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HPV-specific antibodies at the oral cavity up to 30 months after the start of vaccination with the quadrivalent HPV vaccine among mid-adult aged men ☆

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ABSTRACT

Background: HPV-16 and HPV-18 cause most oropharyngeal cancers, which are increasing in incidence among males. Although HPV vaccines are highly effective against a number of HPV-associated cancers, efficacy for oropharyngeal cancers has not yet been demonstrated. In addition, the level of antibodies required for protection against oral HPV infection is unknown.
Methods: 150 men ages 27–45 years from Tampa, FL, USA, and Cuernavaca, Mexico, received Gardasil at Day 1, Months 2, and 6. Then, sera and oral gargles were collected one month, 12 months, and 24 months after completion of the three doses (Month 7, 18 and 30 of the study) and tested for anti-HPV-16 and HPV-18 IgG antibody levels by a L1 VLP ELISA.
Results: All participants developed detectable serum anti-HPV-16 and anti-HPV-18 antibodies and most had detectable antibodies in oral gargles at Month 7 (HPV-16: 93.2%; HPV-18: 72.1%). By months 18 and 30, oral antibodies were detectable in a lower number of participants (HPV-16, 39.8% and 29.6%; HPV-18, 10.7% and 4.6% of individuals, respectively). Overall, oral HPV-16- and 18-specific antibody levels, normalized to total IgG at months 7, 18, and 30, correlated with serum levels (HPV-16, $R^2 = 0.93$; HPV-18, $R^2 = 0.91$).
Conclusions: Reduced detectability of oral and serum HPV-16 and HPV-18 antibodies was observed at months 18 and 30 after initiation of the quadrivalent vaccination. However, when detectable, serum and oral HPV-16 and HPV-18 antibody levels were strongly correlated.
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1. Introduction

Human papillomavirus (HPV) is one of the most common sexually transmitted viruses. There are over 100 types of HPV, 40 of which infect mucosal epithelia, and approximately 15 are considered oncogenic [1]. HPV-16 is known to induce the majority of oropharyngeal cancers, which is found at a higher prevalence in men compared to women [2]. Vaccination against HPV-types 6, 11, 16 and 18 with Gardasil is highly efficacious in preventing HPV-related genital lesions and anal diseases in young males [3]. Mid-adult men, who are highly susceptible to HPV-related cancers, have been shown to develop a strong antibody response to Gardasil [4]. The high efficacy of the Gardasil vaccine is in agreement with a robust systemic antibody response [5,6].

It is not yet known whether HPV vaccination is as efficacious in protection from HPV-related oral cancers, as with other HPV-associated cancers. Recently published data from our group demonstrated that Gardasil induced HPV-specific antibodies at detectable levels in the oral cavity, one-month post- dose three of the vaccine series [7]. However, antibody levels at the oral cavity were significantly lower than in serum. In prior vaccine trials, serum HPV antibodies were shown to consistently peak soon after completion of the 3 doses of vaccine and then plateau by 18–24 months after vaccination [8]. HPV antibody levels at the oral cavity, when plateau levels are achieved in serum, remain unknown. Therefore, we characterized the longevity of the

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immune response to vaccination at the oral cavity. Accordingly, we conducted a follow-up study to extend our previous oral and serum HPV antibody observations at 7 months (1 month after three doses of vaccine) to 18 and 30 months following the start of vaccination.

2. Materials and methods

2.1. Study population

The *Mid-Adult Men Trial Study* (www.clinicaltrials.gov, NCT01432574) is a single-arm intervention trial that enrolled, vaccinated, and assessed the circulating antibody response to Gardasil in men, ages 27–45 [9]. Men were solely selected for this study due to the 3–4-fold higher incidence of oropharyngeal cancer amongst males compared to females [2]. Briefly, subjects were vaccinated intramuscularly with Gardasil at Day 1 of the study and at Months 2 and 6. A total of 150 men from Tampa, FL, USA, and Cuernavaca, Mexico who met eligibility criteria (male, 27–45 years, completed four years of follow-up in the *HPV Infection in Men (HIM) Study*) were enrolled and received at least one dose of vaccine. All individuals with samples tested at Day 1, Month 7, 18, and 30 received three doses of vaccine. Each participant underwent a clinical examination on Day 1 and at Months 7, 18 and 30. At the Day 1 and Months 7, 18 and 30 study visits, blood and oral specimens (oral gargle) were collected. The institutional review boards at each participating center (University of South Florida in the U.S. and Instituto Nacional de Salud Publica in Mexico) approved the protocol, and informed consent was obtained from all subjects. The study was conducted in conformance with applicable country or local requirements regarding ethical committee review, informed consent, and other statutes or regulations regarding the protection of the rights and welfare of human subjects participating in biomedical research.

2.2. Specimens

10 mL of blood was collected in a red top tube (BD Cat# 366430). Following centrifugation, sera was aliquoted into cryovials and stored at −80 °C until testing. Oral fluid from each participant was collected in oral gargles. Oral gargle specimens were collected by use of a 30-second rinse/gargle with 15 mL of mouthwash solution [10]. Supernatants were stored at −80 °C prior to antibody testing. A subset of vaccine recipients had oral gargles (n = 108) and serum (n = 108) available for HPV antibody testing at Day 1, Month 7, Month 18, and Month 30. These collection methods and validated assays have been used in a number of clinical epidemiologic studies [10–12].

2.3. Direct L1 VLP ELISA

Anti-HPV IgG antibodies were detected by an enzyme-linked immunosorbent assay (ELISA), as previously described [13–15]. This ELISA measures total levels of HPV-16 and 18-specific IgG antibodies (both neutralizing and non-neutralizing), and is amenable for use in large epidemiologic and clinical studies. The HPV-16 and HPV-18 ELISAs are specific for the detection of HPV-16 antibodies or HPV-18 antibodies, respectively. As part of the assay qualification procedure, specificity of the Virus-Like Particles (VLP) is assessed using HPV-16 or HPV-18 monoclonal antibodies. HPV-16 VLPs are only recognized by HPV-16 monoclonals and not by HPV-18 monoclonals and vice versa. The WHO International HPV-16 and HPV-18 Standards have also been tested in the ELISAs with no evidence for cross-reactivity. The assay is highly reproducible, with a reported overall coefficient of variation (CV) of 11.4% [13]. Briefly, polystyrene flat-bottom microtiter plates (MaxiSorp, high binding; Nunc, Cat# 439454 Thermo Fisher Scientific, USA) were coated with HPV-16 or HPV-18 L1 VLPs and incubated at 4 °C. Prior to use, the plates were washed with a phosphate-buffered saline containing 0.05% Tween 20 (VWR, Cat# EM-PX1296-1). After blocking the plates with blocking buffer containing 4% skim milk (BD, Cat# 232100) and 0.2% Tween 20 in phosphate-buffered saline (Gibco, Cat# 14190-136), the plates were washed again. Serum (starting dilution 1:100) and oral fluids (oral gargle, starting dilution 1:2) from participants were serially diluted in the blocking solution in two-fold increments in the assay plate. The plates were incubated for one hour at room temperature. After washing four times, a solution of peroxidase-labeled goat anti-human IgG (KPL, Inc., Cat# 214-1002) was added for one hour at room temperature. Plates were then developed with a tetramethylbenzidine (TMB) substrate solution (KPL, Inc., Cat# 50-76-03), for 25 min in the dark at room temperature. Next, the reaction was stopped with 0.18 M H_2SO_4 (J. T. Baker, Cat# 4700-01), and the absorbance measured with a microtiter plate reader (Spectramax M5; Molecular Devices, Sunnyvale, CA). Antibody levels, expressed as ELISA units (EU)/mL, were calculated by interpolation of OD values from the standard curve by averaging the calculated concentrations from all dilutions that fall within the working range of the standard curve. The seropositivity lower cut points for serum were set at 19 EU/mL for anti-HPV-16 and 18 EU/mL for anti-HPV-18 antibodies [16]. Cut points for oral gargles were set at 0.042 EU/mL for anti-HPV-16 and 0.032 EU/mL for anti-HPV-18.

2.4. Total IgG ELISA

Total human IgG levels were measured in duplicate for each specimen type (serum and oral gargle) using an ELISA according to the manufacturer's protocol (Bethyl Laboratories, Cat # E80-104 Montgomery, TX, USA) [11]. Total IgG levels in each different sample type (serum and oral gargle) were used to normalize levels of HPV specific antibodies across different biological specimens and to compare levels between different collection time points.

2.5. Statistical analysis

An intent-to-treat analysis (ITT) was conducted, and included all men in the trial, regardless of HPV DNA and antibody status at enrollment. The proportion of men who had measurable serum or oral HPV-16/18 IgG and the 95% confidence interval (CI) were estimated. Geometric mean concentrations (GMTs) and 95% CIs for HPV-16 and 18 antibody levels were calculated and reported by categories of the covariates examined. GMTs were compared across groups using the Wilcoxon rank-sum test. GMTs were compared across months and country using Mann-Whitney test. Correlations between serum- and oral-specific IgG levels were determined by Spearman correlation coefficients. Among trial participants with detectable antibodies in both serum and oral gargle, HPV-specific antibody levels were normalized to the total IgG level in the serum or oral specimen, respectively, and reported as ratios of the HPV-specific IgG concentrations/total concentrations of IgG.

3. Results

Percent seropositive and antibody concentrations (GMT) were measured and compared in oral gargle and serum samples at three time points following vaccination (Fig. 1A and B). At day 1 (prior to vaccination), less than 21% of participants had detectable HPV-16 or HPV-18 antibodies in serum and less than 5% of participants had detectable HPV-16 or HPV-18 antibodies in oral gargles (Table 1). As previously reported, at month 7, 100% of participants

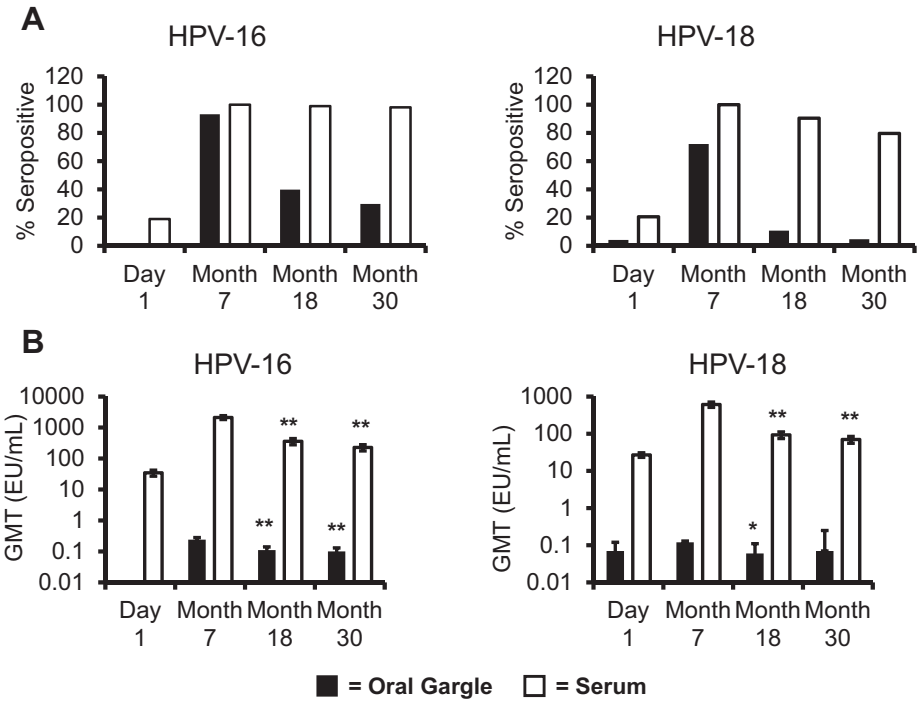

Fig. 1. HPV-16 and HPV-18 antibody detectability and levels in oral gargles and serum from vaccinated individuals. (A) % seropositive among samples from serum and oral gargle amongst US and Mexico samples. (B) Geometric mean concentration (GMT) between positive samples. Error bars display confidence interval (95% CI). 18 and 30 month values were compared to the corresponding 7 month value. Statistical significance was determined by Wilcoxon rank sum test. * = P < 0.01, ** = P < 0.0001.

Table 1
Individuals with detectable anti-HPV-16 and HPV-18 antibodies in oral fluids and serum prior to and up to Month 30 post-vaccination.

				Day 1		Month 7			Month 18[c]			Month 30[d]	
HPV type	Specimen Type	Clinic	N	AB positive (%)[a]	GMT[b] (95% CI)	AB positive (%)[a]	GMT[b] (95% CI)	N	AB positive (%)[a]	GMT[b] (95% CI)	N	AB positive (%)[a]	GMT[b] (95% CI)
16	Oral gargle	U.S.	72	0 (0.0)	–	62 (86.1)	0.17 (0.14, 0.20)	55	18 (32.7)	0.11 (0.08, 0.15)	58	16 (27.6)	0.08 (0.06, 0.10)
		Mexico	75	0 (0.0)	–	75 (100.0)	0.33 (0.27, 0.41)	48	23 (47.9)	0.11 (0.08, 0.16)	50	16 (32.0)	0.13 (0.07, 0.22)
		Total	147	0 (0.0)	–	137 (93.2)	0.24 (0.21, 0.28)	103	41 (39.8)	0.11 (0.09, 0.14)	108	32 (29.6)	0.1 (0.07, 0.13)
	Serum	U.S.	51	7 (13.7)	35.40 (19.66, 63.71)	51 (100.0)	2078.34 (1640.51, 2633.02)	56	55 (98.2)	381.31 (282.83, 514.09)	58	56 (96.6)	236.4 (174.9, 319.52)
		Mexico	75	17 (22.7)	34.09 (27.68, 41.98)	75 (100.0)	2148.40 (1836.81, 2512.84)	48	48 (100.0)	336.52 (252.52, 448.46)	50	50 (100.0)	218.5 (164.17, 290.82)
		Total	126	24 (19.0)	34.46 (28.27, 42.01)	126 (100.0)	2119.76 (1858.22, 2418.11)	104	103 (99.0)	359.74 (293.03, 441.63)	108	106 (98.1)	227.78 (185.5, 279.7)
18	Oral gargle	U.S.	72	1 (1.4)	–	45 (62.5)	0.08 (0.07, 0.10)	55	4 (7.3)	0.05 (0.03, 0.07)	58	0 (0)	–
		Mexico	75	5 (6.7)	0.07 (0.03, 0.13)	61 (81.3)	0.15 (0.12, 0.18)	48	7 (14.6)	0.07 (0.03, 0.18)	50	5 (10.0)	0.07 (0.02, 0.25)
		Total	147	6 (4.1)	0.07 (0.04, 0.12)	106 (72.1)	0.12 (0.10, 0.13)	103	11 (10.7)	0.06 (0.04, 0.11)	108	5 (4.6)	0.07 (0.02, 0.25)
	Serum	U.S.	51	5 (9.8)	27.21 (20.11, 36.81)	51 (100.0)	603.74 (466.19, 781.86)	56	50 (89.3)	91.05 (69.92, 118.55)	58	44 (75.9)	70.82 (54.33, 92.3)
		Mexico	75	21 (28.0)	26.81 (22.88, 31.41)	75 (100.0)	617.02 (512.43, 742.96)	48	44 (91.7)	96.19 (73.77, 125.44)	50	42 (84.0)	69.49 (52.66, 91.69)
		Total	126	26 (20.6)	26.89 (23.57, 30.67)	126 (100.0)	611.61 (526.41, 710.6)	104	94 (90.4)	93.42 (77.71, 112.30)	108	86 (79.6)	70.16 (58.16, 84.65)

[a] AB positive represents the number (%) of subjects with an antibody response above the cutoff.
[b] GMT represents the geometric mean concentrations amongst positive values (above cutoff).
[c] Month 18 represents 12 months following the last dose of HPV vaccine.
[d] Month 30 represents 24 months following the last dose of HPV vaccine.

had measurable levels of anti-HPV-16 and anti-HPV-18 antibodies in serum and 93.2% and 72.1% of participants had measurable levels of anti-HPV-16 and anti-HPV-18 antibodies, respectively, in oral gargles (Table 1). At months 18 and 30, the percentage of participants with detectable anti-HPV-16 and anti-HPV-18 antibody levels dropped considerably in oral gargles, but to a lesser extent in serum. At month 18, oral anti-HPV-16 and anti-HPV-18 antibody levels were detected in 39.8% and 10.7% of participants, respectively, while serum anti-HPV-16 and anti-HPV-18 antibody levels remained detectable in 99.0% and 90.4% of participants, respectively. By month 30, oral anti-HPV-16 and anti-HPV-18 antibody levels were detectable in 29.6% and 4.6% of participants, while serum anti-HPV-16 and anti-HPV-18 antibodies were detectable in 98.1% and 79.6% of participants, respectively (Table 1).

At month 18, oral HPV-specific antibody levels, among those with detectable levels, were 1557-3270-fold lower compared to serum levels (HPV-16, 0.11 vs 359.74 EU/mL; HPV-18, 0.06 vs 93.42 EU/mL). At month 30, oral HPV-specific antibody levels in oral gargles were 1002-2278-fold lower compared to serum levels (HPV-16, 0.1 vs 227.78 EU/mL; HPV-18, 0.07 vs 70.16 EU/mL) (Table 1).

To adjust for differences in the amount of oral fluid collected, antibody levels were normalized to the corresponding total IgG, determined in each sample. At month 7, normalized anti-HPV-16 and anti-HPV-18 antibody concentrations were 1.49-fold and 1.03-fold higher in serum compared to oral gargles (Table 2). At month 18, normalized anti-HPV-16 and anti-HPV-18 antibody concentrations were 1.86-fold and 2.83-fold higher in oral gargles compared to serum (Table 2). At month 30, normalized anti-HPV-16 and anti-HPV-18 antibody levels were 2.11-fold and 2.47-fold higher in oral gargles compared to serum (Table 2). The number of seropositive oral gargles decreased over time, as antibody levels naturally decayed after reaching peak levels at month 7. Therefore, the number of oral samples that contributed to the oral gargle GMT was only a subset of the serum samples at months 18 and 30. When serum GMTs were restricted to the samples that were oral gargle seropositive, the antibody level differences between oral gargles and serum, at months 18 and 30, were consistent with what was found at month 7. Levels of anti-HPV-16 antibodies were 1.20 (Month18) and 1.31 (Month 30) fold higher in serum vs oral gargles, and 1.10 (Month 18) and 1.38 (month 30) fold higher in serum vs oral gargles for HPV-18 (Table 3).

Normalized anti-HPV-16 and anti-HPV-18 antibody levels over the 30-month time period followed a similar pattern of reduction by participant country of residence (Fig. 2). Overall, men residing in Mexico had lower, but non-statistically significant, normalized oral anti-HPV-16 and anti-HPV-18 antibodies at month 7. By month 18, the USA cohort had significantly higher oral anti-HPV-16 antibodies than the Mexico cohort. In regression analyses, strong correlations were observed between oral and serum anti-HPV-16 antibodies at months 7, 18, 30, and across all the months combined (R^2 = 0.90, 0.90, 0.83, and 0.93, respectively) (Supplementary Fig. 1). Similar oral-serum correlations were observed for anti-HPV-18 antibodies (R^2 = 0.89 at month 7, 0.94 at month 18, 0.80 at month 30, and 0.91 across all months tested) (Supplementary Fig. 2). As time progressed after vaccination, the number of participants with detectable oral anti-HPV-18 antibodies was reduced compared to anti-HPV-16 antibodies.

4. Discussion

Gardasil has demonstrated robust efficacy against HPV infection and related disease at the external genital skin and anal canal of men, and the cervix, vulva, and vagina among women [3,5,17]. Trials to determine the efficacy of Gardasil against oral HPV infections and associated diseases have not been conducted. In a Phase III Trial among young men, and a Phase II trial of mid-adult men, Gardasil was shown to induce strong peak serum antibody responses one month after completion of a 3-dose regimen [7]. In a post-hoc analysis of females vaccinated with Cervarix, the vaccine was shown to have efficacy against oral HPV infections [18]. Recently, we published that the quadrivalent HPV vaccine induces HPV-specific antibodies in the oral cavity at 7 months after the first vaccine dose. However, the longevity and the concentration of antibodies at later follow-up times was unknown, as well as their correlation with systemic antibody levels over longer periods of time. Findings from this study indicate that anti-HPV-16 and anti-HPV-18 antibody levels at 18 and 30 months following the start of vaccination are reduced compared to the levels observed with peak responses at month 7 (1 month following administration of the three doses of vaccine). In serum, nearly 100% of males seroconverted, and the majority of individuals had detectable levels of anti-HPV-16 and anti-HPV-18 antibodies in serum up to month 30 (98.1% and 79.6%). In contrast, over the same time period most individuals lost detectable anti-HPV-16 and anti-HPV-18 antibodies in oral gargles (29.6% and 4.6% positive for anti-HPV-16 and anti-HPV-18 antibodies, respectively). It remains unknown

Table 2
IgG normalized-HPV-16 and HPV-18 antibody levels post vaccination in oral fluids and serum.

HPV type	Specimen Type	Country	Month 7 N[a]	Month 7 GMT[b] (95% CI)	Month 18[c] N[a]	Month 18[c] GMT[b] (95% CI)	Month 30[d] N[a]	Month 30[d] GMT[b] (95% CI)
16	Oral gargle	U.S.	62	192.17 (159.85, 231.01)	18	125.80 (84.73, 186.77)	16	88.42 (54.68, 142.99)
		Mexico	75	165.55 (139.34, 196.69)	23	56.03 (38.73, 81.06)	16	51.57 (34.48, 77.13)
		Total	137	177.11 (156.31, 200.67)	41	79.91 (59.89, 106.63)	32	67.53 (49.47, 92.18)
	Serum	U.S.	51	251.92 (195.41, 324.77)	55	51.25 (37.58, 69.89)	56	39.26 (28.32, 54.42)
		Mexico	75	272.68 (233.09, 319.01)	48	35.22 (26.22, 47.29)	50	25.34 (19.05, 33.69)
		Total	126	264.08 (230.29, 302.83)	103	43.03 (34.71, 53.34)	106	31.93 (25.64, 39.76)
18	Oral gargle	U.S.	45	83.91 (68.06, 103.46)	4	34.85 (11.91, 102.00)	0	–
		Mexico	61	66.93 (55.94, 80.07)	7	29.59 (11.26, 77.74)	5	24.48 (10.99, 54.54)
		Total	106	73.67 (64.32, 84.37)	11	31.40 (17.23, 57.23)	5	24.48 (10.99, 54.54)
	Serum	U.S.	51	73.18 (55.72, 96.12)	50	12.19 (9.30, 15.98)	44	12.0 (9.1, 15.82)
		Mexico	75	78.31 (64.97, 94.41)	44	9.95 (7.49, 13.23)	42	8.13 (6.21, 10.64)
		Total	126	76.19 (65.29, 88.92)	94	11.09 (9.13, 13.46)	86	9.92 (8.17, 12.04)

[a] N indicates number of samples with detectable antibody levels.
[b] GMT represents the geometric mean concentrations amongst positive values (above cutoff), and GMT values are normalized to total IgG levels in the corresponding sample type.
[c] Month 18 represents 12 months following the last dose of HPV vaccine.
[d] Month 30 represents 24 months following the last dose of HPV vaccine.

Table 3
IgG normalized-HPV-16 and HPV-18 antibody levels in oral gargles and serum by country at Month 18 and Month 30 restricted to samples with detectable levels of antibodies in both serum and oral gargle.

HPV type	Specimen Type	Country	Month 7 N[a]	Month 7 GMT[b] (95% CI)	Month 18[c] N[a]	Month 18[c] GMT[b] (95% CI)	Month 30[d] N[a]	Month 30[d] GMT[b] (95% CI)
16	Oral gargle	U.S.	42	221.46 (181.04, 270.91)	18	125.80 (84.73, 186.77)	16	88.42 (54.68, 142.99)
		Mexico	75	165.55 (139.34, 196.69)	23	56.03 (38.73, 81.06)	16	51.57 (34.48, 77.13)
		Total	117	183.78 (160.94, 209.86)	41	79.91 (59.89, 106.63)	32	67.53 (49.47, 92.18)
	Serum	U.S.	42	341.76 (281.89, 414.34)	18	150.76 (104.90, 216.66)	16	123.71 (74.56, 205.25)
		Mexico	75	272.68 (233.09, 319.01)	23	67.25 (48.48, 93.27)	16	63.72 (42.06, 96.52)
		Total	117	295.71 (261.76, 334.06)	41	95.85 (73.53, 124.95)	32	88.78 (63.74, 123.65)
18	Oral gargle	U.S.	29	91.79 (74.38, 113.28)	4	34.85 (11.91, 102.00)	0	–
		Mexico	61	66.93 (55.94, 80.07)	7	29.59 (11.26, 77.74)	5	24.48 (10.99, 54.54)
		Total	90	74.10 (64.40, 85.25)	11	31.40 (17.23, 57.23)	5	24.48 (10.99, 54.54)
	Serum	U.S.	29	130.63 (104.44, 163.38)	4	37.83 (12.05, 118.75)	0	–
		Mexico	61	93.37 (77.99, 111.78)	7	32.73 (14.87, 72.04)	5	33.90 (11.65, 98.67)
		Total	90	104.04 (90.17, 120.04)	11	34.50 (20.53, 57.97)	5	33.90 (11.65, 98.67)

[a] N indicates number of samples with detectable antibody levels.
[b] GMT represent the geometric mean concentrations amongst positive values (above cutoff), and GMT values are normalized to total IgG levels in the corresponding sample type.
[c] Month 18 represents 12 months following the last dose of HPV vaccine.
[d] Month 30 represents 24 months following the last dose of HPV vaccine.

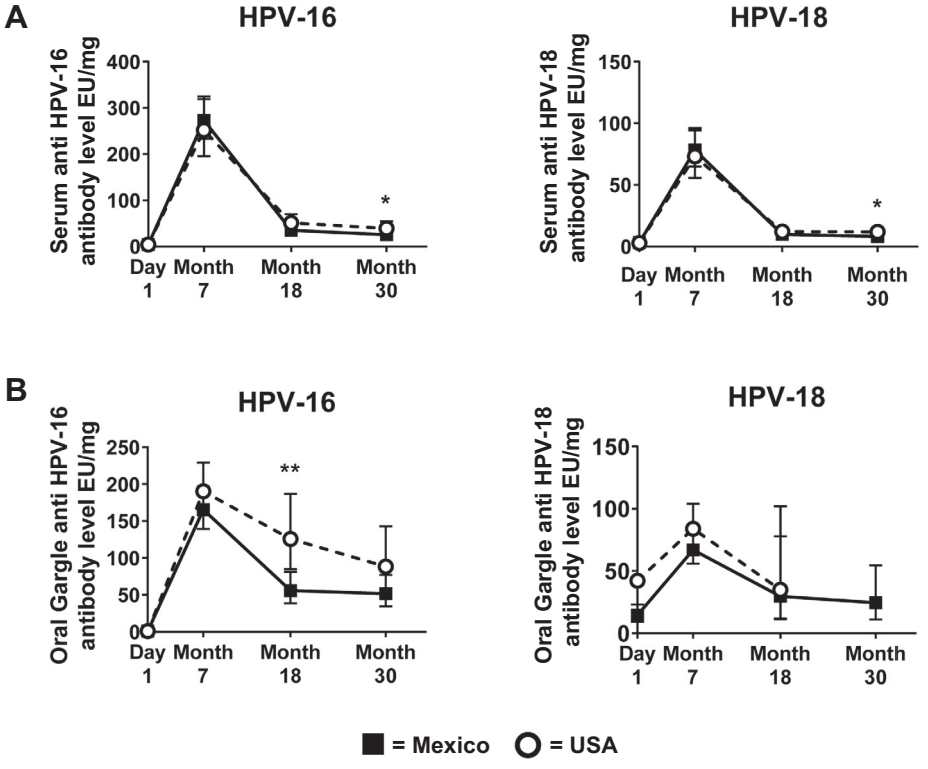

Fig. 2. Kinetics of HPV-16 and HPV-18 antibody levels in serum and oral gargles post vaccination. (A) Serum and (B) oral gargles from participants that received three doses of Gardasil were tested for anti-HPV-16 and HPV-18 antibodies and total immunoglobulin (IgG) levels. Antibody levels were normalized to total IgG levels in the respective samples. The values are represented as geometric mean concentrations amongst samples with detectable levels of antibodies. Error bars display confidence interval (95% CI). Comparisons between each country were conducted at each time point via Mann-Whitney test. * = P = 0.03, ** = P = 0.0008.

whether the lack of detectability with current antibody assays reflects a true lack of antibody presence or insensitivity of current assays to detect low levels of antibody that may be present. Development of more sensitive antibody assays are needed to address this question.

Despite the reduced levels of anti-HPV-16 and anti-HPV-18 antibodies, the correlation between serum and oral anti-HPV-16 and anti-HPV-18 antibody levels was evident in those with detectable oral anti-HPV levels. Overall, oral HPV-16 and HPV-18 antibodies were 1000-3000-fold lower in oral gargles compared to serum samples at months 18 and 30. This finding can be explained by the fact that the majority of HPV-specific antibodies detected in the oral mucosa likely transudate from the peripheral blood, as described in the context of other vaccines, and only a small percentage of HPV-specific antibodies are detectable in the oral mucosa [19,20]. However, when antibody levels were normalized by total IgG amounts and restricted to specimens that had antibodies detected both in oral gargles and in serum, the antibody levels in oral gargles were approximately 1.4-fold higher than serum samples. The levels of oral IgG detected in our study were similar to what have been previously reported [11,21,22]. Due to the variability in oral collection volumes, total IgG in serum and oral

gargles was used as a normalization factor to better understand the relative abundance of HPV antibodies in the oral cavity as compared to serum. The fact that normalized levels of HPV antibodies in serum and oral gargles are relatively similar suggests that oral antibody levels reflect serum levels, and the HPV-specific antibodies detected in the oral cavity are likely to be transudated from the peripheral blood.

Data presented in this study demonstrate that both HPV-16 and HPV-18 antibodies are present at the oral mucosa in most individuals at the time of peak immune response to vaccine (Month 7), and the levels decrease considerably over time, with levels in serum reaching a plateau phase as has been previously described in other vaccine trials [8]. The observed loss in oral antibody detectability may be related to assay sensitivity issues, as even in serum, only 80% of individuals had detectable responses to vaccination. Prior Gardasil trials among women found a loss of measurable anti-HPV-18 antibody years following vaccination; however, there was no breakthrough disease observed from 12 years of follow up [23,24]. This observation suggests that the currently used antibody assays may not have sufficient sensitivity to detect the low levels of antibody circulating long-term in serum or at the oral cavity. Further work is warranted to develop assays with increased sensitivity to be able to detect low antibody levels, such as those likely present at the oral cavity and after years post-vaccination in sera to inform antibody protection against disease long term. In addition, further research is needed to identify new potential circulating and local surrogate markers of protection against infection and disease.

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6. Potential Conflicts of interest

ARG reports receiving grants from Merck to her institution during the conduct of the study and other grants from Merck outside the submitted work. All other authors reported no potential conflicts.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.vaccine.2019.03.064.

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