APPENDIX A

Sample size calculation by PASS program

One-Way Analysis of Variance F-Tests

Numeric Results

Means: 2.5   3.5 5.5

Power	Average n	G	Total N	K	Std Dev of Means σm	Standard Deviation σ	Effect Size	Alpha
0.8367	12.00	3	36	1.00	1.25	2.20	0.5669	0.0500

References

Desu, M. M. and Raghavarao, D. 1990. Sample Size Methodology. Academic Press. New York.

Fleiss, Joseph L. 1986. The Design and Analysis of Clinical Experiments. John Wiley & Sons. New York.

Kirk, Roger E. 1982. Experimental Design: Procedures for the Behavioral Sciences. Brooks/Cole. Pacific Grove, California.

Report Definitions

Power is the probability of rejecting a false null hypothesis. It should be close to one.

n is the average group sample size.

G is the number of groups.

Total N is the total sample size of all groups combined.

K is the group means multiplier.

σm is the standard deviation of the group means under the alternative hypothesis.

σ is the within group standard deviation.

The Effect Size is the ratio of σm and σ.

Alpha is the probability of rejecting a true null hypothesis. It should be small.

Summary Statements

In a one-way ANOVA study, sample sizes of 12, 12, and 12 are obtained from the 3 groups whose means are to be compared. The total sample of 36 subjects achieves 84% power to detect differences among the means versus the alternative of equal means using an F test with a 0.0500 significance level. The size of the variation in the means is represented by their standard deviation which is 1.25. The common standard deviation within a group is assumed to be 2.20.

APPENDIX B

Categories of the sampled farms

Farms		A	B	C	D	E	F	G	H	I	J	K
Location	GT					x	x	x	x	x		
	BT	x	x	x	x						x	x
Farm scale	S (<500)			x	x	x				x	x	
	M (500 - <1300)	x	x				x	x	x			x
Purpose of production	B (meat-type)	x		x	x	x	x	x	x			
	L (layer)		x	x							x	x

APPENDIX C

Data from r program computing

Codes for general least-squares

```
> f <- gls(Titre ~ Time + Farm, corr=corAR1( , form= ~1|ID), data= L3)
> anova(f)
Denom. DF: 483
            numDF  F-value p-value
(Intercept)     1 4607.690  <.0001
Time            2  464.731  <.0001
Farm           10    4.500  <.0001
```

Codes for calculating the overall geometric mean titres of each times of vaccination

```
> library(dplyr)
> gm <- L3 %>% group_by(Time) %>%
+    summarize(mean=mean(Titre, na.rm=T), sd=sd(Titre, na.rm=T), n=n()) %>%
+    mutate(se = sd/sqrt(n))
> gm
# A tibble: 3 x 5
   Time2      mean       sd       n         se
  <fctr>     <dbl>    <dbl>   <int>      <dbl>
1      0 2.018072 0.133616     166 0.01037061
2      1 5.295181 1.846432     166 0.14331089
3      2 6.475610 1.703303     164 0.13300564
```

Codes for multiple comparisons between GMTs of times of vaccination

```
> library(lsmeans)
> lsmeans(f, pairwise ~ Time, adjust = c("tukey")) $lsmeans
 Time2   lsmean        SE  df lower.CL upper.CL
 0      2.020854 0.1081309 483 1.808389 2.233319
 1      5.297963 0.1081309 483 5.085498 5.510428
 2      6.473828 0.1087793 483 6.260089 6.687567

Results are averaged over the levels of: Farm
Confidence level used: 0.95

$contrasts
 contrast  estimate        SE  df t.ratio p.value
 0 - 1    -3.277108 0.1441452 483 -22.735  <.0001
 0 - 2    -4.452974 0.1524292 483 -29.213  <.0001
 1 - 2    -1.175866 0.1446409 483  -8.130  <.0001

Results are averaged over the levels of: Farm
P value adjustment: tukey method for comparing a family of 3 estimates
```

Codes for multiple comparisons between GMTs of farms

```
> library(lsmeans)
> lsmeans(f, pairwise ~ Farm, adjust = c("tukey"))
$lsmeans
 Farm   lsmean        SE df lower.CL upper.CL
 A    4.661510 0.2254442 483 4.218537 5.104483
 B    3.675677 0.2231265 483 3.237259 4.114096
 C    5.219240 0.2231265 483 4.780821 5.657659
 D    4.914176 0.2231265 483 4.475757 5.352594
 E    4.803821 0.2231265 483 4.365402 5.242239
 F    4.893566 0.2231265 483 4.455148 5.331985
 G    4.135743 0.2181436 483 3.707115 4.564370
 H    4.824380 0.2231265 483 4.385961 5.262798
 I    3.975652 0.2231265 483 3.537234 4.414071
 J    4.921785 0.2231265 483 4.483366 5.360203
 K    4.547484 0.2231265 483 4.109065 4.985902

Results are averaged over the levels of: Time2
Confidence level used: 0.95

$contrasts
 contrast       estimate        SE  df t.ratio p.value
 A - B      0.985832709 0.3171714 483   3.108  0.0722
 A - C     -0.557729974 0.3171714 483  -1.758  0.8038
 A - D     -0.252665555 0.3171714 483  -0.797  0.9994
 A - E     -0.142310566 0.3171714 483  -0.449  1.0000
 A - F     -0.232056089 0.3171714 483  -0.732  0.9997
 A - G      0.525767288 0.3136831 483   1.676  0.8472
 A - H     -0.162869777 0.3171714 483  -0.514  1.0000
 A - I      0.685857837 0.3171714 483   2.162  0.5329
 A - J     -0.260274747 0.3171714 483  -0.821  0.9992
 A - K      0.114026240 0.3171714 483   0.360  1.0000
 B - C     -1.543562683 0.3155171 483  -4.892  0.0001
 B - D     -1.238498264 0.3155171 483  -3.925  0.0047
 B - E     -1.128143275 0.3155171 483  -3.576  0.0167
 B - F     -1.217888798 0.3155171 483  -3.860  0.0060
 B - G     -0.460065421 0.3120237 483  -1.474  0.9276
 B - H     -1.148702485 0.3155171 483  -3.641  0.0133
 B - I     -0.299974872 0.3155171 483  -0.951  0.9972
 B - J     -1.246107456 0.3155171 483  -3.949  0.0043
 B - K     -0.871806469 0.3155171 483  -2.763  0.1757
 C - D      0.305064419 0.3155171 483   0.967  0.9968
 C - E      0.415419408 0.3155171 483   1.317  0.9657
 C - F      0.325673885 0.3155171 483   1.032  0.9945
 C - G      1.083497262 0.3120237 483   3.472  0.0236
 C - H      0.394860197 0.3155171 483   1.251  0.9760
 C - I      1.243587811 0.3155171 483   3.941  0.0044
 C - J      0.297455227 0.3155171 483   0.943  0.9974
 C - K      0.671756214 0.3155171 483   2.129  0.5569
 D - E      0.110354989 0.3155171 483   0.350  1.0000
 D - F      0.020609466 0.3155171 483   0.065  1.0000
 D - G      0.778432843 0.3120237 483   2.495  0.3101
 D - H      0.089795778 0.3155171 483   0.285  1.0000
 D - I      0.938523392 0.3155171 483   2.975  0.1039
 D - J     -0.007609192 0.3155171 483  -0.024  1.0000
 D - K      0.366691795 0.3155171 483   1.162  0.9861
 E - F     -0.089745523 0.3155171 483  -0.284  1.0000
 E - G      0.668077854 0.3120237 483   2.141  0.5483
```

```
E - H     -0.020559210 0.3155171 483   -0.065  1.0000
E - I      0.828168403 0.3155171 483    2.625  0.2389
E - J     -0.117964181 0.3155171 483   -0.374  1.0000
E - K      0.256336806 0.3155171 483    0.812  0.9993
F - G      0.757823377 0.3120237 483    2.429  0.3504
F - H      0.069186312 0.3155171 483    0.219  1.0000
F - I      0.917913926 0.3155171 483    2.909  0.1231
F - J     -0.028218659 0.3155171 483   -0.089  1.0000
F - K      0.346082328 0.3155171 483    1.097  0.9911
G - H     -0.688637064 0.3120237 483   -2.207  0.5009
G - I      0.160090549 0.3120237 483    0.513  1.0000
G - J     -0.786042035 0.3120237 483   -2.519  0.2959
G - K     -0.411741048 0.3120237 483   -1.320  0.9652
H - I      0.848727613 0.3155171 483    2.690  0.2075
H - J     -0.097404971 0.3155171 483   -0.309  1.0000
H - K      0.276896016 0.3155171 483    0.878  0.9986
I - J     -0.946132584 0.3155171 483   -2.999  0.0975
I - K     -0.571831597 0.3155171 483   -1.812  0.7725
J - K      0.374300987 0.3155171 483    1.186  0.9838
```

Results are averaged over the levels of: Time
P value adjustment: tukey method for comparing a family of 11 estimate
s

APPENDIX D

Equipment, materials and reagents

1)   Materials for collecting blood samples:

70° alcohol for hand disinfection

Boot covers

Cooling box

Cotton ball

Face masks

Gloves

Ice gel packs

Leg bands for duck identification

Marker pens

Needles (23 gauge and 25 gauge)

Syringes (3 mL and 5 mL)

2)   Equipment and Materials for laboratory analyzing:

20 mL-, 50 mL-, 100 mL-, 200 mL- glassware

Autoclave (steam sterilization)

Centrifuge for 10 mL – 15 mL tubes

Cooling chamber at $+4^0$C

Deep Freezer at - 20^0C

Electronic balance

Haematocrit Centrifuge

Marker pens

Microtitre plate caps

Multichanel Micropipettes for 25 µl – 50 µl, 50 µl – 300 µl

pH mater

Pipette tips

Reservoirs

Single-chanel Micropipettes for 25 μl – 50 μl, 100 μl – 1000 μl

Test tube racks

Timer

Titer Plate Shaker

U- and V- bottomed plastic microtitre plates

Vortex mixer

Water Bath

3) **Preparation of Alsever's solution:**

Glucose: 2.5 g

NaCl: 0.42 g

Citrate Natri: 0.8 g

Distilled water: 100ml

Adjust pH = 6.1, steam sterilization by an autoclave

4) **Preparation of phosphat buffer saline (PBS) solution:**

NaCl: 8 g

KCl: 0.2 g

Na_2HPO_4: 1.15 g

KH_2PO_4: 0.2 g

Double-distilled water: 1 L

Adjust pH = 7.3 ± 0.2, steam sterilization by an autoclave at 110 ^{0}C for 15 min.

APPENDIX E

Sampling sites, sample collection

1) Pictures of some sampled farms involved in this study

Samples were collected from 11 village/household farms in 2 districts in Ben Tre province in the Mekong Delta -Vietnam, representing a variety of breeds, flock sizes (100 - < 1,500 ducks/farm), and purpose of production (meat-type ducks and layer ducks).

2) Vaccination and blood sample collection:

Vaccinations were performed intramuscularly in the breast using automatic syringes either by competent farm workers or by local veterinarians following the guidelines of the national mass vaccination program against H5N1 HPAI for poultry regarding vaccine strain and schedule of immunization.
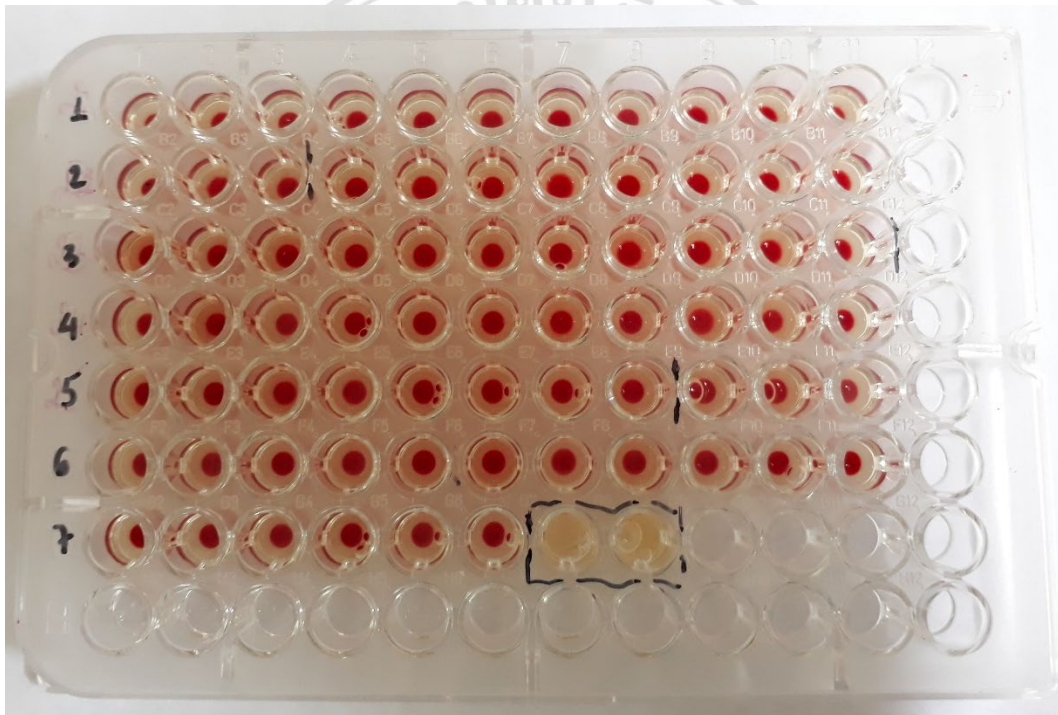

In each flock, 20 ducks were selected to be identified individually with leg bands and were blood sampled. Approximately 1 to 2 mL of blood was taken from each individual duck via the medial metatarsal vein.

APPENDIX F

Laboratory analyzing

### 1) Pre-treatment of duck serum samples with 10% RBCs

Duck sera were heat-inactivated at 56°C for 30 min then treated with 10% roosters' RBC suspension to remove nonspecific inhibitors and prevent nonspecific HA reaction that might otherwise occur during HI assay in the sera of species other than chickens. The reagent plate was kept for 60 min at room temperature, waiting for the 10% RBCs suspension to settle. The supernatant was aspirated to be tested by the HI assay.

2)   Titration of 1 Haemagglutination Unit of the Antigen suspension
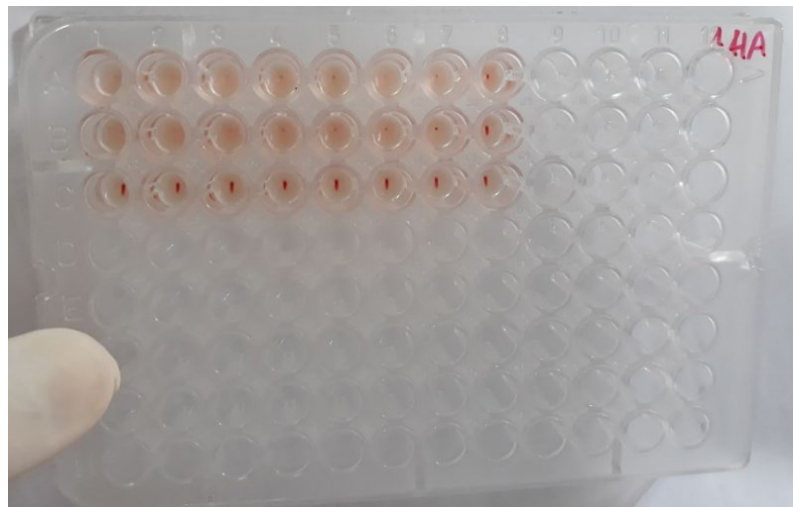
To ensure that the correct amount of antigen will be used in the HI test, a plate was prepared for the titration of the 1 HAU antigen (the reagent is 1 mL lyophilized in a glass ampoule which were reconstituted with 1 mL sterile distilled water before use).

3)   Reading HI test result:
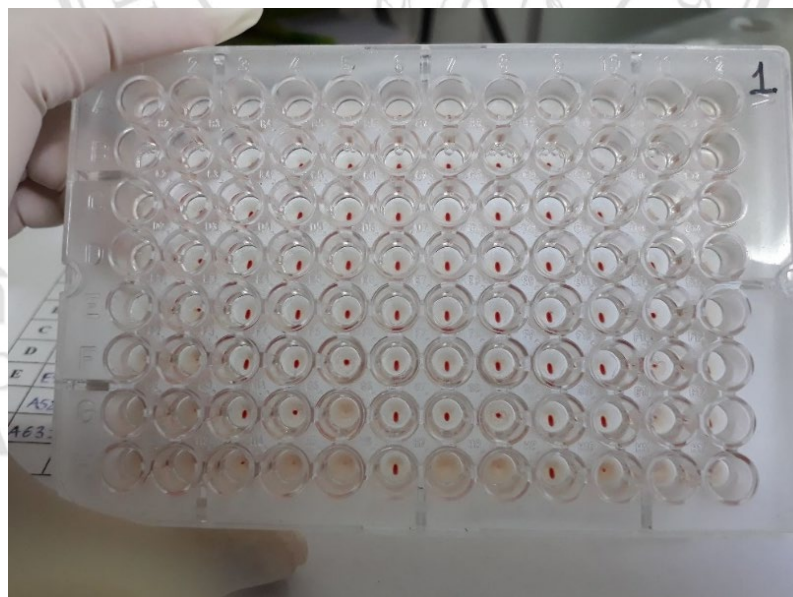
Plates are read after 40 – 45 min at 4°C when the RBCs in control wells have settled and assumed a button shape. The samples are read by tilting the plate at approximately 45 degrees, using a white background and observing the appreance of tear-shaped streaming of the RBCs.

CURRICULUM VITAE

Author's Name   Ms. Huynh Thi Thanh Hoa

Date of Birth   30 April 1992

Place of Birth   Vietnam

Education

2010 – 2016   Bachelor's Degree, Doctor of Veterinary Medicine (advanced program), the Faculty of Animal Science & Veterinary Medicine, Nong Lam University – Ho Chi Minh City, Vietnam.

2007 – 2010   High school education, Trung Vuong Senior High School, Ho Chi Minh City, Vietnam.

2003 – 2007   Secondary school education, Tran Van On Junior High School, Ho Chi Minh City, Vietnam.

1998 – 2003   Primary school education, Nguyen Binh Khiem Primary School, Ho Chi Minh City, Vietnam.

Scholarship

2016-2018   USAID EPT2 One Health Workforce Project

Publication

Huynh Thi Thanh Hoa, Ho Thi Kim Hoa, Truong Thi Bich Nguyen, Le Thanh Hien, Van Thi Yen Khang, "Screening lactic acid bacteria of swine origin for Probiotic use", Journal of Agricultural science and technology, 04/2015, ISSN: 1859 – 1523.

Experience

2016   Research & Develoment personnel, MEBIPHA animal and aquatic animal pharmaceutical manufacture & trade Co., Ltd., Ho Chi Minh city – Vietnam,

Other

2015   Intership for a research project in Biosensor titled "*Preliminary study of one-pot immunoassay based on gold nanoparticles and methylene blue doped-silica particles conjunction for the detection of Samonella spp.*", School of Bioresources and Technology, King Mongkut's University of Technology Thonburi, Bangkok - Thailand.