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Salivary and Serum Antibody Response Against *Neisseria meningitidis* After Vaccination With Conjugate Polysaccharide Vaccines in Ethiopian Volunteers

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Abstract

Meningococcal conjugate vaccines induce serum antibodies crucial for protection against invasive disease. Salivary antibodies are believed to be important for hindering meningococcal acquisition and/or clearance of established carriage. In this study, we measured salivary IgA and IgG antibodies induced by vaccination with a monovalent serogroup A conjugate vaccine or a tetravalent A, C, W and Y conjugate vaccine, in comparison with antibody levels in serum. Saliva and serum samples from Ethiopian volunteers (1–29 years) collected before and eight times on a weekly basis after receiving the serogroup A conjugate vaccine, the tetravalent serogroup A, C, W and Y conjugate vaccine, or no vaccine (control group), were analysed using a multiplex microsphere immunoassay for antibody detection. Serogroup-specific IgG antibody levels in saliva increased significantly after vaccination with both vaccines. The monovalent serogroup A vaccine also induced an increase in salivary IgA antibodies. A strong correlation between serogroup-specific IgG antibodies in saliva and serum, and a somewhat lower correlation for IgA, was observed for all serogroups. There was also a strong correlation between specific secretory IgA and IgA antibodies in saliva for all serogroups. Meningococcal conjugate vaccines are able to elicit salivary antibodies against serogroup A, C, W and Y correlating with antibody levels in serum. The strong correlation between saliva and serum antibody levels indicates that saliva may be used as a surrogate of systemic antibody responses.

Introduction

Neisseria meningitidis is an obligate human commensal, commonly colonizing the oropharynx of asymptomatic carriers [1]. Studies in industrialized countries have shown that around 10% of the population carry meningococci in their throat [2, 3], with prevalence peaking in late teenage and early adulthood [4]. In Africa, carriage rates are lower, peaking at a younger age, in late childhood and early teenage [5]. The high incidence of carriage is not reflected in the much lower frequency of disease, but the bacterium has the potential to invade the body in susceptible individuals and cause large-scale epidemics. Such epidemics have occurred all over the world [6], with the worst affected area being the sub-Saharan meningitis belt, stretching from Senegal to Ethiopia [7].

Meningococcal vaccines have successfully been used to control epidemics and prevent disease [8, 9]. Vaccines based on capsular polysaccharides from meningococcal serogroups A (MenA), C (MenC), W (MenW) and Y (MenY), four of the five major disease-causing serogroups [1, 3], have largely been replaced by conjugated vaccines, where the polysaccharides are coupled to carrier proteins. In contrast to plain polysaccharide vaccines, conjugate vaccines are able to induce B cell memory and protective antibodies in young children [10, 11]. Another important feature of conjugate vaccines is the ability to induce herd protection, an indirect protection of non-vaccinated individuals by decreasing transmission of the bacterium. This has been shown for both MenC [12, 13] and MenA [14, 15] conjugate vaccines.

The immunological mechanisms behind herd protection are not yet fully known, but the local immune response in

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the oropharynx and saliva is believed to be of importance. The meningococcal vaccines' induction of serum immunoglobulin G (IgG) antibodies is the most important mechanism in preventing invasive disease, but the importance of mucosal antibodies in affecting carriage is not yet clear.

In saliva, immunoglobulin A (IgA) is the most abundant Ig class. The vast majority of IgA is produced by local plasma cells in salivary glands and transported into salivary fluids, contrary to salivary IgG which is considered to be mainly derived from serum by passive diffusion, through gingival crevices [16]. Some IgG may also be locally produced by plasma cells in the gingiva and salivary glands. Locally produced IgA is found mainly as dimers with a stabilizing secretory component attached and is designated secretory IgA (sIgA). sIgA exhibits its action primarily by binding to the pathogens, thereby blocking their access to epithelial receptors and facilitating their removal [17], while IgG at the mucosal level is preventing colonization by agglutination and thereby clearance of bacteria [18, 19].

Only a few studies have looked at the salivary IgA and IgG antibody responses after vaccination with meningococcal conjugate vaccines [20–23], and none were performed in a population in sub-Saharan Africa – the population with highest disease incidence. Salivary IgA antibody responses to MenW and MenY after vaccination have to our knowledge not been studied previously. To gain more knowledge on the effect of conjugate polysaccharide vaccines on meningococcal carriage, we conducted a study in Ethiopia to investigate the relationship between the kinetics of salivary IgG and IgA antibody responses and carriage following vaccination. The salivary antibody response was compared to antibody levels in serum.

Materials and methods

Ethics statement. The study obtained ethical approval by the Regional Committee for Medical Research Ethics, South-East Norway, the World Health Organization (WHO), the Armauer Hansen Research Institute (AHRI)/All-Africa Leprosy, Tuberculosis and Rehabilitation Training Centre (ALERT) Ethics Review Committee, and the National Research Ethics Review committee, Ethiopia. Study information was given in the local language, and written informed consent in Amharic language was obtained from all participants, or by parents in the case of children (<18 years).

Study vaccines. MenAfriVac, a monovalent serogroup A conjugate vaccine (MenA-TT) (Serum Institute of India, Pune, India), contains 10 μg of purified MenA capsular polysaccharide conjugated to 10–33 μg of tetanus toxoid and adsorbed to aluminium phosphate. Menveo is a tetravalent conjugate serogroup A, C, W and Y conjugate vaccine (MenACWY-CRM) (Novartis Vaccines and Diagnostics, Siena, Italy) and contains 10 μg of MenA and 5 μg each of MenC, MenW and MenY capsular polysaccharides, all conjugated to a mutant diphtheria toxin, CRM_{197}.

MenAfriVac was obtained locally through the Ministry of Health in Ethiopia. Menveo was imported from Norway with permit from the Food, Medicines and Health Care Administration and Control Authority of Ethiopia (FMHACA). The vaccines maintained appropriate temperature during shipment and storage.

Study design and sampling. A cross-sectional carriage study was performed between 18 March 2014 and 1 October 2014, in 1–29-year-old volunteers from four kebeles (the smallest administrative unit within a district) in Arba Minch, Southern Ethiopia (manuscript in preparation). All participants were registered as part of the national, community-based Demographic Surveillance System (DSS) in Ethiopia who was in charge of recruitment in collaboration with community leaders and collection of demographic data.

Oropharyngeal swab samples were taken in the field, and the samples were analysed at the local laboratory in Ethiopia. Analyses included bacterial identification by culture and enzymatic testing [24], as well as determination of presumptive serogroup of *N. meningitidis* using conventional slide agglutination. Sixty-five individuals detected as carriers of encapsulated meningococci in Ethiopia were included in a follow-up study. Individuals presumptively carrying serogroup A meningococci were randomized to either receiving the MenA-TT vaccine group or a control group, and those carrying presumptively serogroup W or Y meningococci were randomized to the MenACWY-CRM vaccine group or a control group. Individuals in the control groups were offered the MenA conjugate vaccine at the end of the study period.

Saliva samples, serum samples and throat swabs were collected from these 65 individuals at inclusion and weekly for another eight consecutive weeks. Serum samples were collected by venipuncture into tubes without anticoagulant (Vacutainer serum tubes, BD, Franklin Lanes, NJ, USA) and transported at ambient temperature to Arba Minch General Hospital. Saliva samples were collected using OraSure® (OraSure Technologies, Bethlehem, PA, USA), consisting of an absorbent cotton pad which was placed between the lower gum and cheeks for 2 min, and then transferred to a tube containing approximately 0.8 ml proprietary solution with preservatives. Saliva samples were immediately kept cool (maximum 4 °C) in temperature-monitored boxes. All samples reached the laboratory within 6 h. At the laboratory, saliva and serum samples were centrifuged and aliquoted into Nunc cryotubes (Thermo Fisher Scientific Inc., Waltham, MA, USA) before storage at −80 °C.

Serum, saliva samples and bacteria were transported to Norway on dry ice for analysis at the Norwegian Institute

of Public Health (NIPH). On analysis of the bacterial isolates at NIPH, using cultures, enzymatic testing and slide agglutination [24], as well as further characterization using molecular methods [25], most presumptively encapsulated meningococci were found to be non-groupable meningococci lacking the capsule gene. In the MenA-TT vaccine group, there was one carrier of MenY, and in the MenACWY-CRM vaccine group, three individuals were confirmed as carriers of MenW and two as carriers of MenY. In the control group, there were four carriers of

Table 1 Characteristics of study population.

		Total	MenA-TT	MenACWY-CRM	Controls
n		65	18	15	32
Age	1–4	10	5	3	2
	5–10	31	6	9	16
	11–19	14	3	1	10
	20–29	10	4	2	4
Sex	Female	31	8	7	16
	Male	34	10	8	16

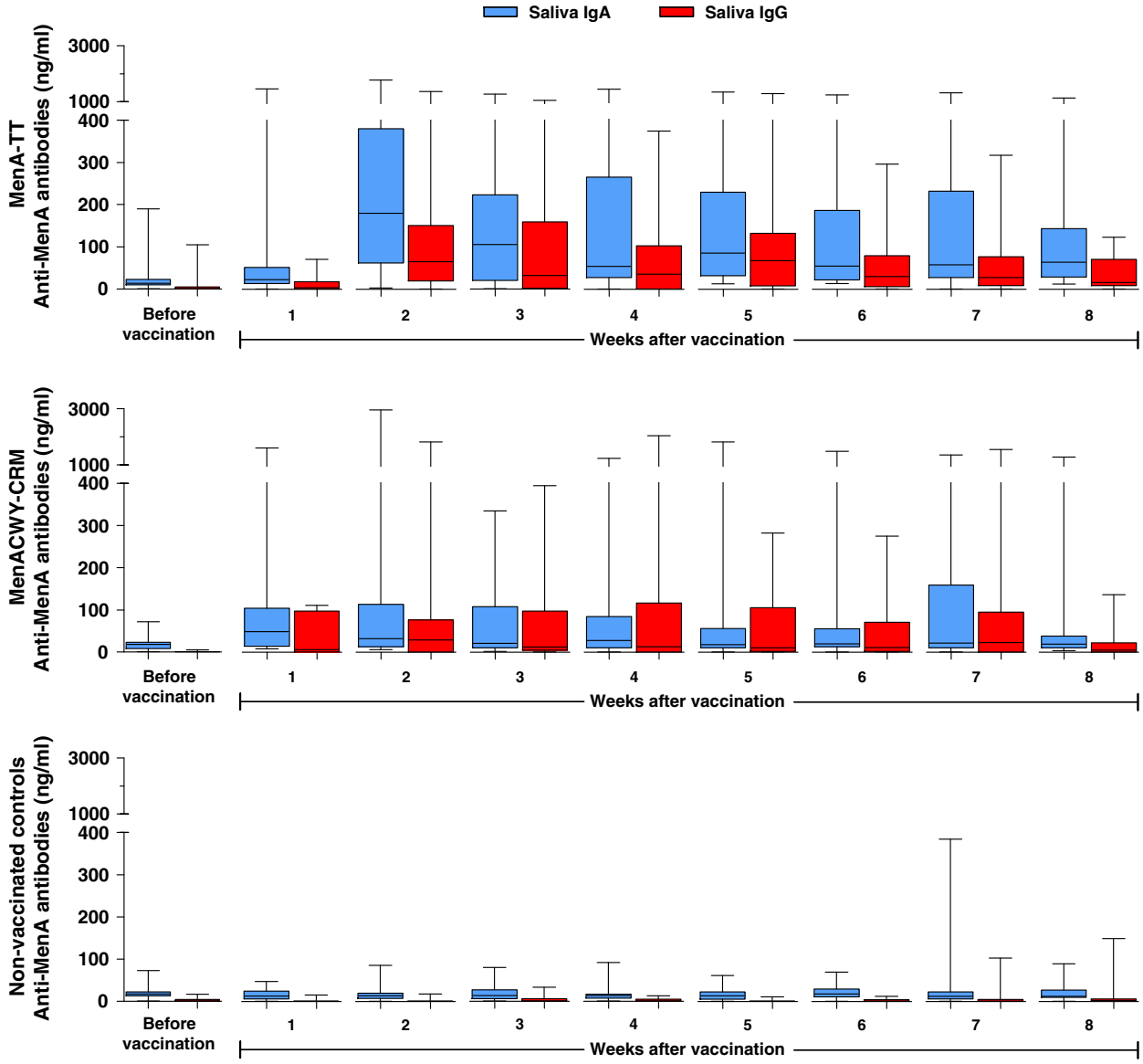

Figure 1 Antimeningococcal serogroup A polysaccharide antibodies in saliva (ng/ml) before and eight consecutive weeks after vaccination with a monovalent serogroup A meningococcal conjugate vaccine (MenA-TT, $n = 18$) (top graph), a tetravalent meningococcal serogroup A, C, W and Y conjugate vaccine (MenACWY-CRM, $n = 15$) (middle graph), and in non-vaccinated controls ($n = 32$) sampled at the same time point as the vaccinated individuals (bottom graph). Boxplot with median line and whiskers represent range (min–max). Blue bars represent salivary IgA antibodies, and red bars represent salivary IgG antibodies.

MenW and three carriers of MenY. Due to the low number of carriers, analysis of the effect of conjugate vaccines on carriage was not possible, and thus, the two control groups were combined to one.

Multiplex assay. The multiplex assay used for simultaneous detection of serogroup A, C, W and Y capsular polysaccharide-specific antibodies has been developed at NIPH and described previously [26]. Purified *N. meningitidis* capsular polysaccharides from different serogroups were conjugated to different magnetic microspheres. For detection of IgG antibodies, R-phycoerythrin (PE)-conjugated anti-human IgG (Sigma-Aldrich, St. Louis, MO, USA) was used. For detection of IgA antibodies, monoclonal mouse anti-human IgA (Sigma-Aldrich) and secondary R-PE-conjugated goat anti-mouse IgG (Jackson ImmunoResearch Inc., West Grove, PA, USA) detection antibodies were used. A monoclonal mouse anti-human secretory component (Sigma-Aldrich) and a secondary PE-conjugated goat anti-mouse IgG (Jackson ImmunoResearch) were used for the detection of sIgA. To determine Ig concentrations in the samples, fluorescent intensity of unknown samples was analysed against the known concentrations of the standard meningococcal reference serum CDC 1992 (NIBSC, UK). For quantitation of sIgA in saliva, no commercial reference is available and the standard meningococcal reference serum contains too low amounts of secretory IgA to serve as sIgA standard. An in-house sample of pooled saliva, obtained from healthy volunteers vaccinated with Menveo, was used for reference. Samples were tested in duplicate, and all samples from one individual were analysed in the same run.

Samples <2 positive standard deviations above the background were assigned a value of 1 ng/ml, previously determined as the lower limit of detection for the method used [26]. Serum IgG and IgA antibody levels were reported in μg/ml and salivary IgG and IgA antibodies in ng/ml. sIgA antibody levels were reported in arbitrary units per ml (AU/ml).

Statistical analysis. Results were analysed using IBM SPSS statistics 22 and GRAPHPAD PRISM version 5. Geometric mean concentrations (GMC) were calculated for each serogroup, isotype and vaccine group at each time point. Comparisons between vaccine groups were carried out using Mann–Whitney *U*-test and between the different time points using Wilcoxon signed-rank test. For correlation analysis, antibody levels were logarithmically transformed (log 10) and correlations made with Spearman's rank correlation coefficient, *r* test. Two-sided tests were used, and a *P*-value <0.05 was considered statistically significant.

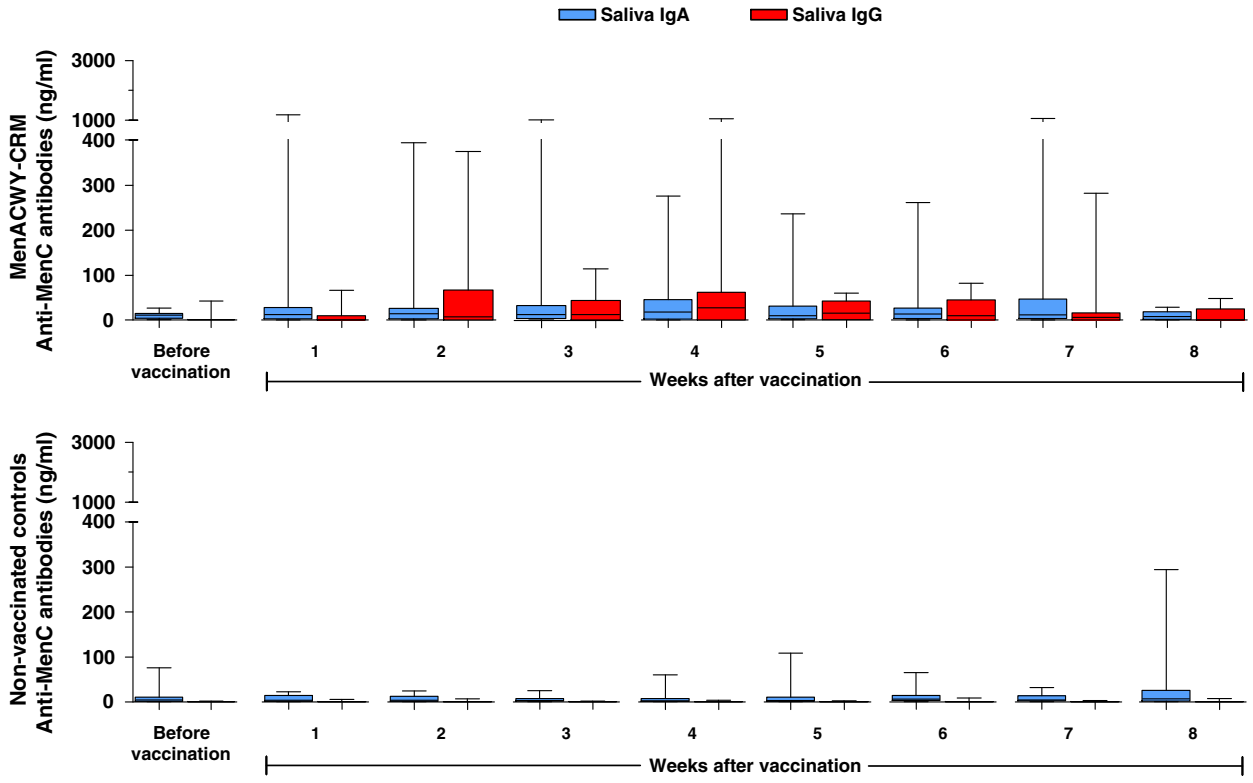

Figure 2 Antimeningococcal serogroup C polysaccharide antibodies in saliva (ng/ml) before and eight consecutive weeks after vaccination with a tetravalent meningococcal serogroup A, C, W and Y conjugate vaccine (MenACWY-CRM, $n = 15$) (top graph) and in non-vaccinated controls ($n = 32$) sampled at the same time point as the vaccinated individuals (bottom graph). Boxplot with median line and whiskers represent range (min–max). Blue bars represent salivary IgA antibodies, and red bars represent salivary IgG antibodies.

Results

Study population

The median age among the 65 participants was 9 years (range 1–29 years), and 63% of the individuals were 10 years or younger. There was no significant difference in gender and age distribution between the vaccine and control groups. There were not sufficient participants in each age group to make a valid comparison of antibody responses according to age.

Eighteen participants received the MenA-TT vaccine, 15 received the MenACWY-CRM vaccine, and 32 individuals served as controls (Table 1).

Anti-MenA antibody responses in saliva

Prior to vaccination, there was no significant difference in salivary antibody levels between the vaccine and control groups against serogroup A (Fig. 1) or any other serogroup (Fig. 2–4). Both vaccines elicited a rise in both IgA and IgG salivary antibodies against MenA in most vaccines already 1 week after vaccination (all $P < 0.01$). The amount of salivary IgA induced was significantly higher in the MenA-TT group (GMC = 139.8 ng/ml after 2 weeks) than in the MenACWY-CRM group (GMC = 53.2 ng/ml after 2 weeks), whereas there were no significant differences between the two vaccines regarding salivary IgG (Table S1). Although the antibody levels gradually declined during the following weeks, they were still significantly higher at 8 weeks post-vaccination than before vaccination in both vaccine groups (all $P < 0.05$). Salivary IgA levels were significantly higher for the MenA-TT group after 8 weeks than for the MenACWY-CRM vaccine ($P = 0.03$) (Fig. 1). The MenA-TT group showed significantly higher IgA and IgG antibody levels than the control group at all time points after vaccination (all $P < 0.05$) (Fig. 1). For those receiving the MenACWY-CRM vaccine, salivary IgA levels were generally higher compared to the control group, but the difference was only significant at week 1, whereas for IgG, significantly higher antibody levels were found after vaccination at all time points, except for week 8 where antibodies had declined to the same level as found in the control group.

Anti-MenC antibody responses in saliva

For those receiving the MenACWY-CRM vaccine, a small increase in anti-MenC salivary IgA antibodies was

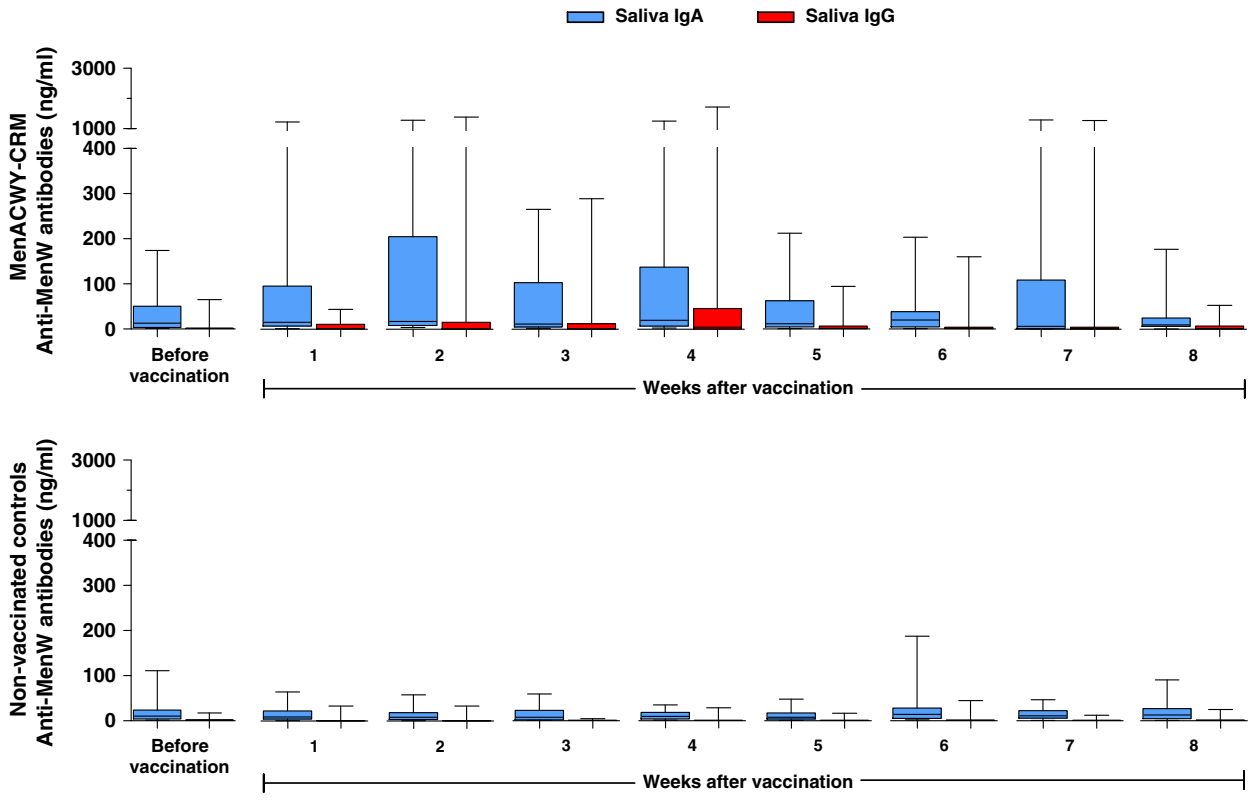

Figure 3 Antimeningococcal serogroup W polysaccharide antibodies in saliva (ng/ml) before and eight consecutive weeks after vaccination with a tetravalent meningococcal serogroup A, C, W and Y conjugate vaccine (MenACWY-CRM, $n = 15$) (top graph) and in non-vaccinated controls ($n = 32$) sampled at the same time point as the vaccinated individuals (bottom graph). Boxplot with median line and whiskers represent range (min–max). Blue bars represent salivary IgA antibodies, and red bars represent salivary IgG antibodies.

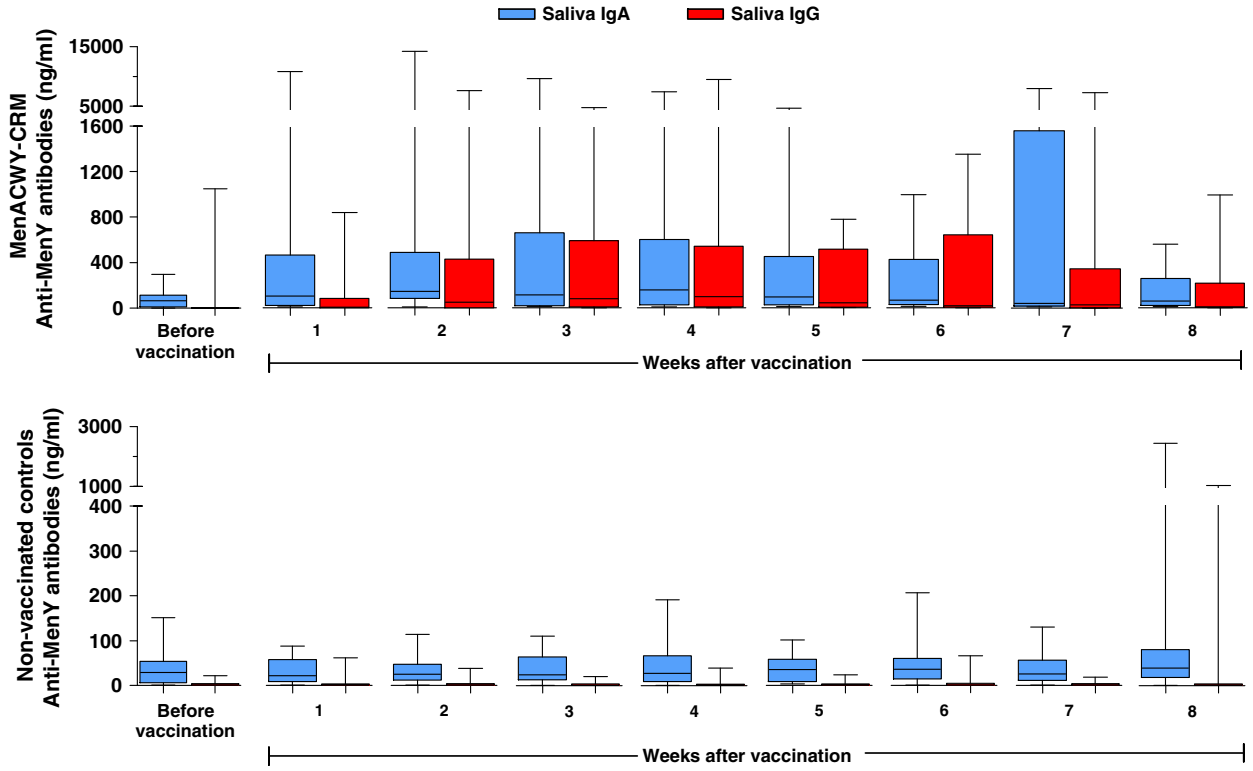

Figure 4 Antimeningococcal serogroup Y polysaccharide antibodies in saliva (ng/ml) before and eight consecutive weeks after vaccination with a tetravalent meningococcal serogroup A, C, W and Y conjugate vaccine (MenACWY-CRM, $n = 15$) (top graph) and in non-vaccinated controls ($n = 32$) sampled at the same time point as the vaccinated individuals (bottom graph). Boxplot with median line and whiskers represent range (min–max). Blue bars represent salivary IgA antibodies, and red bars represent salivary IgG antibodies. Note the different scale on the *y*-axis for top and bottom graphs.

observed, but the increase was only statistically significant 4 weeks after vaccination ($P = 0.01$) (Fig. 2). A small, but statistically significant increase for anti-MenC IgG was found after 1 week ($P = 0.02$), but declined to non-significant levels after 6 weeks (Table S2).

Anti-MenW antibody responses in saliva

We observed a significant increase in anti-MenW salivary IgA up to 7 weeks after MenACWY-CRM vaccination (all $P < 0.01$) (Fig. 3). A small increase, which was not observed in the control group, was detected in salivary IgG at 4 ($P = 0.05$) and 6 weeks ($P = 0.03$) after vaccination (Fig. 3, Table S3), but the increase was not significant from prevaccination levels throughout the study period.

Anti-MenY antibody responses in saliva

The highest IgA and IgG salivary antibody response after MenACWY-CRM vaccination was found for MenY. There was a significant increase in MenY-specific salivary IgA already 1 week after MenACWY-CRM vaccination ($P = 0.01$) and an increase in salivary IgG after 2 weeks ($P = 0.02$) (Fig. 4, Table S4). No salivary IgG was found in the control group (Fig. 4).

Secretory IgA in saliva

As a measurement of locally produced IgA, serogroup-specific secretory IgA was analysed and compared with IgA levels for all serogroups. A strong correlation between IgA and sIgA antibodies in saliva was found for all serogroups (Fig. 5), indicating that the majority of IgA produced after vaccination is locally produced.

Anti-MenA antibody responses in serum

Prior to vaccination, there was no difference between the vaccine and control groups in neither IgA nor IgG antibody levels against MenA or any of the serogroups (Table 2). A significant increase in anti-MenA IgA and IgG antibody levels in serum was seen in both vaccine groups after vaccination and stayed significantly elevated as compared to prevaccination levels at all time points for both the MenA-TT and MenACWY-CRM vaccine (all $P < 0.01$) (Table 2). IgG antibodies dominated the response in serum. Although the antibody levels generally were higher in the MenA-TT group

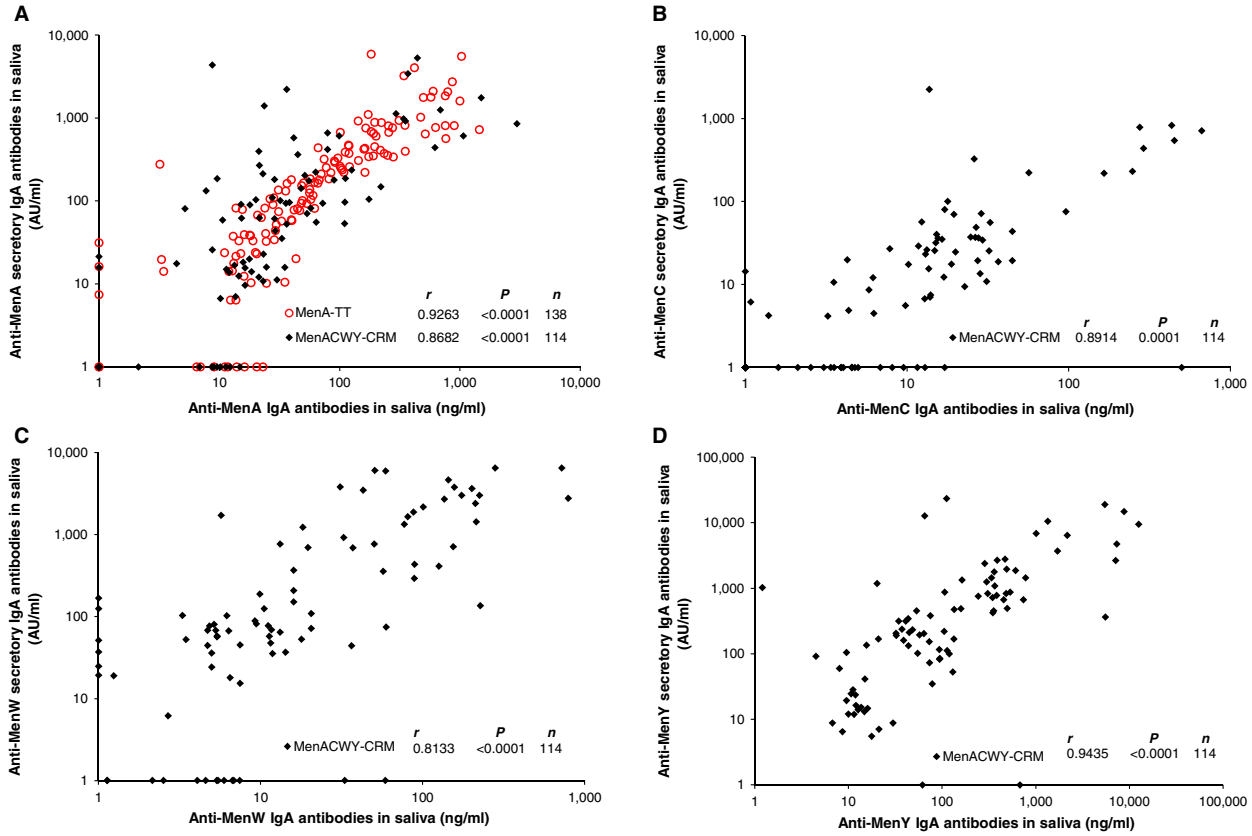

Figure 5 Correlation between serogroup-specific antimeningococcal secretory IgA antibodies (AU/ml) and IgA antibodies (ng/ml) in saliva before and eight consecutive weeks after vaccination. Panel (A) shows correlation for antiserogroup A antibodies after vaccination with a monovalent serogroup A meningococcal conjugate vaccine (MenA-TT) (red circles) or a tetravalent serogroup A, C, W and Y meningococcal conjugate vaccine (MenACWY-CRM) (black squares). Panels (B), (C) and (D) show correlation for antibodies against serogroup C, W and Y, respectively, after vaccination with the MenACYW-CRM vaccine. Spearman's rank correlation coefficient (*r*) and statistical significance were calculated using GraphPad Prism version 5, using all time points for the analysis.

from 2 weeks onwards, the difference between the two vaccine groups was not statistically significant (Table S1).

Anti-MenC antibody responses in serum

For MenC, there was a significant rise in both IgA ($P = 0.001$) and IgG ($P = 0.001$) in serum already after 1 week in the group receiving the MenACWY-CRM vaccine, and anti-MenC IgA and IgG levels in serum stayed significantly elevated compared to prevaccination levels at all time points (all $P < 0.01$) (Table 2). Serum IgG levels were considerably lower than for MenA (Table 2).

Anti-MenW antibody responses in serum

A significant rise in anti-MenW antibody levels, both in IgA ($P = 0.004$) and IgG ($P = 0.02$) serum antibodies, was observed already after 1 week in the group receiving the MenACWY-CRM vaccine (Table 2). Anti-MenW IgG serum antibody levels were significantly different from prevaccination levels at all time points (all $P < 0.05$) except 8 weeks post-vaccination. Anti-MenW IgA serum antibody levels were significantly elevated at all time points (all $P < 0.05$) except for after 5 weeks (Table S3).

Anti-MenY antibody responses in serum

The highest IgA and IgG serum antibody response after MenACWY-CRM vaccination was found for MenY. A significant rise in IgA ($P = 0.001$) and IgG ($P = 0.003$) anti-MenY antibody levels in serum was seen already after 1 week in the MenACWY-CRM vaccine group (Table 2). Anti-MenY IgA and IgG antibody levels stayed significantly elevated from prevaccination levels at all time points (all $P < 0.05$), except for IgG antibody levels 8 weeks post-vaccination which was not significantly different from prevaccination levels.

Comparison of antibody levels in serum versus saliva

A strong correlation for IgG antibody levels in saliva and serum was observed for MenA in both vaccine groups, as well as for the other three serogroups in the MenACWY-CRM vaccine group (Fig. 6). A strong, but somewhat weaker correlation was also observed for IgA antibodies in saliva and

Table 2 Geometric mean concentration (GMC) of antibodies (μg/ml) in serum after vaccination.

		MenA-TT				MenACWY-CRM					
		anti-MenA		anti-MenA		anti-MenC		anti-MenW		anti-MenY	
Vaccine		IgA	IgG	IgA	IgG	IgA	IgG	IgA	IgG	IgA	IgG
Prevaccination	No. analyzed	16	16	14	14	14	14	14	14	14	14
	GMC	**0.1**	**3.3**	**0.0**	**4.7**	**0.1**	**0.7**	**0**	**1**	**0.7**	**3.8**
	95% CI	0.0–0.6	1.0–11.5	0.0–0.3	3.0–7.4	0.0–0.4	0.3–1.4	0.0–0.3	0.4–2.7	0.1–8.5	1.0–14.7
1 week post-vaccination	No. analyzed	15	15	14	14	14	14	14	14	14	14
	GMC	**1.9**	**11.5**	**4.2**	**13.7**	**1.1**	**2.4**	**1.7**	**2.4**	**18.7**	**27.4**
	95% CI	0.5–7.4	6.4–20.7	1.2–14.7	7.0–27.0	0.4–2.8	0.8–7.2	0.5–5.6	0.8–6.6	5.1–67.7	8.1–93.0
4 weeks post-vaccination	No. analyzed	15	15	13	13	13	13	13	13	13	13
	GMC	**3.8**	**50.4**	**3.6**	**40.8**	**1.4**	**6.8**	**1.9**	**6.2**	**22.8**	**151.8**
	95% CI	0.8–17.2	22.7–112.2	1.3–10.1	15.0–111.1	0.4–4.6	2.0–22.7	0.8–4.3	1.7–22.9	5.4–96.9	43.5–530.2
8 weeks post-vaccination	No. analyzed	13	13	13	13	13	13	13	13	13	13
	GMC	**3.3**	**50.6**	**1.6**	**37.4**	**1.5**	**7.2**	**1.4**	**5.4**	**11.9**	**80.3**
	95% CI	0.7–16.4	24.6–103.9	0.3–8.2	14.6–95.4	0.5–4.2	2.2–23.3	0.5–3.8	1.6–18.5	3.6–39.3	20.5–314.5

Bold indicates the most important GMC values..

serum for all serogroups (Fig. 7). In general, the level of IgA antibodies reached its highest level around 2 weeks post-vaccination, slightly earlier in saliva than in serum, while for IgG, the peak levels were reached after about 3 weeks, slightly later in saliva than in serum (Table S1–4).

Discussion

Our study showed that meningococcal conjugate vaccines elicit salivary antibodies against serogroup A, C, W and Y, as well as serum antibodies. This is the first study to report both salivary and serum IgA and IgG antibody levels against all four serogroups after vaccination with meningococcal conjugate vaccines in an African population. Additionally, very few studies have reported specific IgG and IgA antibody levels in saliva or serum induced after vaccination with MenACWY-CRM vaccine. Due to low numbers of carriers of encapsulated meningococci, we were not able to investigate the relationship between carriage and antibodies, but we present here the first analysis of salivary and serum antibodies simultaneously in the same individuals and over time.

Of the two vaccines, only MenA-TT elicited a significant increase in anti-MenA salivary IgA over time, compared to the control group, and the amount of salivary IgA induced was considerably higher in the MenA-TT group than in the MenACWY-CRM group. This suggests that MenA-TT is better in eliciting a mucosal antibody response. The potential difference between the two vaccines in their ability to elicit salivary IgA antibody responses may be explained by difference in their composition; monovalent versus polyvalent, different carrier proteins (TT versus CRM) and the addition of adjuvant in MenA-TT.

Low salivary antibody responses were observed against MenC as compared to the other serogroups after vaccination with the MenACWY-CRM vaccine. This was also found in a small study by Clarke *et al.* [27] where one dose of a MenACWY-CRM vaccine in naïve individuals gave no significant rise in salivary IgG against MenC. Salivary IgA and IgG antibody responses against MenC were similar to what was found after vaccination with another tetravalent meningococcal conjugate vaccine conjugated to a diphtheria toxoid (MenACWY-DT) using similar methodology [20].

Salivary IgA antibody levels against serogroup W and Y have not been reported previously. We found a significant IgA and IgG antibody response in saliva against MenW and MenY after MenACWY-CRM vaccination. Of all the four serogroups, by far the highest IgA and IgG salivary antibody response after MenACWY-CRM vaccination was found for MenY. The high levels of anti-MenY antibodies in this study might be explained by cross-reactive antibodies or previous exposure to serogroup Y in this population leading to a possible 'booster' response after

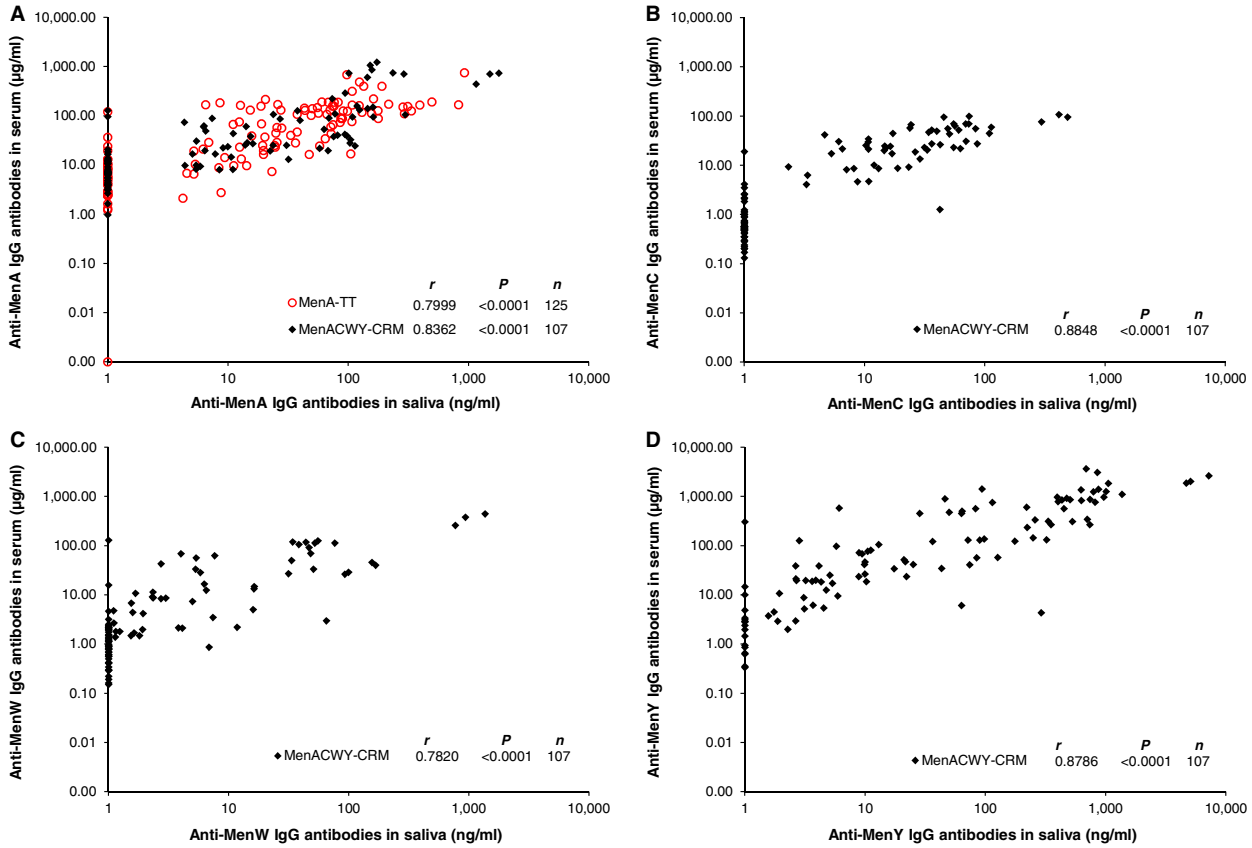

Figure 6 Correlation between serogroup-specific antimeningococcal IgG antibodies in saliva (ng/ml) and serum (μg/ml) before and eight consecutive weeks after vaccination. Panel (A) shows correlation for antiserogroup A antibodies after vaccination with a monovalent serogroup A meningococcal conjugate vaccine (MenA-TT) (red circles) or a tetravalent serogroup A, C, W and Y meningococcal conjugate vaccine (MenACWY-CRM) (black squares). Panels (B), (C) and (D) show correlation for antibodies against serogroup C, W and Y, respectively, after vaccination with the MenACYW-CRM vaccine. Spearman's rank correlation coefficient (r) and statistical significance were calculated using GraphPad Prism version 5, using all time points for the analysis.

vaccination. Higher levels of MenY antibodies compared to the other serogroups were also found in our non-vaccinated control group at all time points.

IgG in saliva is mainly derived from serum by passive diffusion, preferentially through gingival crevices [16], and we found, as others, a strong correlation between specific IgG levels in serum and saliva for all four serogroups (Spearman r = 0.78–0.88) [21, 22, 28]. It is believed that IgG at the mucosal level takes part in the host defence by clearing of bacteria by agglutination in an Fc-independent manner [19]. Increased serum IgG has shown to reduce carriage of pneumococci [29], and individuals with gammaglobulin deficiency have an increased incidence of respiratory tract infections [18], suggesting that salivary IgG is important. Bactericidal antibodies in serum that are able to activate the complement system are crucial in the defence against systemic meningococcal disease [30, 31]. Complement factors are found in saliva, especially along the gingival margin and in periodontal inflammation, but saliva also contains several factors downregulating the complement system [32]. It is not known whether the salivary anti-meningococcal polysaccharide IgG antibodies are able to elicit the complement system in saliva and whether the complement system is functionally active; this might be an interesting aspect to investigate in the future.

IgA in saliva is believed to mainly be locally produced and has previously been found to correlate poorly with IgA levels in serum [23, 27]. However, we found a correlation between IgA antibodies in serum and saliva for all serogroups, as also shown recently by Stoof *et al.* [21]. This difference might be explained by different compositions of the vaccines or variation in saliva sampling methods. Independent of the mechanisms and origin of the antibodies, the increase of both IgG and IgA in saliva, is an indication that parenterally administered vaccines can induce immunity at the salivary level. The strong correlation between secretory IgA and IgA supports the evidence that IgA in saliva indeed is produced locally [21, 22, 27, 33]. Salivary IgA is thought to be beneficial to the host by inhibiting microbial adhesion and by agglutination [34], possibly also by facilitating phagocytosis by binding to FcαRI receptor for IgA on granulocytes, monocytes and macrophages. The secretory component in sIgA, however, sterically hinders binding to the Fc

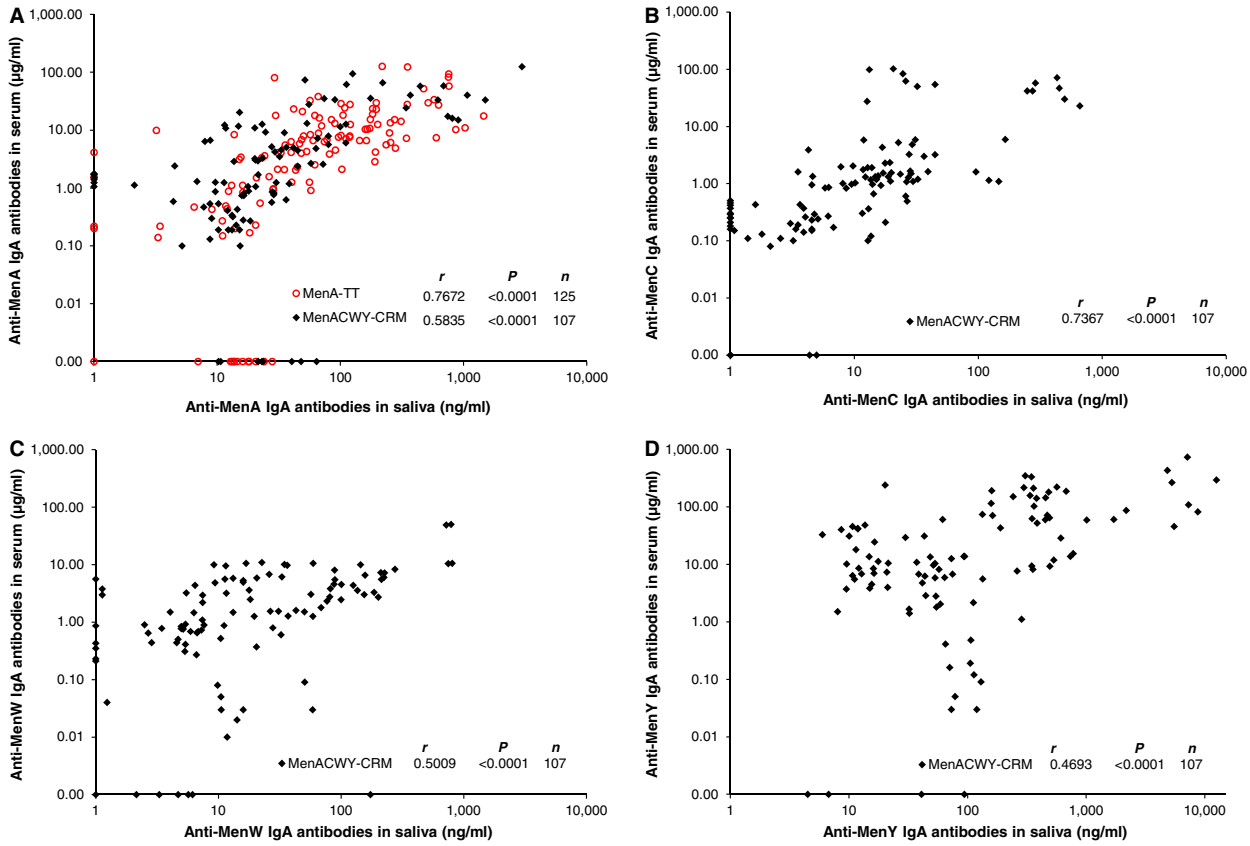

Figure 7 Correlation between serogroup-specific antimeningococcal IgA antibodies in saliva (ng/ml) and serum (µg/ml) before and eight consecutive weeks after vaccination. Panel (A) shows correlation for antiserogroup A antibodies after vaccination with a monovalent serogroup A meningococcal conjugate vaccine (MenA-TT) (red circles) or a tetravalent serogroup A, C, W and Y meningococcal conjugate vaccine (MenACWY-CRM) (black squares). Panels (B), (C) and (D) show correlation for antibodies against serogroup C, W and Y, respectively, after vaccination with the MenACYW-CRM vaccine. Spearman's rank correlation coefficient (r) and statistical significance were calculated using GraphPad Prism version 5, using all time points for the analysis.

receptor, and binding is less efficient than for IgA molecules lacking the secretory component [35]. Conversely, *N. meningitidis* produces an IgA protease that cleaves IgA1 [36], the IgA subclass dominating in saliva, leaving the meningococcus decorated with IgA1 fragments that are not able to bind to Fc receptors and prevents binding of more potent IgG antibodies. Thus, induction of IgA might also benefit the meningococcus by leading to enhanced colonization and invasion [16].

Although the level of anti-MenA salivary IgA was significantly higher than prevaccination throughout the study period, there was a continuous decline in antibody levels. This decline was even more pronounced for the other serogroups, suggesting that the induced rise in salivary antibodies after vaccination might be of short duration.

Significant IgG and IgA antibody responses in serum were found against serogroup A after MenA-TT vaccination and against all four serogroups after MenACWY-CRM vaccination. No significant difference was seen between the MenA-TT-vaccinated individuals and the control for serogroup C, W and Y (data not shown), showing that the antibody response is specific against the serogroup included in the vaccine.

Carriage is an immunizing process, found to induce specific antimeningococcal antibodies in both serum and saliva [37, 38]. The clinical role of mucosal antibodies on carriage is not known, but individuals who are non-secretors of ABO blood group antigens are more prone to meningococcal colonization, and this is believed to be due to a deficiency of immunoglobulins in secretions [39]. An antibody concentration of 2 µg/ml in serum has been shown to correlate with clinical protection against serogroup A disease [40], but no such antibody correlate has been established in saliva for protection against carriage. It is not known whether a certain concentration of antibodies is needed to have an effect on carriage, or if other laboratory measures are better correlates of protection against carriage, like serum bactericidal antibody activity is for investigating protection against systemic disease [41, 42].

It would have been of great interest to investigate whether the conjugate vaccines are able to induce a direct

effect on carriage, but due to the low numbers of confirmed carriers of encapsulated meningococci, we were not able to do so in this study. In the few participants found to be carriers of MenW and MenY, we did not observe significant differences or obvious trends in antibody levels and vaccine response against the serogroup carried (data not shown). The miscategorization of serogroups, which occurred at the primary laboratory, reflects the challenge of conducting a study in a low-resource setting where local staff has limited experience. Implementing microbiological methods, such as preparation of media, enzymatic testing and seroagglutination, in a laboratory where little or no microbiological investigations were carried out routinely, was particularly challenging. Due to logistical and time constraints, confirmatory analysis in Norway was not possible prior to inclusion as the volunteers had to be included in the follow-up study as soon as carriage was detected. Additionally, there was a time limitation on the inclusion of participants in this study due to mass introduction of MenAfriVac in the region in October 2014. Thus, for future studies of this aspect, confirmatory molecular methods should be set up locally.

In conclusion, we have shown that meningococcal conjugate vaccines are able to elicit IgA and IgG antibodies in saliva and serum. Only MenA-TT was able to elicit a sustained increase in anti-MenA IgA in saliva, whereas MenACWY-CRM was able to elicit transient increases in anti-MenC, MenW and MenY salivary IgA. The dynamics of the antibody response and the strong correlation of IgA and secretory IgA in saliva provide further evidence that most IgA in saliva is produced locally, whereas IgG is derived from serum. The strong correlation of serum and saliva antibodies indicates that saliva might be used for studies and surveillance of systemic antibody responses in the future. However, the clinical role of these salivary antibodies in the defence against carriage and disease, and a potential correlate for elimination of or protection against carriage, is yet not known and should be investigated further.

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Conflict of Interest

We confirm that the authors have no conflict of interests to declare. The manuscript has been reviewed and approved by all authors.

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Supporting Information

Additional Supporting Information may be found online in the supporting information tab for this article:

Table S1 Geometric mean concentration (GMC) of anti-MenA antibodies in saliva and serum after vaccination.

Table S2 Geometric mean concentration (GMC) of anti-MenC antibodies in saliva and serum after vaccination.

Table S3 Geometric mean concentration (GMC) of anti-MenW antibodies in saliva and serum after vaccination.

Table S4 Geometric mean concentration (GMC) of anti-MenY antibodies in saliva and serum after vaccination.