Title: The rise and fall of pneumococcal serotypes carried in the PCV era

Vanessa T. Devine[1§], David W. Cleary[1§], Johanna M.C. Jefferies[1,2], Rebecca Anderson[1], Denise E. Morris[1], Andrew C. Tuck[1], Rebecca A. Gladstone[1,3], Grace O' Doherty[1], Priyasharmila Kuruparan[1], Stephen D. Bentley [3], Saul N. Faust[1,2,4]* and Stuart C. Clarke[1,2]*

[§] Denotes joint first authors; * denotes equal final authors.

[1] Faculty of Medicine and Institute for Life Sciences, University of Southampton, Southampton, UK, SO17 1BJ.

[2] Southampton NIHR Respiratory Biomedical Research Unit, University Hospital Southampton Foundation NHS Trust, Southampton, UK, SO16 6YD.

[3] Pathogen Genomics, Wellcome Trust Sanger Institute, Hinxton, UK, CB10 1SA.

[4] NIHR Wellcome Trust Clinical Research Facility, University Hospital Southampton Foundation NHS Trust, Southampton, UK, SO16 6YD.

Corresponding author email: s.c.clarke@soton.ac.uk

Infectious Disease Epidemiology Group, University of Southampton, Mailpoint 814, Level C, Sir Henry Wellcome Laboratories, South Block, University Hospital Southampton Foundation NHS Trust Southampton SO16 6YD, UK

Office +44 (0)2381 206652

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Abstract

Streptococcus pneumoniae is a major cause of meningitis, sepsis and pneumonia worldwide. Vaccination using pneumococcal conjugate vaccines (PCV) has therefore been part of the UK's childhood immunisation programme since 2006. Here we describe pneumococcal carriage rates in children under five years of age attending the paediatric department of a large UK hospital in response to vaccine implementation over seven winter seasons from 2006 to 2013. *S. pneumoniae* (*n* = 696) were isolated from nasopharyngeal swabs (*n* = 2267) collected during seven consecutive winters, October to March, 2006/7 to 2012/13. This includes the period immediately following the introduction of the seven-valent pneumococcal conjugate vaccine (PCV7) in 2006 in addition to pre- and post-PCV13 introduction in 2010. We show a decrease in PCV13 vaccine serotypes (VT) in the three years following PCV13 vaccine implementation (2010/11 to 2012/13). Serotype 6A represented the only observed VT following PCV13 implementation with all others (including PCV7 serotypes) absent from carriage. Overall pneumococcal carriage, attributable to non-VT (NVT), was consistent across all sampling years with a mean of 31·1%. The ten most frequently isolated NVTs were 6C, 11A, 15B, 23B, 15A, 21, 22F, 35F, 23A and 15C. Fluctuations in the prevalence of each were however noted. Comparing prevalence at 2006/07 with 2012/13 only 15A was shown to have increased significantly (*p* value of 0·003) during the course of PCV implementation. These data support the increasing evidence that the primary effect of PCVs is due to population immunity by reducing or eliminating the carriage of invasive VT serotypes. With IPD being increasingly attributed to non-vaccine serotypes, surveillance of carriage data continues to act as an early warning system for vaccine design and public health policy that require continual data of both carried pneumococcal serotypes and IPD attributed serotype data.

Introduction

Invasive pneumococcal disease (IPD) is a cause of substantial morbidity and mortality worldwide. In the UK, over 5,000 cases of IPD are currently reported per year[1] and, in the USA, almost 28,000 cases of IPD were reported in 2014 resulting in over 2,900 deaths that year.[2] Individuals become transiently colonised with *Streptococcus pneumoniae* and other nasopharyngeal flora during the first months of life and this colonisation is a pre-requisite for disease. For example, higher proportions of children presenting with acute otitis media (AOM) have been shown to be pneumococcal carriers[3], and for IPD where concordance between disease and carriage strains within individuals has been demonstrated.[4] The age of pneumococcal colonisation varies and may be affected by environmental factors such as having siblings, attending daycare or geographical location.[5,6] For IPD, the capsule type has been shown to be a stronger predictor of invasive potential than of genotype.[7]

The first pneumococcal conjugate vaccine, Prevenar 7™ (PCV7) was added to the UK's national immunisation programme in September 2006 and included serotypes 4, 6B, 9V, 14, 18C, 19F and 23F.[8] After the introduction of PCV7, the UK and several other countries reported a decrease in vaccine-type (VT) IPD and an increase in non-vaccine type (NVT) IPD.[9–12] In April 2010, Prevenar13™ (PCV13) replaced PCV7 which included additional serotypes 1, 3, 5, 6A, 7F and 19A. IPD decreased across all age groups in the UK from eight cases per 100,000 in the PCV7 era to seven cases per 100,000 post-PCV13.[13] In the under twos age group this reduction was from 29 to 14 per 100,000 with IPD caused predominately by NVTs.[13] Reductions in IPD across all age groups have also been observed in the USA.[14] Although use of the PCV13 vaccine has resulted in a reduction in carriage and IPD of VT pneumococci, IPD attributed to PCV13 NVTs remains a clinical problem.

In addition to lowering incidence of IPD, PCVs have also had a dramatic effect on both penicillin and multi-drug resistance within circulating pneumococcal serotypes. In the USA, penicillin non-susceptible IPD fell from 6.3 to 2.7 cases per 100 000, with multi-drug resistance similarly falling from 4.1 to 2.7 per 100 000, attributable to the removal of PCV7 serotypes in the four years following vaccination.[15] A combination of capsule switch and clonal expansion of non-VT serotypes led however, post-PCV7, to the expansion of serotype 19A lineages which in 1999 accounted for 0.8% of high level (≥ 1 μg/ml) penicillin non-susceptible IPD cases increasing to 90.3% in 2009.[16] Similar trends, though modest in comparison to 19A, have been observed post-PCV13 with non-VTs 35B(ST558), 15B/C (ST3280) and 23B(ST1373); that have also been identified with an increased prevalence in paediatric IPD.[17]

We have previously described pneumococcal serotype carriage during and after the introduction of PCV7 and the first year following PCV13 (2006/07 to 2010/11).[18,19] Here we report an additional two years of pneumococcal carriage data after the introduction of PCV13 in the UK. Changes in pneumococcal carriage and IPD from VT to NVT highlight the need for continued surveillance in the post-PCV13 era. Using seven years of carriage data from a UK paediatric population, we describe the change from a primarily VT carried population in 2006/07 to a population dominated by NVTs in 2012/13, with no significant change in the overall proportion of children colonised with *S. pneumoniae*.

Methods

Nasopharyngeal swabs were collected from children aged 4 years and under during seven consecutive winters, October to March, 2006/7 to 2012/13. Research ethics approval was granted by the Southampton and South West Hampshire Research Ethics Committee B (NHS Research Ethics 06/Q1704/105). The initial five years of the study have been described previously.[18,19] Briefly, sample size and power calculations were based on the lowest expected carriage rate of 10%. A minimum sample size of 100 isolates per year was selected to enable the detection of an estimated 50% relative reduction in carriage with 80% power at a 5% significance level. Informed consent was obtained before or after an outpatient appointment, no specific outpatient clinic was targeted. The participant was either the child attending clinic or their sibling. Only one child per family was swabbed. Age was the primary exclusion criteria. Rayon tipped Transwabs (Medical Wire, Corsham, UK) in charcoal Amies media were plated onto Columbia Colistin Naladixic Acid agar within 9 hours of collection at the Health Protection Agency Southampton Laboratory (now part of Public Health England) between 2006/07 and 2011/12 and by technical staff in our research group during 2012/13. Presumptive *S. pneumoniae* were plated on Columbia blood agar with an optochin disc and confirmed with a ≥14 mm diameter inhibition zone. Only one colony of *S. pneumoniae* per participant swab was selected for further analysis. Sampling continued with the aim of collecting 100 *S. pneumoniae* each winter. Illumina sequencing was performed at the Wellcome Trust Sanger Institute with 75 bp paired-ends. Data is deposited in the European nucleotide database with accession numbers: ERR044859: ERR044953, ERR044955: ERR044987, ERR044989: ERR048210, ERR048212: ERR045394, ERR045396: ERR045400, ERR045402: ERR045413, ERR045415: ERR045431, ERR045433, ERR045434, ERR045436: ERR047110, ERR047112: ERR047149, ERR047151: ERR047163, ERR657177: ERR657183, ERR657186: ERR657252, ERR657272: ERR657278, ERR657281: ERR657347, ERR775445:

ERR775462, ERR775449, ERR775451, ERR775453, ERR775455, ERR656989: ERR657041, ERR656990: ERR656993, ERR657083: ERR657087, ERR657092: ERR657135, ERR657253: ERR657271, ERR657184, ERR657185, ERR657279, ERR657280, ERR657348: ERR657366, ERR657448, ERR657450, ERR657452, ERR657454 and ERR657456. *De novo* assembly and analysis was performed as described previously,[19] using Velvet assembler,[20] and Velvetoptimiser.[21] Serotype was inferred from whole genome data using an *in silico* adaptation of a PCR serotyping method and sequence mapping of capsule types as previously described.[19] Phenotypic serotyping was used if the genetic basis for serotype was unknown. PCV7 serotypes were 4, 6B, 9V, 14, 18C, 19F and 23F. PCV13 serotypes were those of PCV7 with 1, 3, 5, 6A, 7F and 19A identified as PCV13 only VTs. All others were identified as non-VT serotypes. Presumptive pneumococcal serotype prevalence's were estimated with 95% confidence intervals (CI). Statistical analysis was done using Fisher's exact tests with Bonferroni correction for multiple testing where appropriate.

Results

A total of 2267 nasopharyngeal swabs were taken, between 223 and 399 each year, resulting in a total of 696 *S. pneumoniae* isolates, between 77 and 111 each year (Table S1). PCV7 VTs initially accounted for 53·4% of all carried pneumococci but decreased after the introduction of PCV7. By 2011/12 no PCV7 VTs were observed in carriage (Figure 1). PCV13 only VTs decreased from 20% of the total serotypes observed in 2009/10 to 2·6% in 2012/13 following introduction of PCV13; a statistically significant reduction (p = 0.001). Serotype 6A was the VT accountable for the remaining incidence (Figure 2). Whilst VTs have decreased, the overall carriage rate has remained steady, with an average of 31·1% pneumococcal positive swabs over the seven-year period. A steady rise in NVT carriage was observed, from 30% of carriage serotypes in 2006/07 to 94·8% in 2012/13.

No PCV7 VT carriage was observed in 2011/12 and PCV13 VT carriage was reduced from 16·5% in 2006/07 to 8% (Figure 2). A small increase in carriage of serotype 3 was seen however and accounted for 5% of the pneumococci isolated that year. The most frequently encountered PCV13 NVTs were 11A, 23B and 15A, representing 12·1%, 11·1% and 10·1% of total carried serotypes respectively.

During 2012/13, of the serotypes included in PCVs only 6A was observed (2·6% of total pneumococci). Serotypes 15A, 15B, 23B and 35B were the most commonly isolated NVTs at 13·0%, 13·0%, 9·1% and 9·1% respectively. Serotype 15B was detected in all previous study years but the greatest incidence was during study year 2012/13. Although not detected during study years 2006/07, 2007/08 and 2009/10, serotype 35B had increased from 1·9% in 2008/09 to 9·1% in 2012/13. Combining 15B and 15C prevalence, as these serotypes together represent a single phylogenetic clade, the proportion accounted for by these serotypes was 15·6%.

Examining the cumulative serotype distribution over all years however it should be noted that the NVTs observed most frequently in 2012/13 were not consistent across all years. In rank order NVT 15B was the third most isolated, with 23B fourth, 15A fifth, and 35B twelfth. This is in contrast to the NVTs 6C and 11A which were detected more frequently during the seven year study. These yearly fluctuations are shown in Figure 3 for the ten most frequently isolated serotypes - 6C, 11A, 15B, 23B, 15A, 21, 22F, 35F, 23A and 15C. Clear fluctuations can be seen, in particular for serotype 6C that exhibited an expansion in 2007/08 and serotype 21 in 2009/10 and 2010/11. However, comparing prevalence at 2006/07 with 2012/13 only serotype 15A was shown to have increased significantly (*p* values of 0·003).

Discussion

Carriage studies are now considered an important facet for the introduction of PCV's and have yielded useful data on post-introduction pneumococcal epidemiology.[22,23] Our carriage study began in 2006/07 when PCV7/PCV13 VTs were dominant in pneumococcal carriage. Our study has shown that the carriage of PCV7 and PCV13 VTs decreased and then was replaced by the carriage of PCV13 NVTs. By study year 7 (2012/13) carriage was dominated by NVTs, with only PCV13 VT 6A being detected in 2012/13. These data support the increasing evidence that the primary effect of PCVs is due to population immunity reducing or eliminating the carriage of invasive VT serotypes. IPD incidence as reported by Public Health England has also shown that there has been a decrease in IPD due to PCV13 VTs across all age groups, and an increase in IPD in all age groups due to PCV13 NVTs (2006/07 to 2012/13 data).[17,24] The herd protection first demonstrated with PCV7 has continued with the indirect protection from PCV13.[17] The pneumococcal carriage rate remained consistent over the seven-year period, despite the introduction of PCV7 then PCV13. With reductions in both PCV related pneumococcal carriage and IPD, there should now be focus on why PCV NVT carriage and IPD is increasing. Recent data from Public Health England shows that there were almost 200 extra cases of IPD from PCV13 NVTs in both the 5 to 64 years of age group and the over 65 years of age group (2014/15) compared to the numbers that were detected at the same time the previous year (2013/14).[24] This is reflected in our two most recent years of analysis, 2011/12 and 2012/13, where serotypes 11A, 15A, 15B, 23B and 35B were prevalent or rising. Similar to our rate of 31.1%, consistency in carriage rates has also been observed in other European countries and in the USA. In Sweden for example a carriage rate of 30% was reported 4-8 years following vaccine introduction in Stockholm, accounted for by the increase in NVTs with overlap to our findings, namely 11A, 23B, 35F and 21.[25] In the US-based study a stable carriage rate (32%) was also found.[26]. For the period 2010 to 2013 the authors reported the same serotypes as commonly carried as our study.[26] In keeping with data presented here, results of a carriage study in Germany also demonstrated a reduction

189 in PC13 VTs with an increase in IPD caused by 15A and 23B.[27,28] A Norwegian carriage study
190 also reported decreases of PCV13 VTs although there was not the substantial replacement
191 with PCV13 NVTs that has been shown to occur in the UK. Additionally, and in further
192 contrast to this study, the few PCV13 NVTs that did increase following vaccine
193 implementation were described as having a low capacity for causing IPD.[29] Finally in the USA,
194 again PCV13 VT IPD decreased in all age groups but no PCV13 NVT replacement was
195 observed in any age group apart from those aged 50-64 years old.[14] Differences in carriage
196 and IPD rates from various countries could be due to differences in laboratory technique and
197 disease surveillance, vaccine policy, IPD diagnosis and access to health care. However, our
198 study and others suggest that the high uptake rate of PCV's in the UK has led to herd
199 protection and hence individual vaccine status is less important.

200 A number of important caveats to this study should be mentioned. We are deriving a
201 community-level description of serotype prevalence from a proxy-population, that being a
202 paediatric outpatient cohort. Needless to say this introduces geographical limitation and
203 potential biases. However the consistency of our findings with those of various national IPD
204 reporting schemes is encouraging. Although no stratification of participants based on
205 vaccine status, demography, antibiotic usage or co-carriage of other upper respiratory tract
206 pathobionts such as *Haemophilus influenzae*, *Staphylococcus aureus* and *Moraxella*
207 *catarrhalis* has been undertaken, continued monitoring alongside the collection of this detail
208 might enable those covariates to be examined in the future.

209 In summary, the potential for changes in pneumococcal carriage rates of individual
210 serotypes, should be taken into account when determining the impact of PCV's. New
211 extended PCV formulations and the promise of protein-based pneumococcal vaccines will
212 require ongoing surveillance of pneumococcal carriage and disease.

213

214 Figure Legends

215 Figure 1: Prevalence of vaccine and non-vaccine serotypes in *S. pneumoniae* carriage from
216 2006 to 2013. The decreasing purple line shows PCV7 VTs. PCV13-only VTs reduced
217 significantly over the period of the study reported here (2010/11 to 2012/13), $p = 0{\cdot}001$.
218 Non-vaccine serotypes increased steadily from 30 to 94·8%. The blue line shows the overall
219 carriage rate as the proportion of pneumococci isolated from the sampled population of that
220 year.

221 Figure 2: Vaccine and non-vaccine serotype distributions per year. Prevalence is reported as
222 a percentage of the total pneumococci isolated for that year of the study. Serotypes are
223 grouped dependent upon their inclusion/exclusion in PCV formulations. Error bars – 95%
224 confidence interval.

225 Figure 3:Serotype prevalence of the ten most commonly observed NVT in carriage (6C, 11A,
226 15B, 23B, 15A, 21, 22F, 35F, 23A and 15C). The dashed line indicates PCV13
227 implementation. Serotypes 15A was the only serotypes that had increased significantly
228 between the first and last year of the study (*p* values of 0·003).

229 **Author Contributions**

230 SCC and SNF conceived and secured funding for the study with assistance from JMJ. SCC
231 and SNF were the site primary investigators. VTD, RA, DEM, ACT, GO and PK undertook
232 participant recruitment and microbiological isolation. Whole genome sequencing was
233 facilitated by SDB. VTD, RG and DWC analysed the whole genome sequencing data. VTD
234 and DWC wrote the manuscript. SCC, SNF, JMJ and RAG reviewed the manuscript.

235 **Conflict of Interest**

SNF receives support from the National Institute for Health Research funding via the Southampton NIHR Wellcome Trust Clinical Research Facility and the Southampton NIHR Respiratory Biomedical Research Unit. SNF, SCC and JMJ act as principal investigator for clinical trials and other studies conducted on behalf of University Hospital Southampton NHS Foundation Trust/University of Southampton that are sponsored by vaccine manufacturers but receives no personal payments from them. SNF, JMJ and SCC have participated in advisory boards for vaccine manufacturers but receive no personal payments for this work. SNF, SCC and JMJ have received financial assistance from vaccine manufacturers to attend conferences. All grants and honoraria are paid into accounts within the respective NHS Trusts or Universities, or to independent charities. RAG and VTD received PhD studentships from Pfizer. RAG employed for one year on a GSK funded research project in 2012. DWC employed for 18 months on a GSK funded research project in 2014/15. All other authors have no conflicts of interest.

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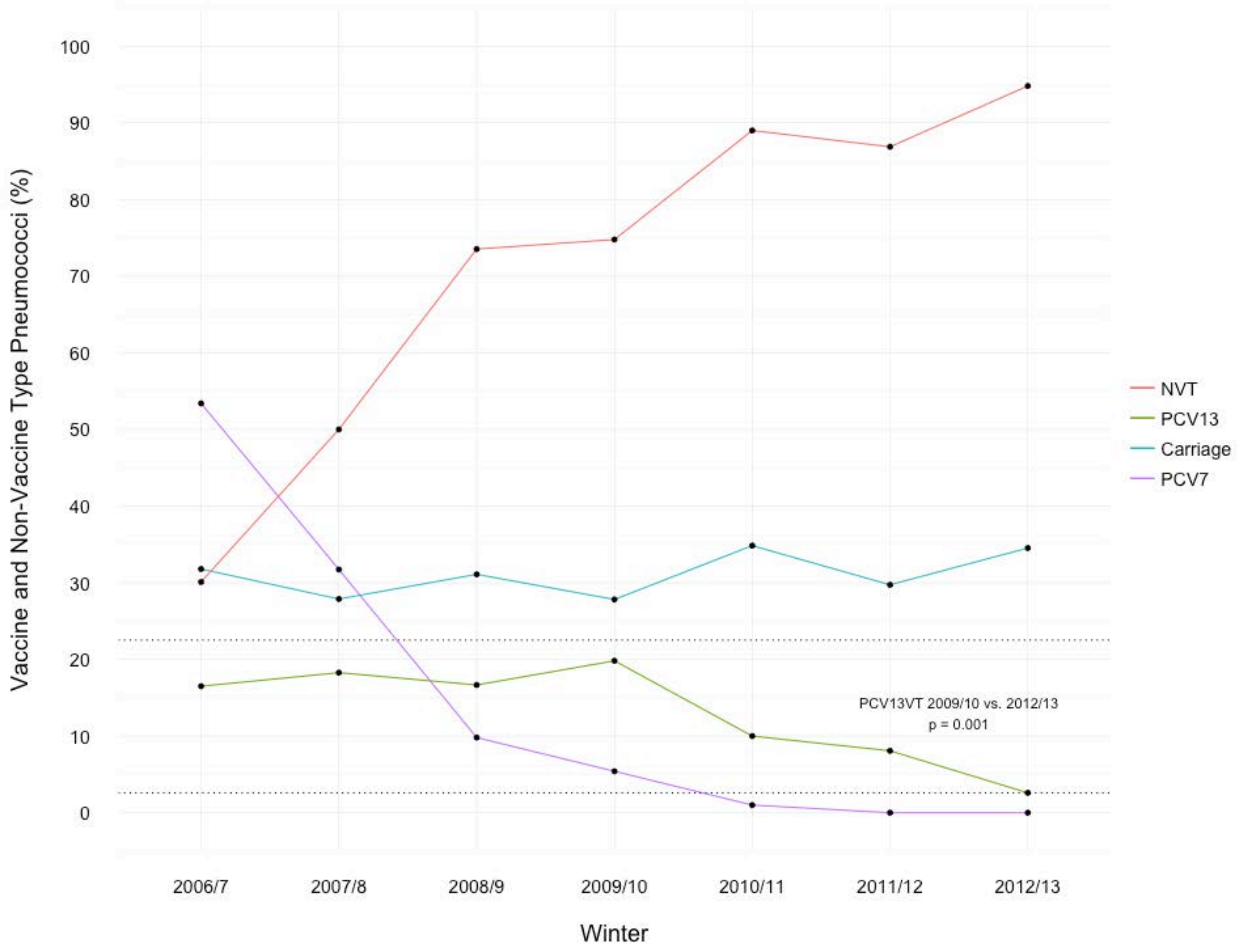
342 Table S1: Serotype prevalence (numbers) over seven years, 2007/07–2012/13

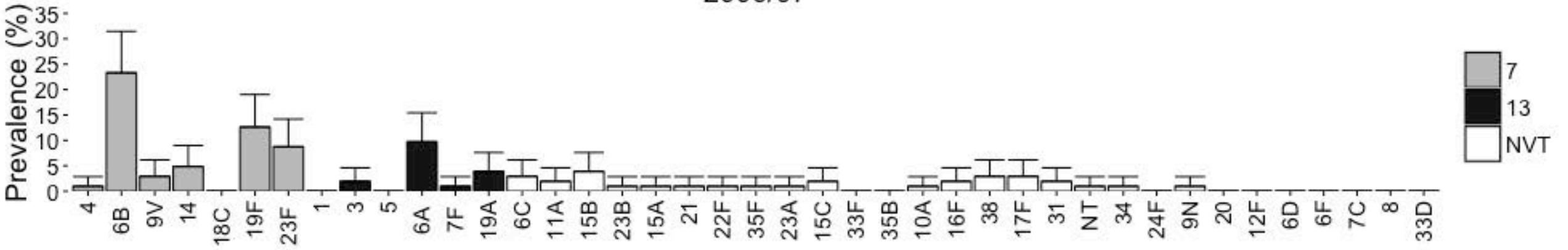
Serotype	2006/07	2007/08	2008/09	2009/10	2010/11	2011/12	2012/13
Swabs taken	324	373	328	399	287	333	223
S. pneumoniae	103	104	102	111	100	99	77
4 (PCV7)	1	0	0	0	0	0	0
6B (PCV7)	24	15	4	2	1	0	0
9V (PCV7)	3	0	1	0	0	0	0
14 (PCV7)	5	2	1	0	0	0	0
19F (PCV7)	13	6	1	0	0	0	0
23F (PCV7)	9	10	3	3	0	0	0
1 (PCV13)	0	0	1	1	1	0	0
3 (PCV13)	2	1	2	2	1	5	0
6A (PCV13)	10	15	6	5	2	1	2
7F (PCV13)	1	0	1	2	1	1	0
19A (PCV13)	4	3	7	12	5	1	0
18C (PCV13)	0	0	0	1	0	0	0
6C	3	14	14	4	9	6	4
11A	2	8	7	13	7	12	5
15B	4	1	7	4	7	5	10
23B	1	2	5	6	8	11	7
15A	1	0	2	4	6	10	10
21	1	2	3	10	13	4	3
22F	1	5	9	9	3	2	2
35F	1	4	2	7	9	4	2
23A	1	3	5	2	4	6	2
15C	2	3	2	5	2	5	2
33F	0	1	2	4	8	1	3

35B	0	0	2	0	3	5	7
10A	1	0	3	0	1	6	4
16F	2	2	0	1	4	1	3
38	3	0	3	2	1	1	1
17F	3	3	1	2	0	1	0
31	2	1	1	0	2	3	1
NT	1	0	4	2	0	0	1
34	1	1	0	2	0	2	1
24F	0	0	0	1	0	1	4
9N	1	0	1	2	0	0	0
20	0	0	1	1	1	0	0
12F	0	0	0	0	1	0	1
6D	0	1	0	0	0	0	0
6F	0	0	0	1	0	0	0
7C	0	1	0	0	0	0	0
8	0	0	0	1	0	0	0
33D	0	0	1	0	0	0	0
Unassigned	0	0	0	0	0	5	2

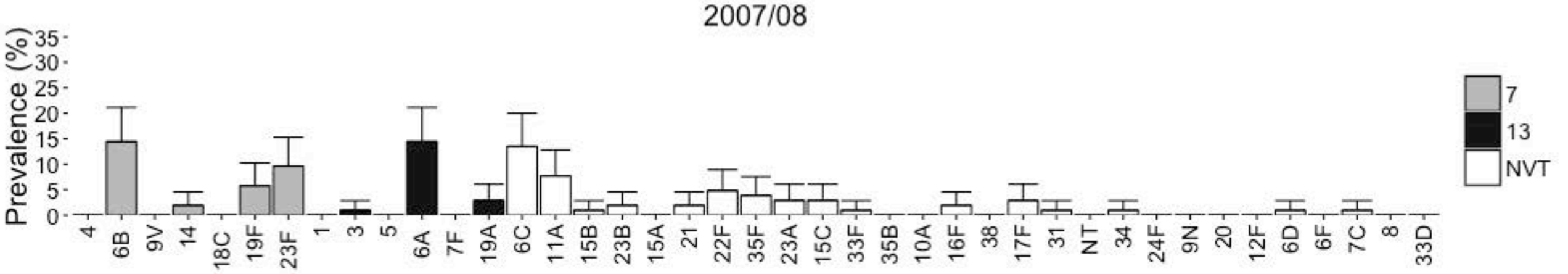
343


Vaccine and Non-Vaccine Type Pneumococci (%)
100
90
80
70
60
50
40
30
20
10
0
2006/7
2007/8
2008/9
2009/10
2010/11
2011/12
2012/13
Winter
NVT
PCV13
Carriage
PCV7
PCV13VT 2009/10 vs. 2012/13
p = 0.001

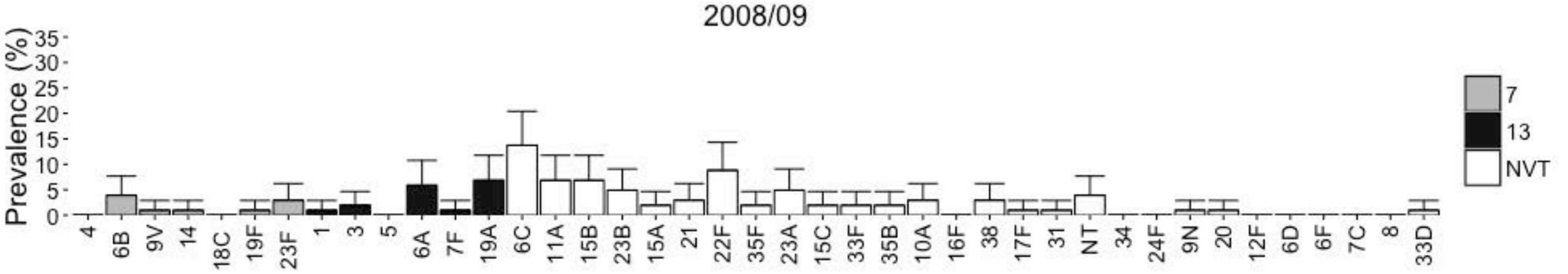
2006/07



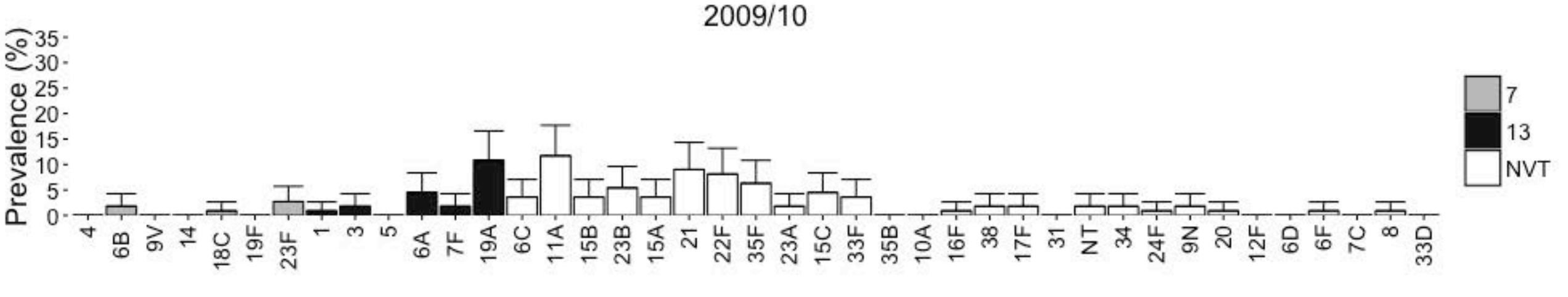
2007/08



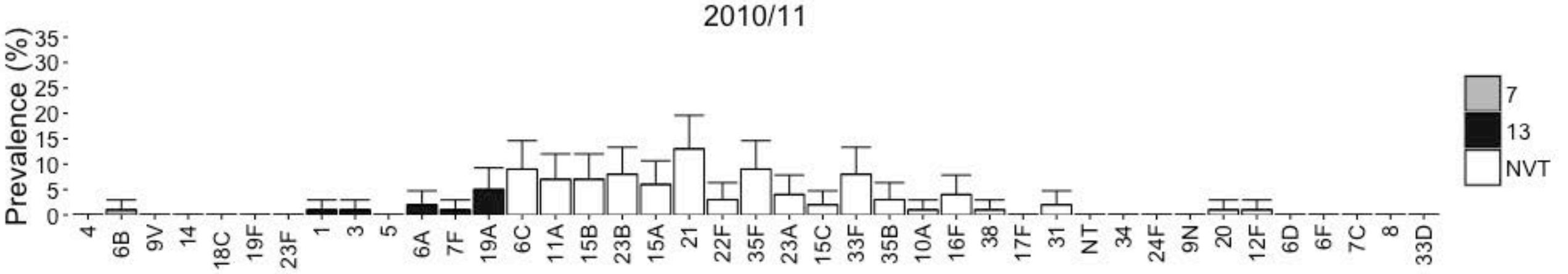
2008/09



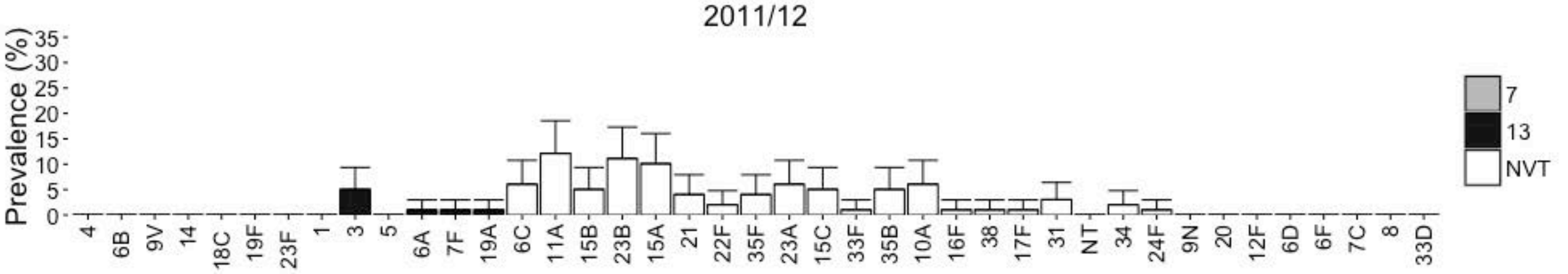
2009/10



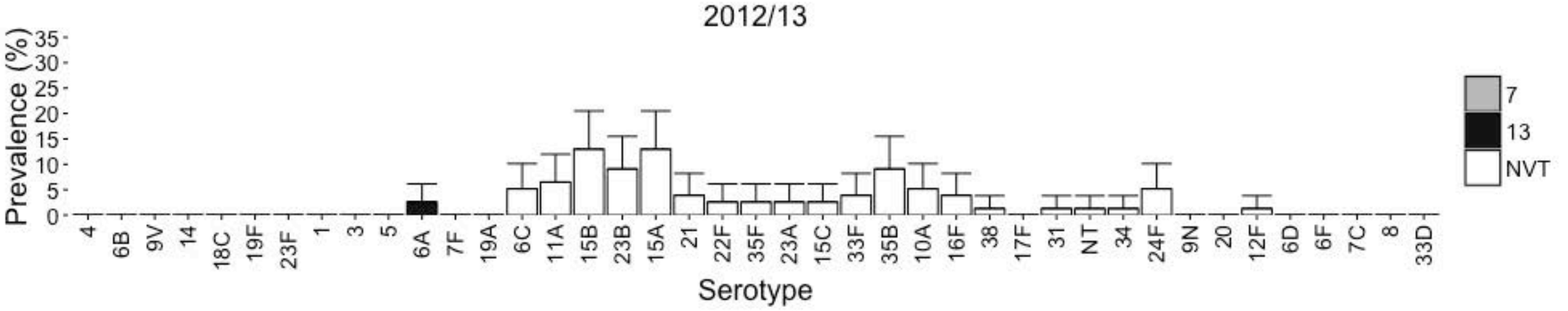
2010/11

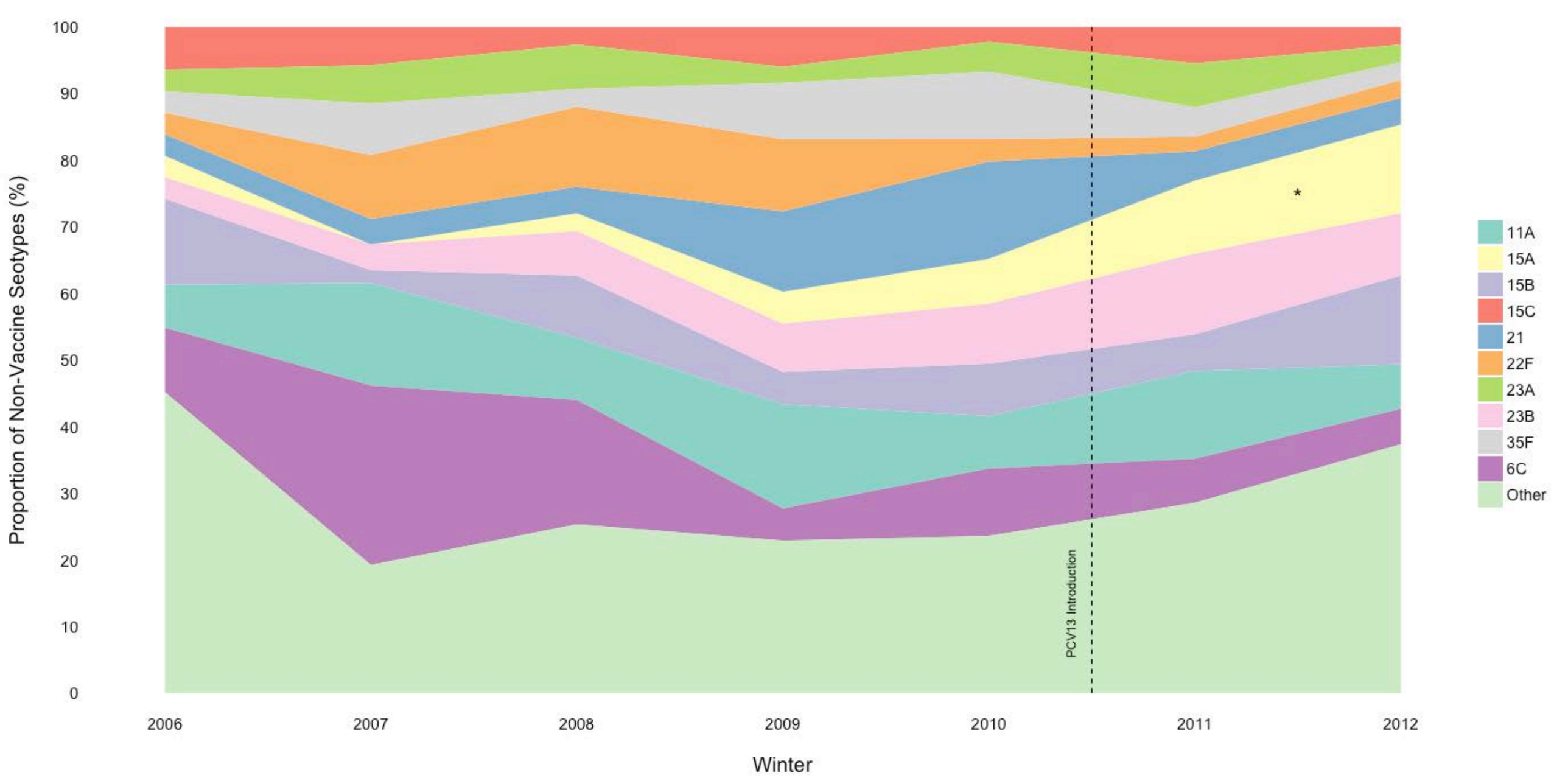


2011/12



2012/13




Proportion of Non-Vaccine Seotypes (%)
100
90
80
70
60
50
40
30
20
10
0
2006
2007
2008
2009
2010
2011
2012
Winter
PCV13 Introduction
*
11A
15A
15B
15C
21
22F
23A
23B
35F
6C
Other