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EVALUATION OF THE IMMUNOGENICITY AND SAFETY OF IVACFLU-A / H5N1 VACCINE IN HEALTHY ADULT VIETNAMESE

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INTRODUCTION

Influenza is one of the infectious diseases with the potential to pose a great threat to people, it is not only the adverse health effects of annual influenza outbreaks, but also the enormous global consequences of these outbreaks or pandemic influenza. During pandemic influenza A / H1N1 (2009) and the spread of influenza A / H5N1 virus among avian influenza transmitted to human populations, this shows the unpredictability of influenza viruses.

Although influenza A / H1N1 (2009) pandemic has subsided and the pandemic virus is considered as seasonal influenza virus, but the threat of a influenza pandemic which cause by avian influenza A / The H5N1, might be still latent and break out suddenly at certain time. Since 1997, the avian influenza A / H5N1 virus has a high pathogenicity that has caused widespread outbreaks in poultry with a very high mortality rate, while it also caused sporadic, severe and fatal illness for people. Southeast Asian countries including Vietnam, have been affected by the influenza A / H5N1. According to WHO report from 2003 to October 2020, there were 861 confirmed cases of influenza A / H5N1, of which 455 died. Southeast Asian countries account for 42% of influenza A / H5N1 virus infections reported since 2003 and A / H5N1 influenza infection in animals is still considered localized in the region curently. As of October 2020, Vietnam had 127 human cases of A / H5N1 influenza, of which over 50% died (64/127) . Therefore, the risk of AH5N1 influenza virus transmission from poultry to humans is still present.

Therefore, research and production of domestic vaccines for prevention of influenza to actively and promptly in the fight against the pandemic, the dependence from the vaccine by foreign suppliers, the appropriate cost of vaccines, the help for increasing in the number of people to have access to influenza vaccine, it is important task for proactively prevent epidemics and minimizing the spread in the community. From that actual, the Ministry of Health has assigned the

Institute of Vaccines and Medical Biologicals (IVAC) to research and produce A / H5N1 influenza vaccine, to hold the initiative source vaccines for domestic demand. IVAC based on the technical and financial support from WHO to build an influenza vaccine production line according to GMP-WHO standards with a potential capacity of up to 3 million doses per year by embryonic technology, that technology currently provides about 80% of the influenza vaccine for using in the world. In order for register of marketing authorisation of a new vaccine product, it is necessary to get data on clinical trials to confirm the safety and immunogenicity of that product. Therefore, a question arises as to whether the vaccine against influenza A / H5N1, commercially known as IVACFLU-A / H5N1, meets the standards of safety and immunogenicity? We conducted the research topic: "***Evaluation of the immunogenicity and safety of IVACFLU-A / H5N1 vaccine in healthy adult vietnamese***". This research consists two following objectives:

1. Evaluation of the immunogenicity of IVACFLU-A / H5N1 vaccine produced by the Institute of Vaccines and Medical Biologicals at a dose of 15mcg.

2. Evaluation of the safety of IVACFLU-A / H5N1 vaccine produced by the Institute of Vaccines and Medical Biologicals at a dose of 15mcg.

The new scientific and practical values contributions of the research:

- The research has topical, practical and urgent significance on the safety and immunogenicity of the avian influenza vaccine named IVACFLU-A / H5N1 produced by Vietnam to hold the initiative in source vaccines for human diseases.

- Currently, vaccine production in Vietnam has been quite proactive and promptly self-produced a number of vaccines to prevent diseases, especially in the Expanded Programme on Immunization, including domestic influenza vaccine, with independence on vaccines supplied by foreign countries. The success of domestic influenza vaccine production has helped for reducing the cost of influenza

vaccine, for increasing in the number of people to be able to access the influenza vaccine, it is contributing to proactively preventing epidemics and limiting disease spread in community.

- After the research results are completed and effectively, Vietnam is going to have a new vaccine for widely applying in the community to prevent influenza A / H5N1 pandemic from poultry to humans.

The structure of thesis:

This thesis consists of 122 pages (excluding references, appendices), include: Introduction (02 pages), Chapter 1: overview (24 pages), Chapter 2: objects and methodology (22 pages), Chapter 3: results (45 pages), Chapter 4: discussion (25 pages), Conclusions (2 pages), Recommendations (1 pages). The thesis includes 30 tables, 11 charts, 03 diagrams, 106 references (including 14 in Vietnamese) and appendices.

Chapter 1
OVERVIEW

1.1. Situation of avian influenza in the world and Vietnam:

Influenza disease: This is a contagious respiratory disease; In humans, it is caused by influenza A virus and influenza B virus (influenza C and D viruses have also been reported). The symptoms associated with influenza virus infection range from a mild respiratory illness (limited to the upper respiratory tract and characterized by fever, sore throat, runny nose, cough, headache, muscle aches, and fatigue) to severe, or fatal pneumonia caused by the influenza virus or leads to secondary infection of the lower respiratory tract in some cases.

Avian influenza: humans can be infected with avian influenza virus, swine influenza and other influenza viruses such as avian influenza A / H5N1, A / H7N9 and swine influenza A / H3N2 ..., these are the common influenza viruses which is spread in animals but can also be transmitted to humans through direct contact with infected animals or contaminated environments.

1.1.1. Influenza A/H5N1 virus

1.1.1.1. The gene of avian H5N1 influenza virus

Influenza virus belongs to the family *Orthomyxoviridae*. There are four types of influenza viruses, such as A, B, C and D. Avian influenza viruses are all classified as influenza A virus. Influenza viruses are classified based on the antigenic properties of their two surface glycoproteins, hemagglutinin (HA) and neuraminidase (NA).

1.1.1.2. Virus resistance

Physical factors such as temperature are believed to be responsible for the decreased activity of the virus, which affects its replication. Some previous reports showed that the A / H5N1 virus could persist for more than 100 days at 4°C but inactivation after 24 hours at 28°C and after 30 minutes at 56°C. Virus was fully inactivated within 30 minutes after exposure to direct sunlight at ambient temperatures of 32 to 35°C but infectivity was retained after 4 days in the shade at 25 to 32°C. Virus was also inactivated after 3 minutes after

exposure at 70°C. The transmission at 4°C can still be detected after 35 days but there are only 2 days in the incubation period at 25°C. H5N1 virus loses its ability to infect at pH below 5 but persists even after 18 hours at pH 5. Thus, H5N1 virus can be inactivated by using high temperatures (eg. 56°C and above), low pH (1-3) or high pH (11-13) of the material which need to be disinfected.

1.1.1.3. Ability to cause disease

Influenza A / H5N1 virus spreads continuously among birds and infrequently among humans, including fatal cases. Although person-to-person transmission between family members has been reported in some cases, but the person-to-person transmission has not been officially confirmed. In order for influenza A / H5N1 virus causes a pandemic, it would have to undergo molecular changes that allow for efficient and sustained transmission in human host. Presently, this species barrier protects human from widespread infection in the community; however, if this barrier is to be broken, a pandemic will occurrence.

1.1.2. Situation of influenza A / H5N1 in humans

1.1.2.1. Epidemiological characteristics

Disease situation in the world:

Disease situation in Vietnam: Since appearance in late 2003 to the end of 2014, there were 127 confirmed cases of influenza A / H5N1 in Vietnam, of which 64 cases died (the rate of death / morbidity was 50.4 %), from 2015 up to now, there were no cases of the disease.

1.1.2.2. Mode of transmission

The migratory birds are one of the sources spreading influenza A / H5N1. Infected birds release influenza A / H5N1 virus in saliva, nasopharynx and feces

The strain of the avian influenza virus can infect many different animals such as birds, pigs, horses, seals, whales, tigers and humans. Avian influenza virus can spread rapidly from one farm to another through mechanical mechanisms by means of transport, clothing, footwear, ... Viruses are found in secretions such as nasopharyngeal

fluid, infected poultry feces, dust and soil. Direct contact with infected birds or utensils contaminated with feces and poultry waste is the main route of transmission. Viruses can be transmitted through the air (droplets of respiratory secretions of infected birds or inhaling the air containing dust from poultry feces and waste) or by eating or drinking (water or food contaminated virus, ...) and contact with contaminated tools and things. Human can be infected by direct contact with infected birds through farming, transporting, slaughtering, processing, eating undercooked or unhygienic infected poultry products.

1.1.1.3. Susceptibility and immunity

In theory, everyone is likely to be susceptible to influenza A / H5N1 virus. In fact, the likelihood of transmitting influenza A / H5N1 virus is very different. Many people are exposed to influenza A / H5N1 viruses, but only a very small number of people get sick. Currently, it is not known what factors increase susceptibility to the virus

Chapter 2
OBJECT AND METHODOLOGY

2.1. Study population, place and time

* Study population: All participants were healthy (based on medical history and physical examination) male and female adults, 18–60 years of age.

* Study time: from Mar. 2016 to Aug. 2019.

* Study location:

- Phase 2: The study was conducted in Ninh Da, Ninh Binh and Ninh Quang communes, Ninh Hoa district, Khanh Hoa province.

- Phase 3: two sites.

+ Cap Tien, Kien Thiet and Hung Thang communes in Tien Lang district, Hai Phong city.

+ Ninh Da, Ninh Binh and Ninh Quang communes, Ninh Hoa district, Khanh Hoa province.

2.2. Methodology

2.2.1. Study design

Phase 2 and 3 double blind, randomized, placebo-controlled clinical trials to assess the safety and immunogenicity of the inactivated influenza A / H5N1 vaccine (IVACFLU) -A / H5N1) produced by IVAC in healthy adults in Vietnam with 15 mcg vaccine dose.

2.2.2 *Sample size and Sampling methods*

Comment [D1]: Không thấy có

Sample size for Phase 2 was 200 participants (each group of 100 vaccine and placebo subjects), and for Phase 3 there were 630 participants (525 subjects for the vaccine group and 105 subjects for the placebo group).

2.3. Contents of the research

2.3.1. Indicators and variables

2.3.1.1 Indicators of immunogenicity

Comment [D2]: Nhiều cái có trong bài không?

- Proportion of subjects with HAI seroresponse of at least a four-fold increase in post-vaccination titer on Day 43.

- Proportion of study subjects with antibody neutralization titer increased at least 4 times on Day 43 as determined by the MN assay.
- Geometric Mean Titer of Day 43 as determined by the HAI and MN assay.
- Geometric Mean Titer Ratio of Day 43/Day 1 as determined by the HAI and MN assay. Single radial hemolyis
- Proportion of subjects with a Single radial hemolyis (SRH) area of ≥25 mm2 (seroprotection) after the second vaccination on Day 43
- Proportion of subjects with SRH area of ≥25 mm2 after immunization in case of negative baseline sample (≤4 mm2) or 50% increase in SRH area if baseline sample is >4 mm2 (seroconversion).
- Geometric Mean Area (GMA) of Day 43 as determined by the SRH test.
- Geometric Mean Area Ratio (GMAR) of Day 43/Day 1 as determined by the SRH test.

2.3.1.2 ***Indicators of Safety***

The safety of IVACFLU-A / H5N1 vaccine was evaluated based on the number and proportion of study subjects having adverse events (AE), which related or not related to research products according to following criteria:

- Immediate reactogenicity: Number and proportion of subjects with immediate solicited local and systemic reactions which occurred within 30 minutes of each injection of research product.

- 7-day reactogenicity: assessed during the 7-day post injection period (Days 1-7) Number and proportion of subjects with solicited local reactions and solicited systemic reactions during the 7-day post injection period (Days 1-7 and Days 22-28).

- Unsolicited Adverse Events: Number and proportion of subjects with unsolicited AEs for 21 days post each injection.

- All serious AEs (SAEs) occurring over the entire study period (through Day 1 and Day 91).

2.3.2. Data collection

2.3.2.1. Data collection tools

- Case report form (CRF) is a dossier that collects all the necessary information for each research subject and can be used by research statistics to synthesize and analyze data. All information should be collected by investigator / authorized person: Name, age, date of birth, health status, administration number, vaccination number, date (time) of vaccination, post-injection reactions (if any), immunological response results.

- Diary card: used to record daily reactions or events of health on the monitoring sheet for 7 days after each injection.

2.3.2.2 Data collection and verification

- After each screening, examination or vaccination, investigator must complete information in the required fields in CRF for each subject

- After the vaccine or placebo has been given, subject was invited to stay for health monitoring at least 30 minutes. During that time, investigator checks whether the participates have any reaction to the injection or not. Simultaneously guide to monitoring and recording the health status on diary cards. Participants were asked to record reactions or phenomena health on the monitoring sheet within 7 days after injection and to return diary cards at the next visit. Seven (7) days after injected research product, participates were invited back to the study site to examine and measure temperature, pulse, blood pressure and conduct a physical examination if they report symptoms, and investigators and participants together review the diary cards to understand and correct what participates recorded in it.

- Before, during and after entering data from CRF, diary cards, staffs of the Contract Research Organization (CRO) conduct review and management of research data each research subject to ensure that quality assurance of data at study site through review CRF, diary cards and check with original documents. For the data entered into the system, a computerized check of the validity of the data was performed

and applied to the database. Regarding the query report missing and misleading data was forwarded to the research coordinator (s) and the study supervisor (s) for resolution. Research database is updated according to resolved query report. In addition, manual reviews are also performed according to a predefined data management plan. All changes to the research database have been recorded.

2.3.2.3 Data management

- All information and data identifying research subjects are encoded with the number of research subjects. These codes are unique and fixed in the research, collection, processing, and analysis of data.

- Data is collected through CRFs, diary cards.

2.4. Statistical analysis

- Data were analyzed using SAS® software

- The data were presented in tabular format by frequency, percentage and 95% confidence interval.

- Chi-squared test, Fisher test were used for statistically significant differences between groups. P-value <0.05 was considered as statistically significant.

2.5. Ethical issue

- The research protocol approved by the Research Ethics Committee's decision No. 5514 / QD-BYT dated December 25, 2015 and agreed to use data from PATH, IVAC and National Institute of Hygiene and Epidemiology. The information is collected in the form of anonymity, ensuring confidentiality and for scientific purposes only.

- The thesis topic was conducted in accordance with approval by the Research Council of Hai Phong University of Medicine and Pharmacy.

- This study was conducted in accordance with the approved protocol as well as good clinical practice regulations of the Ministry of Health and International Conference on I Iamonization of Technical Requiments for Registration of Pharmaceuticals for Human use (ICH), in compliance with the World Medical Association's Declaration of Helsinki.

Chapter 3
RESEARCH RESULTS

3.1. Immunogenicity Results:

3.1.1 HAI Titer

3.1.1.1. Proportion of Subjects with HAI Titer ≥1:40 on Day 43

Table 3.2. Percentage of Subjects with Hemagglutination Inhibition (HAI) Titers >= 1:40 on Days 1 and 43

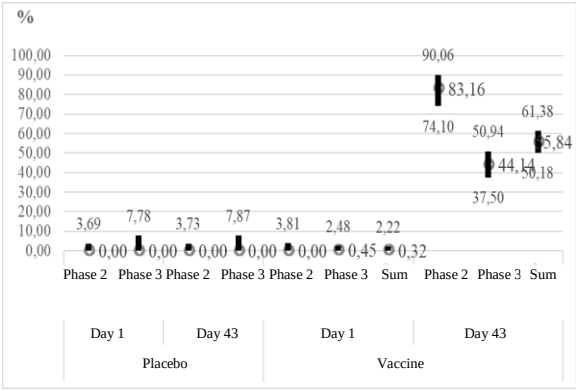
	Date 1				Date 43			
	N	n	%	(95% CI)	N	n	%	(95% CI)
Phase 2								
Placebo	98	0	0,00	(0,00 – 3,69)	97	0	0,00	(0,00 – 3,73)
Vaccine	95	0	0,00	(0,00 – 3,81)	95	79	83,16	(74,10 – 90,06)
Phase 3								
Placebo	45	0	0,00	(0,00 - 7,87)	45	0	0,00	(0,00 – 7,87)
Vaccine	222	1	0,45	(0,01- 2,48)	222	98	44,14	(37,50 – 50,94)
Phase 2/3								
Vaccine	317	1	0,32	(0,0 - 2,22)	317	177	55,84	(50,18 – 61,38)

- The Phase 2: primary immunogenicity endpoint was the proportion of subjects with a HAI titer ≥1:40 on Day 43 (21 days after Injection #2). Seroresponses were positive for 15 mcg vaccine group on Day 43 with 83.16% (95% CI: 74.10 to 90.06%) of subjects in the 15 mcg vaccine group achieving a HAI titer ≥1:40. No subject in the placebo group had a positive seroresponse on Day 43. No subject in the placebo group had a HAI titer ≥1:40 on Day 1.

- The Phase 3: primary immunogenicity endpoint was the proportion of subjects with a HAI titer ≥1:40 on Day 43 (21 days after Injection #2). Seroresponses were positive for the IVACFLU-A/H5N1 vaccine on Day 43 with 44.14% (95% CI: 37.50 to 50.94%) of subjects in the 15 mcg vaccine group achieving a HAI titer ≥1:40. No subject in the placebo group had a positive seroresponse on Day 43.

- Seroresponses were positive for the IVACFLU-A/H5N1 vaccine on Day 43 with 55.84% (95% CI: 50.18 to 61.38%) of subjects in the Phase 2/3 combined 15 mcg vaccine group achieving a HAI titer ≥1:40. One subject (<1.0%) in the 15 mcg vaccine group had a HAI titer ≥1:40 on Day 1.

3.1.1.2. Proportion of Subjects Exhibiting a Seroresponse with at Least a Four-fold Increase in Post-injection Titers



Biểu đồ 3.1. A seroresponse with at least a four-fold increase in post-injection HAI titers on Day 43 compared with baseline (Day 1)

The Phase 2: The proportions of subjects with seroresponse of at least a four-fold increase in HAI titers on Day 43 compared with baseline (Day 1) was achieved for 92.63% of subjects in the 15 mcg vaccine group

The Phase 3: A seroresponse with at least a four-fold increase in post-injection titers on Day 43 with respect to Day 1 was achieved for 67.57% of subjects in the 15 mcg vaccine group. No subject in the placebo group met this criterion.

A seroresponse with at least a four-fold increase in post-injection HAI titers on Day 43 compared with baseline (Day 1) was achieved for 75.08% (95% CI: 69.94 to 79.74) of subjects in the Phase 2/3 combined 15 mcg vaccine group

3.1.1.3. Geometric Mean Titer

Table 3.3. Geometric Mean of HAI Titers on Days 1 and 43

	Day 1			Day 43		
	n	GMT	(95% CI)	n	GMT	(95% CI)
Phase 2						
Placebo	98	5,48	(5,23 - 5,74)	97	5,63	(5,31 - 5,96)
Vaccine	95	5,62	(5,34 - 5,91)	95	62,65	(52,10 - 75,34)
Phase 3						
Placebo	45	5,04	(4,96 - 5,11)	45	5,08	(4,97 - 5,18)
Vaccine	222	5,20	(5,06 - 5,34)	222	27,61	(24,38 - 31,27)
Phase 2/3						
Vaccine	317	5,32	(5,19 – 5,45)	317	35,30	(31,60 – 39,43)

Phase 2: On Day 43, the HAI GMT for the 15 mcg vaccine group was 62.65 compared with 5.63 for the placebo group. The HAI GMTs on Day 43 for the placebo group were similar to baseline.

The Phase 3: On Day 43, the HAI GMT for the 15 mcg vaccine group was 27.61 compared with 5.08 for the placebo group.

The HAI GMT for the Phase 2/3 combined 15 mcg vaccine group on Day 43 was 35.30 (95% CI: 31.60 to 39.43)

3.1.1.4. Geometric Mean Titer Ratio (GMTR)

Table 3.4. Geometric Mean HAI Titers Fold Rise (GMTR) on Day 43 With Respect to Day 1

	Day 1			Day 43		
	n	GMTR	(95% CI)	N	GMTR	(95% CI)
Phase 2						
Placebo	98	1,01	(0,99 - 1,02)	97	1,03	(1,00 - 1,05)
Vaccine	95	5,64	(4,43 - 7,18)	95	11,25	(9,35 - 13,29)
Phase 3						
Placebo				45	1,01	(0,99 - 1,02)
Vaccine				222	5,31	(4,69 - 6,02)
Phase 2/3						
Vaccine				317	6,63	(5,96 – 7,39)

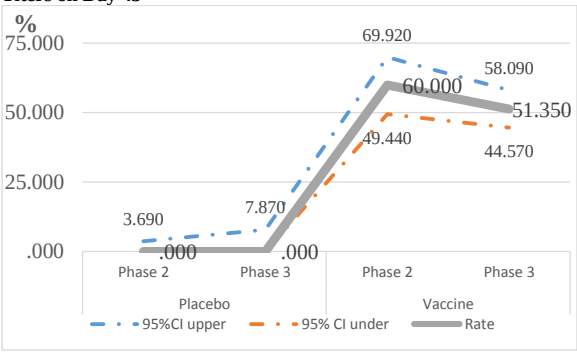
The HAI GMTR for the Phase 2 combined 15 mcg vaccine on Day 43 was 11.25 (95% CI: 9.35 to 12.39)

Phase 3: On Day 43, the HAI GMTR for the 15 mcg vaccine group was 5.31 (95% CI: 4.96 to 6.02) compared with 1.01 for the placebo group.

The HAI GMTR for the Phase 2/3 combined 15 mcg vaccine on Day 43 was 6.63 (95% CI: 5.96 to 7.39)

3.1.2. Neutralizing Antibody Titers

3.1.2.1. Proportion of Subjects Exhibiting a Seroresponse with at Least a Four-fold Increase in Post-injection Neutralizing Antibody Titers on Day 43



Biểu đồ 3.2. Proportion of Subjects Exhibiting a Seroresponse with at Least a Four-fold Increase in Post-injection Neutralizing Antibody Titers on Day 43 with respect to Day 1 – Phase 2 and 3

Phase 2: A seroresponse with at least a four-fold increase in post-injection neutralizing antibody titers on Day 43 compared with baseline (Day 1) was achieved for 60% of subjects in the 15 mcg vaccine group. No subject in the placebo group had a seroresponse of at least a four-fold increase in neutralizing antibody titers on Day 43 compared with baseline (Day 1).

Phase 3: A seroresponse with at least a four-fold increase in post-injection neutralizing antibody titers on Day 43 compared with baseline

(Day 1) was achieved for 51.35% of subjects in the 15 mcg vaccine group. No subject in the placebo group had a seroresponse of at least a four-fold increase in neutralizing antibody titers on Day 43 compared with baseline (Day 1).

3.1.2.2. The neutralizing antibody GMT

Table 3.6 ***Geometric Mean of Neutralizing Antibody Titer on Days 1 and 43*** **phase 2, 3**

Group	Day 1 (N=267)			Day 43 (N=267)		
	n	GMT	(95% CI)	n	GMT	(95% CI)
Phase 2						
Placebo	98	7,15	7,03 – 7,26	97	7,12	7,02 – 7,22
vaccine	95	7,10	7,04 – 7,15	95	29,76	24,47 – 36,20
Phase 3						
Placebo	45	7,07	(, - ,)	45	7,07	(, - ,)
vaccine	222	7,07	(, - ,)	222	26,16	22,66 - 30,20

Phase 2: The neutralizing antibody GMT for the 15 mcg vaccine group on Day 43 was 29,67. The neutralizing antibody GMT on Day 43 for the placebo group was similar to baseline.

Phase 3: The neutralizing antibody GMT for the 15 mcg vaccine group on Day 43 was 26,16. The neutralizing antibody GMT on Day 43 for the placebo group was similar to baseline.

3.1.3. Subjects with Seroprotection of SRH Area

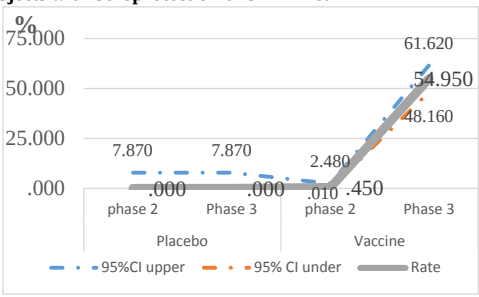


Chart 3.6. Percentage of Subjects with Seroprotection1 of Single Radial Haemolysis (SRH) Area on Days 1 and 43

On Day 43, seroprotection of SRH area (≥25 mm^2) was achieved for 54.95% of subjects in the 15 mcg vaccine group. No subject in the placebo group exhibited seroprotection of SRH area on Day 43.

No subject in the placebo group and one subject (<1.0%) in the 15 mcg vaccine group exhibited seroprotection on Day 1.

3.2. Safety IVACFLU-A/H5N1:

3.2.1. Immediate Reactogenicity (within 30 Minutes Post Each Injection)

Table 3.10. Immediate Reactogenicity (within 30 Minutes Post-injection) – Phase 2 and 3

Local or Systemic Reactogenicity	Placebo			15 mcg vaccine			Giá trị p
	n	(%)	(95% CI)	n	(%)	(95% CI)	
1st injection, n	205			625			
Any local reaction	0	0,0	0,00 - 1,78	2	0,3	0,04 - 1,15	0,567
Any systemic reaction	1	0,5	0,01 - 2,69	1	0,2	0,00 - 0,89	0,433
2nd injection, n	201			615			
Any local reaction	4	2,0	0,54 - 5,02	5	0,8	0,26 - 1,89	0,158
Any systemic reaction	2	1,0	0,12 - 3,55	3	0,5	0,10 - 1,42	0,363

Within 30 minutes following the first injection, solicited local reactions and solicited systemic reactions were reported for <1% of subjects in either group.

Within 30 minutes following the second injection, solicited local reactions and solicited systemic reactions were reported for ≤2% of subjects in either group.

3.2.2.Day Reactogenicity (Over a 7-Day Period Post-injection)

Table 3.20. 7-Day Reactogenicity (over a 7-Day Period Post-injection) – Phase 2 and 3

ocal or stemic eactogenicity	**Placebo**		**15 mcg vaccine**		**Giá trị p**
	n (%)	**(95% CI)**	**n (%)**	**(95% CI)**	
injection, n	205		625		
ny local action	41 (20,0)	(14,75 - 26,14)	492 (78,7)	(75,30 - 81,87)	<0,001
ny systemic action	88 (42,9)	(36,05 - 50,01)	352 (56,3)	(52,33 - 60,25)	0,001
[1] injection, n	201		615		
ny local action	27 (13,4)	(9,04 - 18,94)	271 (44,1)	(40,10 - 48,09)	<0,001
ny systemic action	48 (23,9)	(18,16 - 30,39)	160 (26,0)	(22,59 - 29,67)	0,546

Over the 7-day period following the first injection, solicited local reactions were reported for 78.7% of subjects in the 15 mcg vaccine group compared with 20.0% of subjects in the placebo group. Solicited systemic reactions were reported for 56.3% of subjects in the 15 mcg vaccine group compared with 42.9% of subjects in the placebo group.

Over the 7-day period following the second injection, solicited local reactions were reported for 44.1% of subjects in the 15 mcg vaccine group compared with 13.4% of subjects in the placebo group. Solicited systemic reactions were reported for 26.0% of subjects in the 15 mcg vaccine group compared with 23.9% of subjects in the placebo group.

3.2.3. Brief Summary of Adverse Events

Table 3.27. Unsolicited Adverse Events during 21 Days Post Each Injection – Phase 2 and 3

	Placebo		15 mcg vaccine		Giá trị p
Unsolicited AE	**n (%)**	**(95% CI)**	**n (%)**	**(95% CI)**	
1st injection, n	205		625		
Total number of unsolicited AEs	44		112		
Subjects with at least one AE	37 (18,0)	13,04 - 24,01	93 (14,9)	12,18-7,92	0,279
Subjects with at least one severe AE	3 (1,5)	0,30 - 4,22	0 (0,0)	0,00 - 0,59	0,015
Subjects with at least one serious AE	0 (0,0)	0,00 - 1,78	0 (0,0)	0,00 - 0,59	N/A
Subjects with at least one treatment-related AE	1 (0,5)	0,01 - 2,69	0 (0,0)	0,00 - 0,59	0,247
Subjects with a fatal AE	0 (0,0)	0,00 - 1,78	0 (0,0)	0,00 - 0,59	N/A
2nd injection, n	201		615		
Total number of unsolicited AEs	23		73		
Subjects with at least one AE	22 (10,9)	6,99 - 16,10	66 (10,7)	8,40-13,45	0,932
Subjects with at least one severe AE	2 (1,0)	0,12 - 3,55	2 (0,3)	0,04 - 1,17	0,255
Subjects with at least one serious AE	4 (2,0)	0,54 - 5,02	9 (1,5)	0,67 - 2,76	0,403
Subjects with at least one treatment-related AE	0 (0,0)	0,00 - 1,82	0 (0,0)	0,00 - 0,60	N/A
Subjects with a fatal AE	0 (0,0)	(0,00 - 1,82)	0 (0,0)	(0,00 - 0,60)	N/A

Within 21 days following the first study product injection, unsolicited AEs were reported for 14.9% of subjects in the 15 mcg vaccine group compared with 18.0% of subjects in the placebo group.

No SAEs were reported and there were no deaths. No subject in the 15 mcg vaccine group reported a severe AE or a treatment-related AE compared with 1.5% of subjects who reported a severe AE and <1% of subjects who reported a treatment-related AE in the placebo group.

Within 21 days following the second study product injection, unsolicited AEs were reported for 10.7% of subjects in the 15 mcg vaccine group compared with 10.9% of subjects in the placebo group. Severe AEs were reported for ≤1% of subjects in each group. Serious AEs were reported for 1.5% of subjects in the 15 mcg vaccine group compared with 2.0% of subjects in the placebo group. No treatment-related AEs were reported for any subject in either group. There were no deaths.

Common Adverse Events

The individual unsolicited AEs occurring within 21 days post-Injection #1 are shown by body system, preferred term and severity (mild, moderate or severe) in. No individual unsolicited AE was reported for more than 5% of subjects in the 15 mcg vaccine group. No unsolicited AEs among subjects in the 15 mcg vaccine group had a maximum intensity of severe. All of the reported unsolicited AEs in the 15 mcg vaccine group were considered unrelated to study product by the investigator.

The individual unsolicited AEs occurring within 21 days post-Injection #2 are shown by body system, preferred term and severity (mild, moderate or severe). No individual unsolicited AE was reported for more than 5% of subjects in the 15 mcg vaccine group. Unsolicited AEs and with a maximum intensity of severe were reported for <1.0% of subjects in the 15 mcg vaccine group compared with 1.0% of subjects in the placebo group. All of the reported unsolicited AEs were considered unrelated to study product by the investigator.

3.2.4. Deaths, Other Serious Adverse Events

3.2.4.1. Deaths

There were no deaths reported among subjects who participated in Phase 2 or Phase 3.

3.2.4.2. Other Serious Adverse Events

An SAE was reported for 1.4% of subjects in the 15 mcg vaccine group and 2.0% of subjects in the placebo group ( ). All reported SAEs were grade 2 or 3 and no individual SAE (by preferred term) was reported by more than one subject in a group. No SAE was considered to be related to study product.

Table 3.28. Serious Adverse Events– Phase 2 and 3

latedness to Study Product/ Preferred Term/ Severity	**Placebo (N=205)**				**15 mcg vaccine (N=625)**			
	# of Events	**# of Subjects**	**% of Subjects**	**95% CI**	**# of Events**	**# of Subjects**	**% of Subjects**	**95% CI**
tal	4	4	2,0	0,53 - 4,92	8	8	1,4	0,66 - 2,72
related	4	4	2,0	0,53 - 4,92	8	8	1,5	0,66 - 2,72
dominal adhesion de 2	0	0	0,0	0,00 - 1,78	1	1	0,2	0,00 - 0,89
dominal symptom	1	1	0,5	0,01 - 2,69	0	0	0,0	0,00 - 0,59
pendicitis Grade 2	1	1	0,5	0,01 - 2,69	1	1	0,2	0,00 - 0,89
ppendicitis Grade 3	1	1	0,5	0,01 - 2,69	0	0	0,0	0,00 - 0,59
oncussion Grade 2	0	0	0,0	0,00 - 1,78	1	1	0,2	0,00 - 0,89
verticulitis Grade 2	0	0	0,0	0,00 - 1,78	1	1	0,2	0,00 - 0,89
haryngitis Grade 2	0	0	0,0	0,00 - 1,78	1	1	0,2	0,00 - 0,89
Sebaceous gland infection Grade 2	1	1	0,5	0,01 - 2,69	0	0	0,0	0,00 - 0,59
Tonsillitis Grade 3	0	0	0,0	0,00 - 1,78	1	1	0,2	0,00 - 0,89
al infection Grade 3	0	0	0,0	0,00 - 1,78	1	1	0,2	0,00 - 0,89
Wound độ Grade 2	1	1	0,5	0,01 - 2,69	1	1	0,2	0,00 - 0,89

An SAE was reported for 1.4% of subjects in the 15 mcg vaccine group and 2.0% of subjects in the placebo group. All reported SAEs were grade 2 or 3 and no individual SAE (by preferred term) was reported by more than one subject in a group. No SAE was considered to be related to study product.

Chapter 4
DISCUSSION

4.2. The immunogenicity of IVACFLU-A/H5N1 vaccine:

4.2.1. The immunogenicity of IVACFLU-A/H5N1 vaccine

4.2.1.1. Phase 2:

In phase 2, data on the immune response of research subjects showed that the influenza A / H5N1 vaccine produced by IVAC had well immunogenicity in the study subjects at a dose of 15mcg / 0.5ml and met all the standards defined by the Ministry of Health, including:

+ The proportions of subjects with HAI ≥1:40 was 83.2%, a neutralizing antibody titer ≥1:40 was achieved for 44.21%. According to WHO, a minimum proportion of subjects reaching antibody titres HAI ≥ 1:40 is acceptable at least 70%.

+ The proportions of subjects with seroresponse of at least a four-fold increase in HAI titers on Day 43 compared with baseline were 92.6%.

+ The proportions of GMT at least increases ≥ 2.5 times, but this study reached 11.2.

Comment [D3]: Bàn luận có chính xác?

Comment [D4]: Tiếng Việt viết có chính xác

Comment [D5]: Cần kiểm tra sự chính xác

4.2.1.2. Phase 3:

On Day 43, the HAI GMT for the 15 mcg vaccine group was 27.61. The HAI GMT of the placebo group was similar to that of the baseline before injection. On Day 43, the HAI GMTR for the 15 mcg vaccine group was 5.31 compared with D1. Immunity responses in phase 3 were lower than those in phase 2 studies, but the results met criteria on seroconversion and increased GMT by 4 times. Research results showed that the percentage of subjects with seroconversion was always high, while the percentage of subjects with adverse reactions in the 3 clinical trials was under the control.

4.3. Safety of influenza vaccine A / H5N1 produced by IVAC

4.3.1. Phase 2.

About safety, research results in phase 2 showed that the influenza A / H5N1 vaccine produced by IVAC was well tolerated, among subjects receiving the vaccine after the 1st and 2nd, there did not

Comment [D6]: Tiếng Việt chưa bỏ liều khác

report any moderate or higher immediate reaction within 30 minutes after injection, some cases had local and systemic reactions such as pain at the injection site, fever after injection, all in a mild degree. Neither mortality nor any serious adverse event assessed as related to the product was recorded during the study period. Within 30 minutes of vaccination, injection site and systemic reactions reported approximately <2% of participants across all groups. All responses were mild, and over a period of seven days, after the first and second injections, the rates were higher in participants in the vaccination group.

The study also found that the proportion of subjects with solicited local reactions often accompanied intramuscular injection occurred from 30 minutes to 7 days after the study vaccination. If statistics of subjects with any local reactions after vaccination, the rate in vaccine group higher than in the placebo group, and this difference is statistically significant with p <0.0001. However, the local adverse events (pain at the injection site, pain to the touch, ...) were mild and resolved rapidly within 2-3 days after injection. The solicited systemic reactions, the response to fever was higher in the vaccine group, the proportion of subjects in the vaccine groups had other systemic reactions (eg headache, nausea, fatigue, ...) was similar to the placebo group.

4.3.2. Phase 3

The 15 mcg IVACFLU-A / H5N1 vaccine has been shown to have an immune response and safe as discussed and presented above. In phase 3, the 15 mcg dose compared with the placebo group for an immediate reaction within 30 minutes (table 3.22), after the first injection it showed that the proportion of study subjects with a solicited local reactions was <1% in all groups. None of the subjects had solicited systemic reactions. Within 30 minutes after the second injection, the percentage of study subjects with solicited local and solicited systemic reactions was <2% in all groups. All local and systemic adverse events within 30 minutes after 2nd injection had the

severe severity of degree 1 (mild). The number of study subjects with local and systemic adverse events within 30 minutes after injection of each dose was too small to draw conclusions about the difference between the age groups.

During the 7-day period following 1st injection, the proportion of study subjects with solicited local reactions was 77.9% in the 15 mcg vaccine group in compared to 18.1% in the placebo group. The proportion of study subjects with solicited systemic reactions was 54.5% in the 15 mcg vaccine group in compared to 34.3% in the placebo group. During the 7-day period following 2nd injection, the proportion of study subjects with solicited local reactions was 43.7% in the 15 mcg vaccine group in compared to 14.6% in the placebo group. The proportion of study subjects with solicited systemic reactions was 24.8% in the 15 mcg vaccine group in compared to 18.4% in the placebo group. Most of the solicited adverse events began on day 1 and did not last longer than 7 days in all studied groups.

Data on solicited local reactions after first and second injection collected from day 1 to 7 are presented by name (pain, pain to the touch, redness, swelling, sclerosis). The median of the duration of the events occurring 7 days after each injection was 1 to 3 days. The longest period of solicited adverse events in 21 days. Within 7 days of follow-up after the first injection, the solicited local reactions in severe <1% of study subjects in the 15 mcg vaccine group and no study subjects in placebo group. The severe severity of solicited systemic reactions occurred in 1% of study subjects in both groups. Within 7 days of follow-up after the second injection, neither study subject in either group had a severe of solicited local reactions. The severe severity of solicited systemic reactions occurred in <1% of the 15 mcg vaccine group and no study subjects in the placebo group. Thus, the rate of local and systemic adverse events in 2nd injection was lower than 1st injection.

Within 21 days after the 1st injection, the proportion of study subjects had unsolicited adverse events in the vaccine group was 13.9%

compared with 15.2% in the placebo group. No adverse events in severe severity, serious adverse events, or adverse events related to the study product in all groups. There was no death. Within 21 days after 2nd injection, the proportion of study subjects with unsolicited adverse events in the vaccine group was 10.8% compared with 15.5% in the placebo group. The incidence of the severe adverse event was 1% of study subjects in the placebo group and no study subjects in the vaccine group. The proportion of study subjects with a serious adverse event in the placebo group was 3.9% while that in the vaccine group was 1.5%. No product-related adverse events were reported in all groups. No deaths have been reported. Serious adverse events occurred in 1.5% of study subjects in the 15 mcg dose group and 3.8% in the placebo group. All of these serious adverse events were at degree 2 or 3 and no type of SAE (by encoding dictionary name) occurred in more than one study subject. All SAEs were assessed by the investigator as not related to the study produc.

CONCLUSSION

Comment [D7]: Có chính xác với Kết quả trong bài viết?

1. Immunogenicity:

- The proportions of subjects with HAI ≥1:40 on Day 43 was 55.84%.

- A seroresponse with at least a four-fold increase in post-injection HAI titers on Day 43 compared with baseline (Day 1) was achieved for 75.08%

- The HAI GMT for the Phase 2/3 combined 15 mcg vaccine group on Day 43 was 35.30.

- The HAI GMTR for the Phase 2/3 combined 15 mcg vaccine on Day 43 was 6.63.

- The proportion of subjects with HAI titer ≥1:40 and exhibiting a seroresponse of at least a four-fold increase in HAI titers on Day 43 compared with baseline in 15 mcg vaccine group was 55.52%.

- The proportion of subjects with at least a four-fold increase in neutralizing antibody titers on Day 43 compared with baseline in the 15 mcg vaccine group was 39.13% in phase 2 and 40.99% in phase 3.

- The neutralizing antibody GMT for the 15 mcg vaccine group on Day 43 was 29.76 in phase 2 and 26.16 in phase 3.

- The neutralizing antibody GMTR for the 15 mcg vaccine group on Day 43 was 4.19 in phase 2 and 3.70 in phase 3.

- On Day 43 phase 3, seroprotection of SRH area ≥25 mm^2 was achieved for 54.95% of subjects in the 15 mcg vaccine group.

- On Day 43 phase 3, seroconversion of SRH area was achieved for 55.86% of subjects in the 15 mcg vaccine group

- The geometric mean of SRH areas for the 15 mcg vaccine group on Day 43 phase 3 was 19.99

- The geometric mean of SRH areas ratio on Day 43 with respect to Day 1 for the 15 mcg vaccine group phase was 4.75

2. The safety of IVACFLU-A / H5N1 vaccine:

- The IVACFLU-A/H5N1 doses evaluated were well tolerated in both adult Vietnamese subjects who were 18 to 40 and 41 to 60 years of age.

- Most reactogenicity and unsolicited AEs were mild, transient and judged unrelated to study product.

- No deaths were reported.

RECOMMENDATIONS

1. The results of research have demonstrated the safety and immunogenicity of the IVACFLU-A / H5N1 vaccine at the dose of 15mcg / 0.5ml, which has completed a clinical trial to be ready to serve the needs of outbreak prevention. This information should be posted on websites about outbreak prevention.

2. In this study, study population is disseminated knowledge about influenza prevention with vaccines, it should continue to propagate to the community about general knowledge about influenza prevention and information about the quality of influenza vaccines produced by IVAC to encourage Vietnamese people to use Vietnamese goods, it helps prevention epidemic disease and ensure social security.

LIST OF ANNOUNCED RESEARCH PROJECTS RELATED TO THESIS TOPIC

1. Vu Thi Chau, Tran Nhu Duong, Vu Minh Huong, Le Van Be, Dang Van Chuc, Vu Dinh Thiem. Immunogenicity of influenza A/H5N1 vaccine (IVACFLU - A/H5N1) in healthy Vietnamese adultsVietnam Journal of Preventive Medicine; Volume 29, Issue 14, 2019: 83 – 87.

2. Vu Thi Chau Tran Nhu Duong, Vu Minh Huong, Le Van Be, Dang Van Chuc, Vu Dinh Thiem. Safety of A/H5N1 influenza vaccine (IVACFLU - A/H5N1) in healthy adults Vietnamese Journal of Preventive Medicine; Volume 29, Issue 14, 2019: 88 – 96.