187 Serotype specific IgG GMCs post final dose in comparable infant populations were plotted against each
188 other for the common serotypes 6B, 14, 19F and 23F using data from Daum *et al.*[28], Ahman *et al.*(1998
189 and 1999)[39, 40] and Rupp *et al.*'s formulation A with 250 mcg of aluminium phosphate([37]. Zangwill
190 *et al.*'s [42] IgG GMCs were included for serotype 6B (Figure 2). A dose-response effect was apparent for
191 STs 14, 19F and 23F for the Daum *et al.* [28] and Ahman (1998) *et al.* [39] studies.

192 Confidence intervals around the IgG GMC for serotype 6B and 23F's highest dose in the Ahman (1999)
193 *et al.* [40] study crossed the correlate of protection as well as those for Ahman (1998) *et al.*'s [39] lowest
194 dose for serotype 23F (Figure 2).

195

196 Estimated proportions of infants with IgG GMCs below the correlate of protection (0.35mcg/mL) were
197 calculated for comparable infant studies which reported IgG GMCs and the confidence intervals around
198 these means. Serotype 6B had the highest proportion of infants below the correlate of protection
199 compared to other serotypes (Figure 3). Increasing doses for STs 6B, 14 and 23F seemed to correspond to
200 a decrease in the proportion of infants below the correlate of protection in the Ahman (1998) *et al.* trial
201 [39].

202 Follow-up post primary endpoint in children

203 The longest follow up reported was 36 months after enrolment [39, 40]. A booster dose was administered
204 to children in three studies. All booster doses elicited a strong memory response. Two studies reported
205 that after a polysaccharide vaccine booster, antibody responses post-boost were higher in those who
206 received the lowest vaccine dose in infancy (Table 3).

207

208 Discussion

209 This review aimed to collate evidence on the immunogenicity of varying doses of serotype specific
210 polysaccharide within PCVs. Nine studies were included after a literature search that was limited to
211 studies in humans that reported immunogenicity outcomes for varying doses. It is likely that more
212 information on dose-response exists but lies unpublished by vaccine manufacturers as part of their
213 research and development data. The studies included were all RCTs and graded to be of moderate to high
214 quality evidence. Some of the studies had small sample sizes per trial arm but the effect of this on the
215 statistical power of the results could not be calculated due to limitations in the data reported e.g. no
216 information on loss to follow up and the IgG GMC variance. The included studies were published
217 between 1994 to 2018. Most studies were published before there was an established immune correlate of
218 protection in children, to inform the study results. The most recent study was of a PCV15[37] which is
219 currently undergoing adult and paediatric clinical development.

220

221 Of the seven paediatric studies included, five administered the study vaccine in a schedule of 3 primary
222 doses (3p+0) or a schedule of 3 primary doses plus a booster (3p+1) to infants, starting at 2 months of
223 age i.e. findings may be relevant to current routine infant immunisation schedules. The PCV doses tested
224 ranged between 0.5 and 10 mcg. Only two of these five paediatric studies showed a dose-response where
225 higher ST-specific doses correlated with higher GMCs after the prime vaccinations [37, 39].

226 Paradoxically a clear dose response was not seen for ST6B; however, this serotype is consistently
227 included at higher doses in licensed products than other serotypes, the data supporting this decision is
228 unclear from the available literature.

229

230 When the proportion of children with antibody titres above the established correlate of protection was
231 estimated from the reported GMCs, the confidence intervals around the estimates are wide. Only one of
232 the five studies showed a consistent favourable trend with dose, where the proportion of infants below the
233 correlate of protection (i.e. “unprotected”) decreased with higher doses [39]. The limitations of this
234 approach are acknowledged, the assumption of a normal distribution could be incorrect, despite it being

235 supported by the data visually. Assuming alternative distributions could result in greater or lesser
236 proportions above the correlate of protection. The performance of the assays used by the older studies [2,
237 28, 39, 40] were not standardised. Because of this, it is unclear how their antibody results relate to the
238 0.35 mcg/ml threshold and they may not be accurate at the lower limits. Additionally, the established
239 correlate of protection is thought to overestimate the IgG concentrations needed to protect against
240 invasive pneumococcal disease (IPD) caused by serotypes 6A, 6B, 18C and 23F and underestimate the
241 concentration needed to protect against IPD caused by serotypes 1, 3, 7F, 19A and 19F [45, 46]. Future
242 PCVs may benefit from being evaluated against ST-specific thresholds rather than a common correlate of
243 protection. However, this review provides some evidence that smaller doses than those included in
244 currently distributed PCVs are immunogenic and could be protective in children.

245

246 In all three adult studies, there was a dose response where the highest dose induced the highest immune
247 response [43]. History of pneumococcal polysaccharide vaccine prior to PCV administration could have
248 contributed to the consistently lower IgG GMCs (hypo-responsiveness) in otherwise comparable
249 participants enrolled in the Jackson *et al.* study, compared to the Lode *et al.* study [1, 43]. There is no
250 established correlate of protection for adult populations and therefore the clinical implications of the
251 observed dose-response are unclear.

252

253 Lower priming doses were reported to give a higher GMCs post-boost, regardless of the vaccination
254 schedule, in two paediatric and two adult studies that assessed this [37, 39, 40, 42]. There are some data
255 from studies of other vaccines that indicate smaller prime doses may elicit better memory responses to a
256 booster dose [48, 49]. Although the mechanisms for this are unclear, it is a reminder that measures of
257 immunogenicity one month after the final dose in the series should not be seen in isolation and future
258 studies should assess the impact of dose on immune memory.

259

260 This review is limited by the fact that the observed relationships between dose and immunogenicity are
261 heterogenous and much of this variation may be attributable to factors other than the saccharide dose e.g.
262 the carrier protein, the ratio of polysaccharide to carrier protein, the method of conjugation and the
263 adjuvant of choice[3]. The two Ahman *et al.* studies provide a comparison of two carrier protein
264 conjugates across three saccharide doses. In these studies, the TT conjugates [40] show a varied pattern,
265 whereas the DT conjugates showed a dose-response relationship for some STs [39]. Other important
266 factors are the conjugation technique and dose of adjuvant. For example, the Rupp *et al.* studies evaluated
267 varying doses of PCV15 in two formulations that differed in their conjugation method and amount of
268 aluminium hydroxide. One formulation performed better than the other across all serotypes in adults and
269 infants and was selected for further clinical investigation [37]. Interaction with concurrently administered
270 vaccines can also influence immune responses [47]. Despite reporting a satisfactory immune response to a
271 primary series with OMPC as a carrier protein, Zangwill *et al.* [42], reported a negative effect of
272 concurrent immunization with a homologous carrier protein (Hib conjugate vaccine) on the immune
273 response to PCV. In addition to these factors, development of higher valency PCVs will also need to
274 consider the total polysaccharide and carrier protein content to avoid hypo-responsiveness and immune
275 interference e.g., PCV13 has been shown to induce a lower individual immune response compared to
276 PCV7 and this may be due to the increase in total polysaccharide and carrier protein content[3, 47].

277 Conclusion

278 In conclusion, for some products, the mean antibody concentrations induced against some pneumococcal
279 serotypes increased with increasing doses of the polysaccharide conjugate, but for other serotypes and
280 other products there was no clear dose-response relationship or the dose response curves were negative.
281 Overall, in children, evidence suggests smaller doses of polysaccharide than those in currently distributed
282 formulations are immunogenic and may be protective. However, the carrier protein content, conjugation
283 technique and adjuvant also determine the quality and quantity of the immune response.

284 Since development of higher valency PCVs relies on optimization of the polysaccharide dose while
285 minimizing the total polysaccharide and carrier protein content and adjuvant volume[3], evidence of the
286 immunogenicity of these small doses of polysaccharide may be useful in the development of higher
287 valency vaccines, or dose-sparing delivery.

288

289 **Conflict of interest statement:**

290 All authors declare that they have no known competing financial interests or personal
291 relationships that could have appeared to influence the work reported in this paper.

292

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297

298

299 **Table 1: Candidate Pneumococcal Conjugate Vaccine formulation (pre- and post-licensure)**

Licensure status	Licensed as PCV7							Licensed in PCV10			Licensed in PCV13		Not licensed		Conjugate protein	Adjuvant	
	4	6B	9V	14	18C	19F	23F	1	5	7F	3	6A	19A	22F			33F
Pneumococcal serotype saccharide, dose (µg)																	
Pre-licensure vaccine candidates [Manufacturer, year of earliest appearance in publication]:																	
PCV4 [4] [Merck, 1995]		1		1		1	1										Mening. (B) OMPC Aluminium hydroxide
PCV7 [5, 6] [Merck, 1995]	1	2.5	1	1	1	1	1										Mening. (B) OMPC Aluminium hydroxide
PCV7 [7, 8] [Merck, 1996]	1	3.5	1.5-2	1	1	2- 2.5	1										Mening. (B) OMPC Aluminium hydroxide
PCV5 [9-12] [Lederle, 1996]		10		10	10	10	10										Dip. CRM197 Aluminium hydroxide
PCV5 [13] [Lederle, 1996]		5		5	5	5	5										Dip. CRM197 Aluminium phosphate
PCV4 [14] [Pasteur Merieux, 1997]		3		3		3	3										TT or Dip. Toxoid
PCV7 [15, 16] [Wyeth, 1998]	2	4	2	2	2	2	2										Dip. CRM197 Aluminium phosphate

PCV9 [17-19] [Wyeth-Lederle, 1999]	2	4	2	2	2	2	2	2	2	2							Dip. CRM197	Aluminium phosphate
PCV11 [20-22] [Aventis Pasteur, 2001]	1	10	1	3	3	1	1	1	1	1	3						TT (ST 1, 4, 5, 7F, 9V, 19F, 23F) Dip. Toxoid (ST 3, 6B, 14, 18C)	Aluminium hydroxide
PCV8 [23] [Aventis Pasteur, 2004]	3	3	3	3	3	3	3				3						Dip. Toxoid	
PCV8 [23] [Aventis Pasteur, 2004]	1	1	1	1	1	1	1				1						TT	
PCV11 [24] [GSK, 2008]	1	1	1	1	1	1	1	1	1	1	1						D (NTHib)	
PCV7 [25] [Centre for Bimolecular Chemistry Cuba, 2014]		4		2	2	2	2	2	2								TT	Aluminium phosphate
PCV15 [26, 27] [Merck Sharpe & Dohme, 2015]	2	4	2	2	2	2	2	2	2	2	2	2	2	2	2	2	Dip. CRM197	Aluminium phosphate
Licensed products [year of licensure]:																		
PCV7 (Pfizer/Wyeth; 2000)	2.2	4.4	2.2	2.2	2.2	2.2	2.2										Dip. CRM197	Aluminium phosphate
PCV10 (GSK, 2009)	3.0	1.0	1.0	1.0	3.0	3.0	1.0	1.0	1.0	1.0							D (NTHib), Dip, TT	Aluminium phosphate
PCV13 (Pfizer/Wyeth; 2010)	2.2	4.4	2.2	2.2	2.2	2.2	2.2	2.2	2.2	2.2	2.2	2.2	2.2	2.2	2.2	2.2	Dip. CRM197	Aluminium phosphate

300 Abbreviations: CRM197: non-toxic mutant of Diphtheria toxin; D(NTHib): Protein D of non-typeable Haemophilus influenzae type b; DT: Diphtheria Toxin; OMPC: outer
301 membrane protein complex of *Neisseria meningitidis* serotype B; TT: Tetanus Toxin.
302 ¹PCV10 (GSK) product

303
304

Table 2: Summary of included studies

Reference	Population (age at enrolment)	Vaccine schedule	Total Sample Size	Arms	PCV valency (targeted serotypes)	Manufacturing company	Carrier protein	Adjuvant	Doses tested (mcg) ¹	Timepoint of primary outcome
Steinhoff (1994) [2]	American children (18 – 30 months)	Single dose	118	7	PCV 2 (6B, 23F)	Lederle	DT	Aluminium Phosphate	2, 10	1-month post dose
Daum (1997) [28]	American infants (2 -3 months)	2, 4, 6 months	400	7	PCV 5 (6B, 14, 18C, 19F, 23F)	Wyeth-Lederle	DT	Aluminium Phosphate	0.5, 2, 5	1-month post dose 3
Ahman (1998) [39]	Finnish infants (9 – 13 weeks)	2, 4, 6 months	125	4	PCV 4 (6B, 14, 19F, 23F)	Pasteur Merieux	DT	Not stated	1, 3, 10	1-month post dose 3
Ahman (1999) [40]	Finnish infants (9 – 13 weeks)	2, 4, 6 months	75	3	PCV 4 (6B, 14, 19F, 23F)	Pasteur Merieux	TT	Not stated	1, 3, 10	1-month post dose 3
Zangwill (2003) [42]	American infants (2 months)	2, 4, 6, 12 months	240	3	PCV 7 (4, 6B, 9V, 14, 18C, 19F, 23F)	Merck &Co	OMPC (123 vs 110 mcg)	Aluminium Phosphate	6B: 5, 8 23F: 4 18C, 19F: 2 4, 9V, 14: 1	1-month post dose 3
Anderson (2003) [38]	American children (2 years)	24, 26 months	112	5	PCV 3 (6A, 14, 19F)	Eli Lilly &Co	CRM197	None	6A: 6.7, 15.8 14: 5.3, 12.7 19F: 5, 12.5	1-month post dose 2
Rupp (2019) [37]	American infants (6 – 12 weeks)	2, 4, 6, 12-15 months	404	8	PCV 15 Formulation A ² (1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 22F, 23F, 33F)	Merck & Co	CRM197	Aluminium Phosphate (125 vs 250 mcg)	1, 2, 4 6B: 2, 4, 8	1-month post dose 3
	American infants (6 – 12 weeks)	2, 4, 6, 12-15 months			PCV 15 Formulation B ² (1, 3, 4, 5, 6A, 6B, 7F, 9V, 14,	Merck & Co	CRM197	Aluminium Phosphate (125 vs 250 mcg)	2, 4 6B: 4, 8	1-month post dose 3

					18C, 19A, 19F, 22F, 23F, 33F)					
	American adults (18 – 49 years) with no history of PPV or PCV	Single dose	80	4	PCV 15 Formulation A ² (1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 22F, 23F, 33F)	Merck & Co	CRM197	Aluminium Phosphate (125 vs 250 mcg)	2, 4	1-month post dose
	American adults (18 – 49 years) with no history of PPV or PCV	Single dose			PCV 15 Formulation B ² (1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 22F, 23F, 33F)	Merck & Co	CRM197	Aluminium Phosphate (125 vs 250 mcg)	2, 4	1-month post dose
Lode (2011) [43]	German adults (>70 years) with no history of PPV or PCV	Single dose	443	4	PCV 7 (4, 6B, 9V, 14, 18C, 19F, 23F)	Wyeth Vaccines	CRM197	Aluminium Phosphate (125 vs 250 mcg)	0.44, 2.2, 4.4, 8.8, 6B: 0.88, 4.4, 8.8, 17.6	1-month post dose
Jackson (2007) [41, 44]	Adults (70-79 years) with history of PPV at least 5 years prior	Single dose	220	5	PCV 7 (4, 6B, 9V, 14, 18C, 19F, 23F)	Wyeth Vaccines	CRM197	Aluminium Phosphate (125 vs 250 mcg)	0.44, 2.2, 4.4, 8.8, 6B: 0.88, 4.4, 8.8, 17.6	1-month post dose

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Abbreviations: CRM 197: non-toxic mutant of Diphtheria toxin; DT: Diphtheria Toxin; OMPC: outer membrane protein complex of *Neisseria meningitidis* serotype B; TT: Tetanus Toxin.

¹Doses stated are for all serotypes unless named serotypes are specified.

²The two Rupp *et al.* formulations were conjugated differently. However, each formulation evaluated either 125 or 250 mcg aluminium phosphate adjuvant.

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Table 3: Follow-up post primary series-paediatric studies

Study	Longest follow-up	Booster dose administered	Antibody levels pre-boost	Response to booster dose
Ahman et al (1998) PCV4 with DT carrier protein	36 months	PncPS at 14 months ¹	At 14 months significant waning of IgG GMCs against STs 6B, 14 and 19F but not against 23F. No significant difference in titres by original dose of PCV.	3 to 24-fold increase in IgG GMCs. Booster response was highest in those who received the lowest doses in infancy.
Ahman et al (1999) PCV4 with TT carrier protein	36 months	PncPS at 14 months	At 14 months significant waning of IgG GMCs No significant difference in titres by original dose of PCV.	2.15 to 12-fold increase in IgG GMCs Booster response was highest in those who received the lowest doses in infancy
Zangwill et al (2003) PCV7 with OMPC carrier protein	13 months	PCV at 12 months	Antibody decline was substantial but comparable in all groups	4.3 to 6.5-fold rise, comparable in all groups

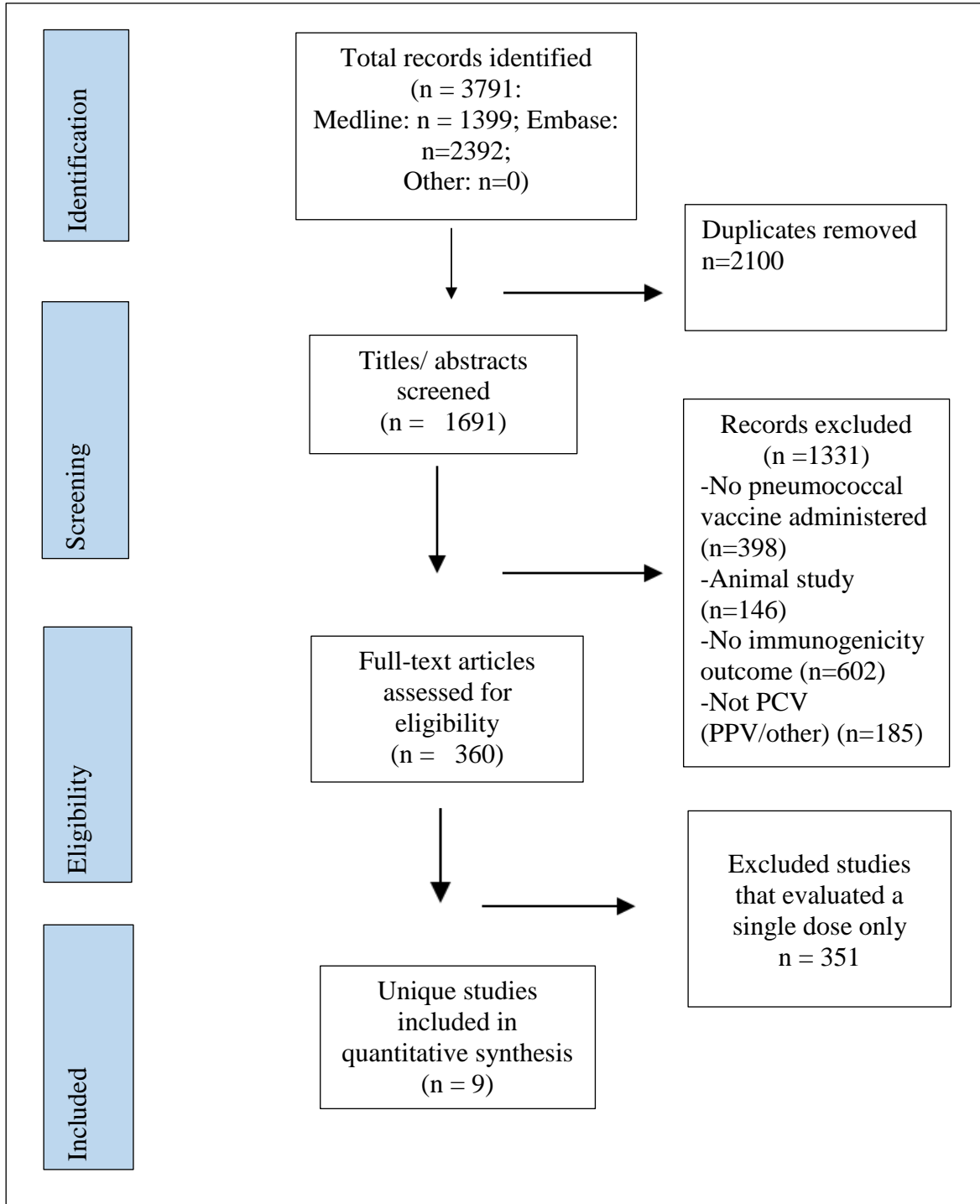
316 Abbreviations: DT: Diphtheria toxoid; GMC: geometric mean concentration; IgG: immunoglobulin; OMPC: outer
317 membrane protein complex of Neisseria meningitidis serotype B; PCV: pneumococcal conjugate vaccine; PncPS:
318 Pneumococcal Polysaccharide Vaccine; ST: serotype; TT: tetanus toxoid

319 ¹Boost dose was administered to all infants who received PCV in infancy (not placebo)
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321 **Figure 1: PRISMA flow diagram**

322 This diagram describes the literature search process and inclusion/exclusion criteria used to identify the studies included in this
323 review

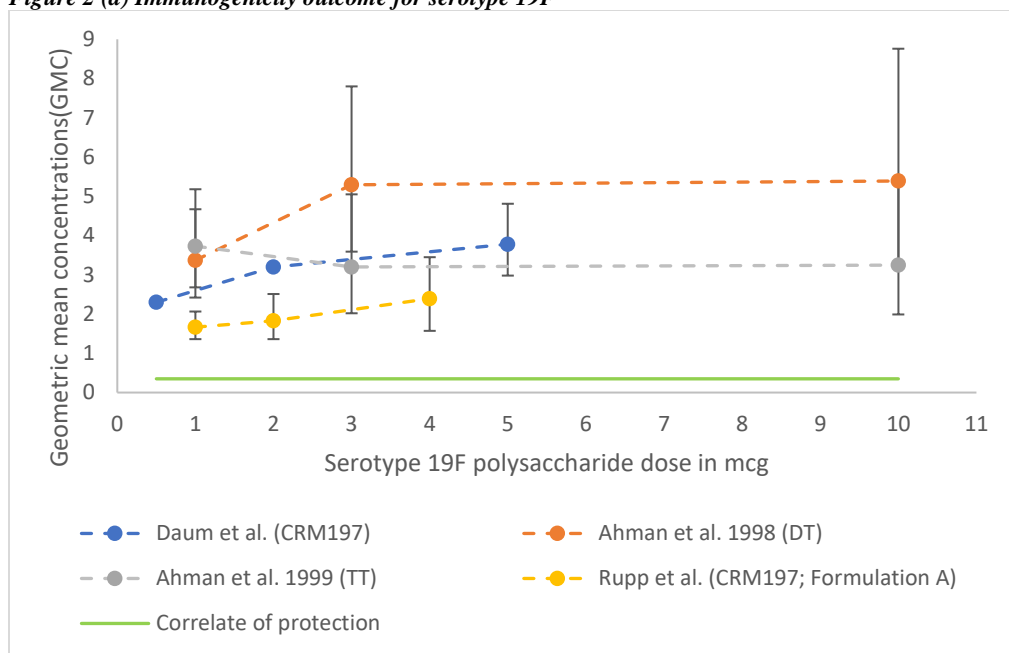
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325 **Figure 2: Immunogenicity outcome in paediatric studies.**

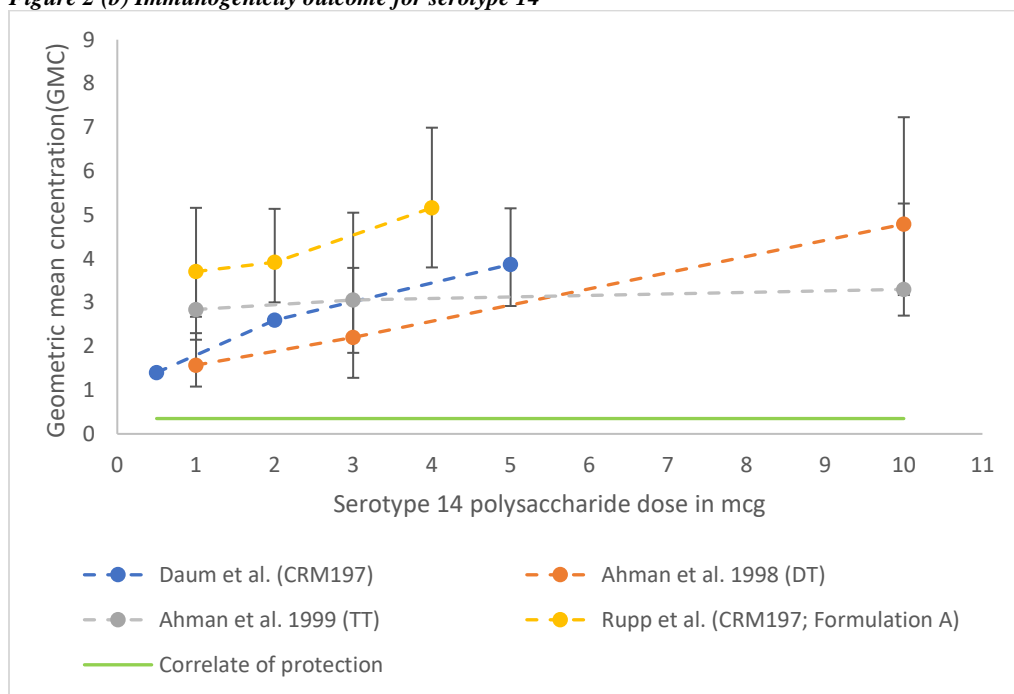
326 These figures illustrate the various immunogenicity outcomes for some of the included paediatric studies. The round dots represent point
 327 estimates i.e. the IgG GMCs reported for each polysaccharide dose evaluated. The limits plotted about the point estimates are margins of
 328 error calculated from the point estimates and their 95% confidence intervals. Note: the scale of the axes for 6B and 23F differ from the
 329 scale for 19F and 14 due to the difference in range of GMCs. Legend: Publication (vaccine carrier protein)

330 **Figure 2 (a) Immunogenicity outcome for serotype 19F**



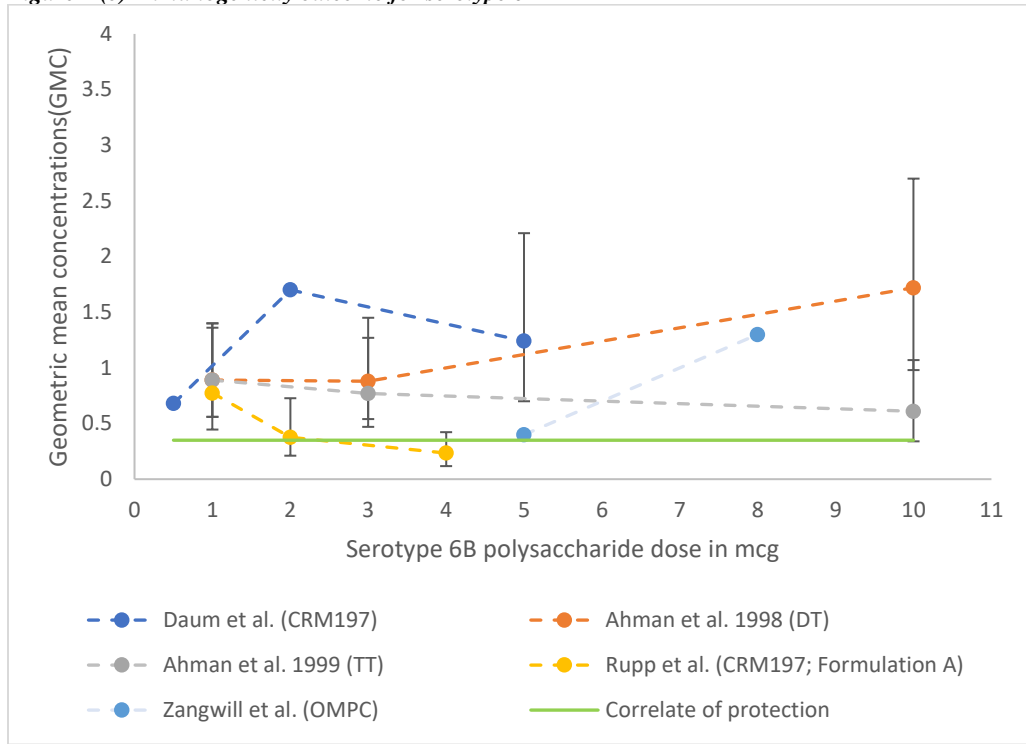
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Figure 2 (b) Immunogenicity outcome for serotype 14

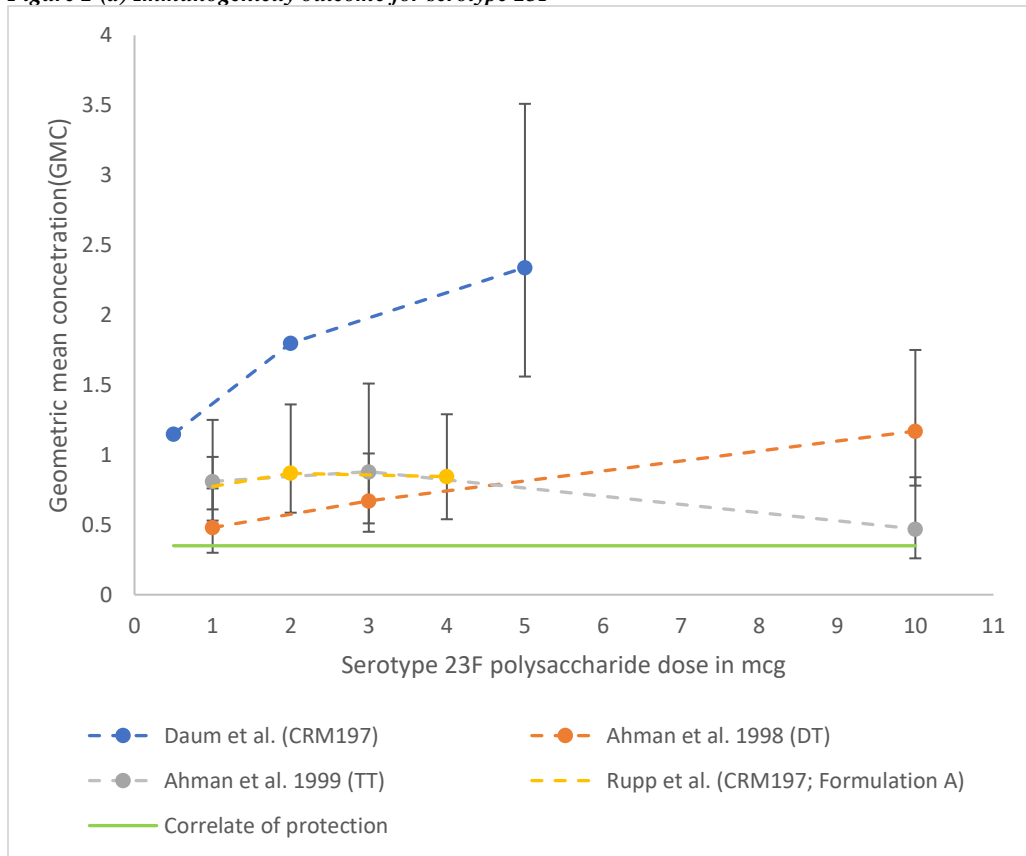


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335 **Figure 2 (c) Immunogenicity outcome for serotype 6B**



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338 **Figure 2 (d) Immunogenicity outcome for serotype 23F**

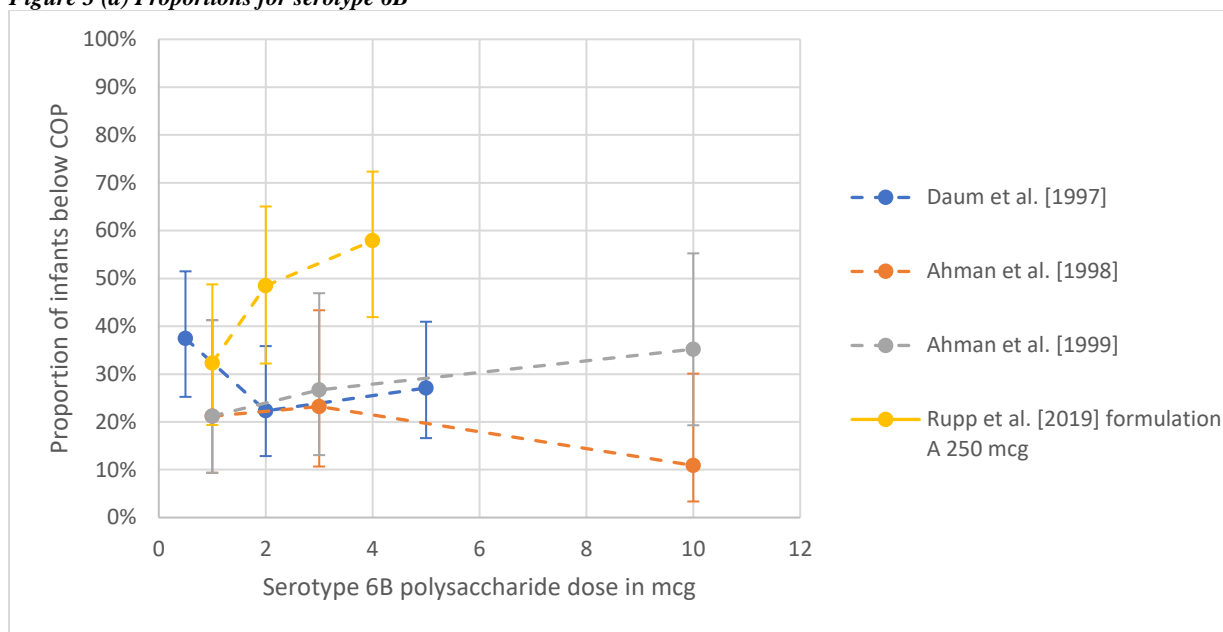


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340 **Figure 3: Estimated proportion of infants below correlate of protection (COP)**

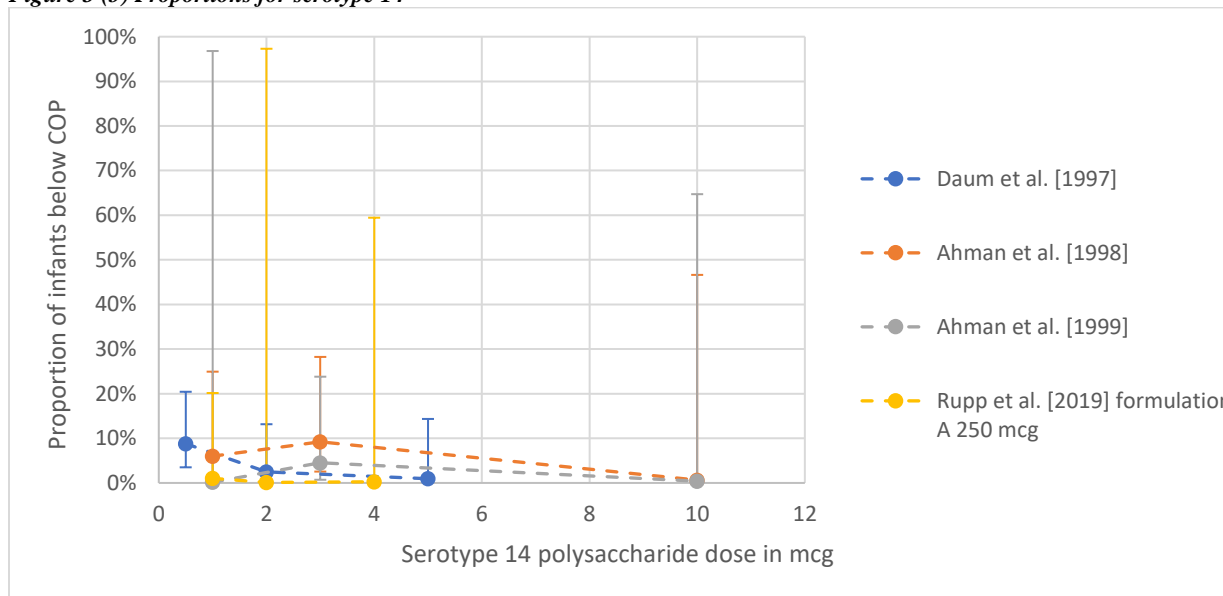
341 These figures illustrate the proportion of infants below the established COP as estimated from the data extracted. The round dots
342 represent point estimates i.e. The estimated proportion below COP. The limits plotted about the point estimates are margins of error
343 obtained from the difference between the 95% confidence intervals and the respective point estimates on either side. Legend: Publication
344 (vaccine carrier protein)

345 **Figure 3 (a) Proportions for serotype 6B**



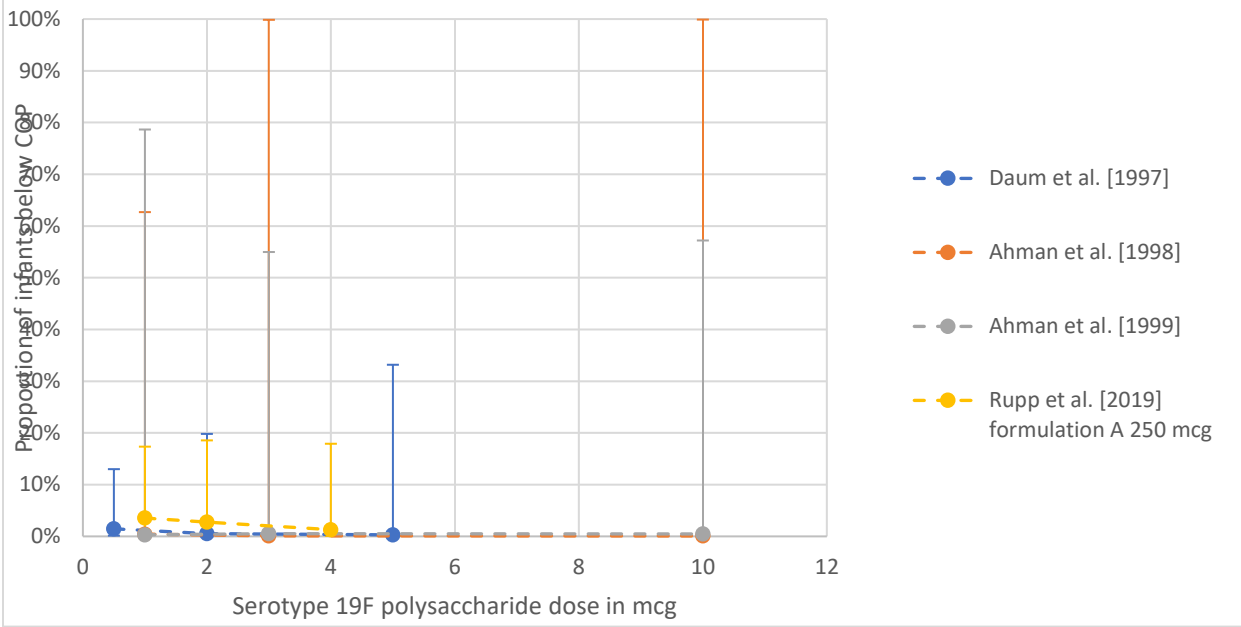
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348 **Figure 3 (b) Proportions for serotype 14**

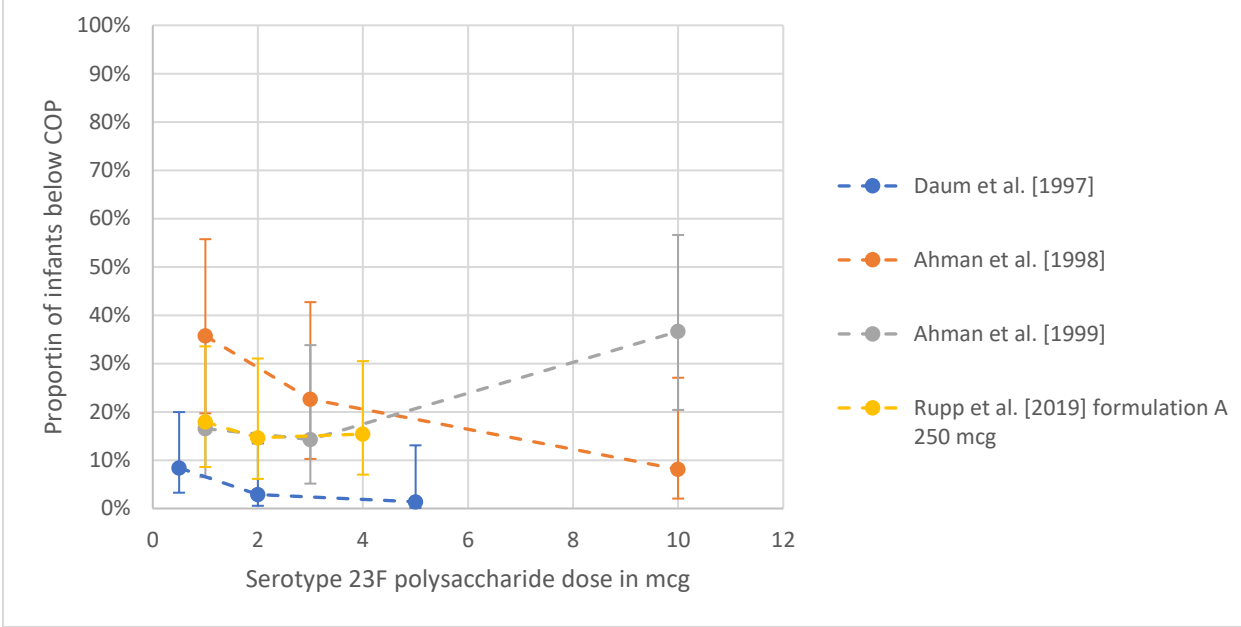


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350 **Figure 3 (c) Proportions for serotype 19F**



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353 **Figure 3 (d) Proportions for serotype 23F**



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