Persistence of vaccine-induced antibody to A/H5N1 after 30 and 36 months of vaccination

Chien Vien Chinh^{1,2}, Viet Phu Quoc³, Loc Huynh Tan⁴, Duoc Nguyen Van¹, Thai Pham Quang^{5,6}, Be Le Van¹

¹ *Institute of Vaccines and Medical Biologicals (IVAC)*

² *TAY NGUYEN Institute of Hygiene and Epidemiology,*

³ *Khanh Hoa Medical Service*

⁴ *Ninh Hoa District Medical Center*

⁵ *National Institute of Hygiene and Epidemiology*

⁶ *Institute of Preventive medicine and Public health, Hanoi medical University.*

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Corresponding author:

Vien Chinh Chien, MD., PhD.
Institute of Vaccines and Medical Biologicals (IVAC),
Address: 9 Pasteur - Nha Trang, Khanh Hoa province - VIET NAM
Email: chienvc66@yahoo.com
ORCID: <https://orcid.org/0000-0002-5626-7762>

INTRODUCTION

H5N1 is a type of influenza virus that causes a highly infectious, severe respiratory disease in birds called avian influenza. The H5N1 bird flu incident in Hong Kong in 1997 was the first known instance of a purely avian virus causing severe human disease and death. Then there was widespread re-emergence in 2003 in Asia and later in Africa, Europe, and the Middle East. Influenza A virus subtype H5N1 (A/H5N1) causes illness in humans and many other animal species. Vietnam is one of six countries marking as an A/H5N1 hotspot at that time. From 2003 to 2009, WHO recorded 861 humans infected with H5N1 and 455 deaths, in which 50,4% of these deaths occurred in Vietnam [1].

Vaccines remain one of our best defense weapons against the influenza virus. In 2006, WHO and the wider international public health community launched the Global Action Plan for Influenza Vaccines to help ramp up the production capacity of vaccination in 14 countries, including Vietnam [2]. In 2012, the Institute of Vaccines and Medical Biologicals (IVAC) successfully produced the influenza vaccine named IVACFLU-A/ H5N1: a whole virion vaccine produced by technology chicken embryo utilization of egg micronutrients, inactivated by formalin, adsorbed aluminum-hydroxide ($Al(OH)_3$) and had no preservatives. Evidence from 1005 Vietnamese voluntary injected people showed that this vaccine produced a high seroconversion rate (75.8%) equivalent to the high level of antibodies after vaccination [3].

Antibody persistence is widely defined as the maintenance of seropositivity and is vital for sustained protection [4]. The persistence of vaccine antibodies has decreased over time and depends on several factors, such as age, sex, vaccines' components, i.e., adjuvants [5]. In our knowledge, there is limited evidence on the relationship between factors related to antibody persistence rate over time. IVACFLU-A/ H5N1 was documented as effective in Vietnam; however, antibody persistence after vaccination has not been well characterized yet, due to the recent licensure of the vaccine. A study in the United States showed that the antibody rate was

76.7% after six months of vaccinated ASO_{3A}-adjuvanted H5N1 [6]; this figure reduced to 30.9% and 8.3% after two years in a vaccine with and without adjuvants, respectively [5]. Another evidence suggested that antibody rates persisted more than two years after H5N1 vaccination [5].

We examined the persistence of antibodies after vaccination and related risk factors in individuals enrolled in phase II with 15 mcg dose, two injections 21 days apart, IVACFLU-A/H5N1 vaccine trials in Ninh Hoa, Vietnam. Our study is one of the first to explore long-term antibody persistence, and the associated characteristics associated with IVACFLU-A/ H5N1 vaccinated individuals.

MATERIAL AND METHODS

Participant Selection and Enrollment

We conducted a longitudinal study without control groups in Ninh Hoa commune, Khanh Hoa province, Vietnam. The phase II clinical trials of a recombinant IVACFLU-A/H5N1 vaccine in 2016 included 194 participants from Ninh Hoa commune, Khanh Hoa province, Vietnam receiving an A/H5N1 vaccine - experimental IVACFLU-A/H5N1 in which 95 people received two doses of 15 mcg and 99 ones received two doses of 30 mcg. At the end of phase II, the dose of 15 mcg was selected for the commerce vaccine. We invited all 95 participants from phase II with a dose of 15 mcg to join this study; 91.6% (87/95) agreed, given the final sample was 86. To be eligible for inclusion into this follow-up, the participants had to be measured anti-A/H5N1 IgG seronegative at baseline. After that, they received all two doses of the vaccine. Blood was drawn at 30- and 36-months after the complete vaccination to assess antibody status. The vaccine is produced from the NIBRG-14 vaccine strain, NIBRG-14 is derived from the recombinant A/Vietnam /1194/2004 strain recombinant with A/PR/8/34 influenza, taking the HA and NA of A/VN/1194/2004 genes which has been removed the toxic components to reduce

virulence. The gene of influenza A/PR/8/34 comes from the National Institute for Biological Standards and Control (NIBSC) of the Health Protection Agency, England.

In 2018 and 2019, at 30-month after the complete vaccination, a 3-mL venous blood draw was taken from each participant, and a designed questionnaire assessing potential A/H5N1 exposures was administered by trained study personnel. After being drawn, the blood was immediately centrifuged, and the resulted serum was separated and frozen at -20°C . All participants received the informed written consent before all follow-up visits. The Ethics Committee of the Khanh Hoa Department of Health approved all procedures for the ongoing follow-up after the vaccine trial.

(Figure 1)

A/H5N1 Virus Antibody Testing

After completing the follow-up appointments, the serum samples were shipped to the Institute of Vaccines and Biological Medical in Nha Trang, Vietnam, where they were tested for anti-A/H5N1 IgG antibodies using the hemagglutination inhibition (HI) assay. This assay titrated the antibody response to a viral infection which is currently the only accepted assay of predicting protection against influenza virus [8]. In addition, this assay takes advantage of some viruses' ability to bind red blood cells. The HI assay was carried out in 96 plates which contain twofold dilution of the sera. Researchers added a known titer of the virus in these plates and incubated them for 30 minutes at 68 to 77°F (20 to 25°C). After that, they added more red blood cells and left them for 30 minutes at the same temperature. The antibodies will bind and prevent the virus from hemagglutinating the red blood cells if they appear in the sera sample cross-reacting with the virus. In this way, we determine the exact titer of the antibodies in the sera [9].

HI antibody parameters included geometric mean titers (GMT) and seroconversion rates (SCR), which is defined as the percentage of subjects achieving an increase in titer from $<1:10$ before

to $\geq 1:40$ after receiving the vaccine, or at least a 4-fold increase in titer from a pre-vaccination titer $\geq 1:10$.

Statistical Methods

We used Epi Info Version 6 for statistical analyses. After completing the anti-A/H5N1 test, we assessed whether each individual persisted 1/10 level of antibodies at 30-month and 36-month after complete vaccination compared to their baseline. Antibody persistence status (persisted 1/10 level of antibodies at 30-month and 36-month) is compared by demographics and exposure risk factors using a univariate logistic regression. One month after receipt of the second vaccine dose was used as the time of seroconversion. We calculated the Geometric mean titer (the average antibody titer) by multiplying all values and taking the 86th root of this number (86 is the number of participants in our study).

Ethical Issue

Khanh Hoa Department of Health's IRB approved this study with accreditation number: 03/HDDD- SYT on 30/8/2018.

RESULTS

In this study, we found that the proportion of 84.9% and 52.3% of participants have persistence at least 1/10 level of A/H5N1 antibodies after taking complete vaccination 30 months and 36 months, respectively. More than half (57%) of participants had the seroconversion at 30-month four times higher than baseline. Meanwhile, this figure at 36-month was 30.2%. The geometric mean titer (the average antibody titer) at 30-month and 36-month were four times and two times higher than baseline, respectively (Table 1).

(Table 1)

Table 2 presents demographics, personal behaviors, medical history, and exposure risk factors of persisting at least 1/10 level of A/H5N1 antibodies at 30-month and 36- months among participants. The proportions of males persisting at least 1/10 level of antibodies after 30 and

36 months after taking full IVACFLU-A/H5N1 vaccination were 73.9% and 43.5%, while these figures in women were 88.9% and 55.6%, respectively. In our study, sex was not associated with persisting at least 1/10 level of antibodies at both 30- and 36-month after vaccination. Older people (41-60 years) had higher odds of persisting at least 1/10 level of A/H5N1 antibodies than younger ones (18-40 years) ($p = 0.02$) (given that age of participants is the age at vaccination day in 2016). In contrast, people who experienced flu-like symptoms in the past 18 months or between 2 visits had lower odds of persisting at least 1/10 level of A/H5N1 antibodies than those who did not ($p = 0.02$).

Our study did not find the association between persisting at least 1/10 level of antibodies and personal behaviors (alcohols use and smoking) and exposure risk factors (having poultry farm, directly contacting poultry, and directly slaughtering and processing poultry).

(Table 2)

DISCUSSION

The overall proportion of persisting at least 1/10 level of A/H5N1 antibodies was 84.9% and 52.3% after 30 months and 36 months of IVACFLU-A/H5N1 vaccination. The geometric mean titer of participants at 30-month and 36-month post-vaccination was 22.3 and 12.0, respectively. Antibody persistence was greater in older people (OR, 95%CI: 5.65; 1.06 - 39.87, $p = 0.02$). However, this estimate of antibody persistence after vaccination was lower in people who experienced flu-like symptoms in the past 18 months or between 2 visits (OR, 95%CI: 0.09; 0.01 - 0.81, $p = 0.02$).

We found most participants (84.9%) persisted at least 1/10 level of A/H5N1 antibodies, and the proportion of those who had SCR after 30 months of vaccination was 57%. After 36 months of vaccination, the former figures decreased to 52.3% and the latter declined to 30.2%. Subjects with a titer of $\geq 1:10$ can be considered seropositive. However, opinions on the protective titers for the influenza vaccine have been widely diverse. Classic research, supported by more recent

studies, has established a correlation between antibody titer evaluated by the HI assay and protection against influenza virus infection, with an HI titer of 1:40 equivalent to 50% protection against infection [10-12]. Lower titers, such as 1:32 or 1:20, have previously been used for HI and neutralizing antibodies to characterize the immune responses to influenza vaccines [13-15]. Meanwhile, other studies argued that there was insufficient evidence to deduce 50% protection against influenza virus infection from an HI titer of 1:40 [16, 17]. Therefore, further study on IVACFLU-A/H5N1 should be conducted to assert a time point for booster vaccination. Nevertheless, there are noteworthy that these results were obtained at 30- and 36-month post-vaccination without booster shots, indicating relatively high persistence of antibodies [18].

On the other hand, our findings on geometric mean titer (the average antibody titer) persisting over time were remarkable compared to previous studies on other H5N1 vaccines. Comparing to a study by Paul Gillard et al. on ASO₃A -adjuvanted heterologous H5N1 vaccines, the geometric mean titer at 36-month post-vaccination was comparable (12.0 versus 10.5). At the same time, our figure for 30 months was notably higher despite longer follow-up time (30 months versus 12, 18, 24 and 30 months) [18]. In another study on ASO₃A-adjuvanted A/H5N1 vaccine, the geometric mean titer for 12 months following vaccination was reported to be 11.8, which is considerably lower than our results in both 30- and 36- month [19]. Aside from the physiological conditions of the study subjects, the differences in the results of immunological indicators between studies might be attributable to the variation in vaccine composition, particularly adjuvants. Adjuvant has been proved to play an important role in enhancing immunogenicity through either physical or chemical association with antigens [20]. Although there has been evidence demonstrating the safety and effective of ASO₃ in boosting the immune responses, aluminum-based adjuvants have remained the “gold standard” for adjuvants due to their long-term success [21]. The results observed in this study suggest future research

comparing the performance of ASO₃ and aluminum-based adjuvants on the persistence of immune responses elicited by the A/H5N1 vaccine.

Regarding factors related to the number of antibodies persisting, the association between older age (OR=5.65), the experience of flu-like symptoms in the past 18 months or between 2 visits (OR=0.09) and the persistence of at least 1/10 level of A/H5N1 antibodies was found to be statistically significant. However, notwithstanding previous findings on the persistence of influenza antibodies in the elderly [22, 23], there has not yet been any specific evidence or explanation for the age group examined in this study (18 – 40 and 41 – 65 years old). Meanwhile, the correlation and mechanism of lowered antibody persistence in people with flu-like symptoms need more investigation.

Clinical recommendations

IVACFLU - A / H5N1 vaccine, after two injections of 15 µg, still has antibody residues for up to 30 months (84.9%) and after 36 months (52.3%), so the repeated dose should proceed after three years or longer. IVACFLU-A / H5N1 does not affect basic DTH traits except it may reduce the risk of infection with common influenza within 18 months.

Limitations and Strengths

The research has been implemented on small sample size, so there are still many inconclusive points. Therefore, longer monitoring of antibody retention time is required and a study with a larger sample size on the epidemiological characteristics after IVACFLU- A/H5N1 vaccination.

CONCLUSIONS

This study highlights the antibody persistence, indicated by seroconversion rate and geometric mean titer at 30- and 36-month post-vaccination, of the IVACFLU-A/H5N1 vaccine. Antibody persistence was more significant in participants aged 41–65 but lower in people who experienced flu-like symptoms in the past 18 months or between 2 visits. We recorded no

differences between A/H5N1 antibodies persistence and the exposure of risk factors such as poultry farm working, poultry contact, slaughtering, or poultry processing.

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Author Contributions

Conceptualization: CVC, BLV. Data curation: VPQ. Formal analysis: CVC, TPQ, LHT, DNV. Funding acquisition: BLV. Methodology: CVC, BLV, TPQ. Project administration: VPQ, LHT. Visualization: DNV, PQT. Writing – original draft: CVC, DNV, TPQ, BLV. Writing – review & editing: CVC, VPQ, LHT, DNV, TPQ, BLV.

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Table 1. Incidence of persisting at least 1/10 amount of A/H5N1 antibodies; increasing seroconversion four times compared to baseline among participants

Time	Persisting at least 1/10 amount of antibodies n(%)	Increasing seroconversion four times n(%)	Geometric mean titer [*]	Geometric mean titer ratio [*]
Baseline [†]	11 (12.8)	--	5.6	1.0
21 days after full vaccination (42 days after 1 st dose)	86 (100.0)	81 (92.4)	66.8	11.8
30 months [⊙]	73 (84.9)	49 (57)	22.3	4.0
36 months [⊙]	45 (52.3)	26 (30.2)	12.0	2.1

^{*} immunology indicators

[†] before taking IVACFLU-A/H5N1 vaccine

[⊙] 30 and 36 months after taking two IVACFLU-A/H5N1 vaccines – full vaccination

Table 2. Demographics, personal behaviours, medical history, and exposure risk factors for persisting at least 1/10 amount of A/H5N1 antibodies at 30 months and 36 months

Characteristics	Total		30 months		36 months		
	n(%)	n(%)	OR (95%CI)*	p-value	n(%)	OR (95%CI)*	p-value
DEMOGRAPHICS							
Sex							
Male	23 (26.7)	17 (73.9)	0.35 (0.09 - 1.40)	0.09	10 (43.5)	0.62 (0.21 - 1.78)	0.32
Female	63 (73.3)	56 (88.9)	1		35 (55.6)	1	
Age groups							
18 – 40	39 (45.3)	37 (94.9)	5.65 (1.06 – 39.87)	0.02	24 (61.5)	1.98 (0.77 - 5.17)	0.12
41 – 65	47 (54.7)	36 (76.6)	1		21 (44.7)	1	
PERSONAL BEHAVIOURS							
Alcohols use							
Yes	17 (19.8)	14 (82.4)	0.79 (0.17 - 4.19)	0.74	10 (58.8)	1.39 (0.42 - 4.64)	0.55
No	69 (80.2)	59 (85.5)	1		35 (50.7)	1	
Smoking							
Yes	14 (16.3)	11 (78.6)	0.59 (0.12 - 3.22)	0.75	6 (42.9)	0.63 (0.17 - 2.29)	0.44
No	72 (83.7)	62 (86.1)	1		39 (54.2)	1	
MEDICAL HISTORY							
Having flu-like symptoms in the past 18 months or between 2 visits							
Yes	33 (38.4)	29 (87.9)	1.19 (0.32 - 4.59)	0.78	1 (11.1)	0.09 (0.01 - 0.81)	0.02
No	53 (61.6)	44 (83.0)	1		44 (57.1)	1	
Having other diseases in the past 18 months or between 2 visits							
Yes	7 (8.1)	7 (100.0)	--	0.53	0 (0.0)	--	0.43
No	79 (91.9)	66 (83.5)	--		45 (53.6)	--	
EXPOSURE RISK FACTORS							
Having a poultry farm							
Yes	53 (61.6)	44 (83.0)	0.7 (0.16 – 2.72)	0.76	30 (56.6)	1.57 (0.6 - 4.12)	0.31
No	33 (38.4)	29 (87.9)	1		15 (45.5)	1	
Directly contacting with poultry							
Yes	49 (57)	41 (83.7)	0.8 (0.20 – 3.06)	0.72	28 (57.1)	1.57 (0.61 - 4.06)	0.30
No	37 (43)	32 (86.5)	1		17 (45.9)	1	
Directly slaughtering and processing poultry							
Yes	5 (5.8)	5 (100.0)	--	0.33	1 (20.0)	0.21 (0.01 - 2.15)	0.30
No	81 (94.2)	68 (84.0)	--		44 (54.3)	1	

* Univariate Logistic Regression Models

Figure 1. Participant flow.