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Antibody Titer of Newcastle Disease in Vaccinated and Non-vaccinated Local Chicken of Cambodia

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Abstract: The experiment was conducted at Veterinary Research Station of Faculty of Veterinary Medicine, Royal University of Agriculture. The experimental period lasted 60 days, starting from October 1st to November 30th 2022. CRD (Completely Randomized Design) was used with 2 treatments/groups, vaccination group and non-vaccination group "control", and 6 replications. The vaccination groups received two times of vaccination by dropping into the ocular at 7 days and 21 days. Meanwhile, blood samples were collected 3 times to detect the antibody level of ND (Newcastle Disease) and contained 21 days old, 35 days old and 49 days old chicks. The ELISA (Enzyme-Linked Immunosorbent Assay) was performed to detect the antibody of those 2 groups. The result of finding showed that the S/P (Sample to Positive) ratio of control at 21 days, was very low, even in 3rd quartile, which was below the threshold. However, the vaccination group was relatively high, even in 1st quartile, which was higher than the threshold. At 35 days, S/P ratio of control group was still very low, but a bit higher than at 21 days. Meanwhile, the vaccination group was still high, even in 1st quartile, and two-time higher than at 21 days, but an increasing number of samples developed less antibody than threshold, accounting for 12.22%. At 49 days, the control group was still very low, even in 3rd quartile, but a bit higher than at 21 days and 35 days, and was close to the threshold. The vaccination group was still relatively high, even in 1st quartile but lower than three times comparing to 35 days. However, in this age, the number of chickens that developed antibody seemed to be increased in the control group, vice versa for vaccination group. The average S/P ratio was different significant ($p < 0.001$), where vaccination had higher S/P ratio than control. It was similar finding for log-titer, the vaccination had higher figure ($p < 0.001$). The risk of infection of ND was higher in control group, but it will reduce by increasing the age of chicken, while vaccination group was decreased by increasing age, especially at 49 days and we need to consider another vaccination to get full protection.

Key words: Antibody, vaccination, control, ND, S/P ratio.

1. Introduction

ND (Newcastle Disease) has strongly affected in the birds, chickens are more susceptible to the disease with high fatality rate. Other birds, such as turkeys and pigeons, are also infected. Ducks are generally resistant to ND, but in some cases ducklings can be infected. Unvaccinated chickens, when infected, are more likely to die quickly. The virulence of ND strains is endemic in poultry in most of Asia, Africa, Mexico and some countries of South America [1] and causes the most important infectious diseases of poultry leading to economic losses. In Cambodia, ND has caused a high fatality rate of chicken in villages and on farms. ND is known in many local names as Dangkorkach and Dangkorek. It is considered one of the deadliest bird diseases worldwide, most occurring during the early rainy season and infecting birds of all ages [2]. The chickens are more vulnerable than other bird/poultry due to infection with the Newcastle virus, which can infect both high and low virulence. Symptoms in chickens vary depending on the type of virus, host species, age of host and infection with other types of organisms, environmental stress and immune status [3]. The clinical spectrum of signs of ND is also similar to those of high pathogenicity avian influenza, as it has devastated the national economy

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through a high percentage of chicken deaths, affecting the local market system [4]. The vaccination was applied as a preventive measure in many countries, but the outbreaks have been reported in vaccinated populations. The low vaccination coverage level or imperfect immunity, allowed the virus to spread in partially vaccinated populations [5]. More compliant to ND vaccination schedule and best practices in poultry farm would enhance ND control [6]. Thus, the combination strategy, vaccination and biosecurity, was an effective measure to prevent ND in Cambodia [7]. However, this study was to evaluate the efficacy of vaccination against ND of local chicken in Cambodia.

2. Research and Methodology

2.1 Location and Duration

The experiment was conducted at the Veterinary Research Station of Faculty of Veterinary Medicine, Royal University of Agriculture. The experimental period last 60 days, starting from October 1st to November 30th 2022.

2.2 Experimental Layout

The experiment was designed by using CRD (Completely Randomized Design), consisted of 2 treatments, with vaccination "experimental group" and without vaccination "control group", and there were 6 replications in each treatment. Fifty chickens were allocated into each replication. All chicken in vaccination group were received two times of vaccination by dropping into the ocular at 7 days and 21 days.

2.3 Experimental Chicken and Feed

The 600 local chicks were purchased from local supplier at 1 day and allocated into each replication as designating. The replications were separated by using the wire net and made into small confinement with litter of rice husk. The commercial feed was used in the whole experiment and all treatment fed the same feed.

2.4 Sample Collection

The blood samples were collected 3 times to detect the antibody level and contained 21 days old, 35 days old and 49 days old chicks. The best location for taking the sample was from the large vein under the wing. The bird was held under the arm of collector, while drawing the blood into the vacuum blood collection tube (no additive tube). Fifteen chickens of each replication were selected randomly for blood sampling.

2.5 ELISA (Enzyme-Linked Immunosorbent Assay) Method for Detecting Antibody

The chicken serum samples were analyzed in the laboratory of the Faculty of Veterinary Medicine at the Royal University of Agriculture, immediately after the collection of samples was completed. Determination of Newcastle antibody levels was performed using the ELISA method. The procedure of analysis has been performed following the procedure of VDPro® NDV AB ELISA CAT.NO. EP-NDV-01 (MEDIAN Diagnostics) [8]. All samples were duplicated on an ELISA microchip to maintain the accuracy and control of any bias that may occur during the test. PC (Positive Control) and NC (Negative Control) samples were also used to verify the experimental results.

2.6 Data Record and Analysis

All the data were recorded in Excel and analised by using descriptive and inferential statistics.

S/P (Sample To Positive) ratio and log titer were calculated by followed the guideline of MEDIAN Diagnostics [8] as below:

$$S/P\ \ ratio = \frac{Sample\ \ OD - NCx}{CPC}$$

$$CPC = PCx - NCx$$

$$Log\ \ titer = 1.8 * (\log s/p) + 3.56$$

where:

S/P ratio: Sample to Positive Ratio.

OD: Optical Density.

CPC: Corrected Positive Control.

PC*x*: Positive Control.

NC*x*: Negative Control.

Interpretation:

Test samples having ≥ 0.2 S/P ratio are positive.

Test samples having < 0.2 S/P ratio are negative.

3. Result

3.1 S/P Ratio at 2 Weeks after First Vaccination (21 Days Old)

The S/P ratio at 21 days of control group was very low, even in 3rd quartile, 0.059, which was below threshold, 0.2. However, the vaccination group was relatively high, even in 1st quartile, 0.638, which was higher than the threshold (Fig. 1).

For the distribution of S/P ratio, there were 6.66% and 96.67% of control and vaccination group, respectively, which was higher than the threshold (Table 1).

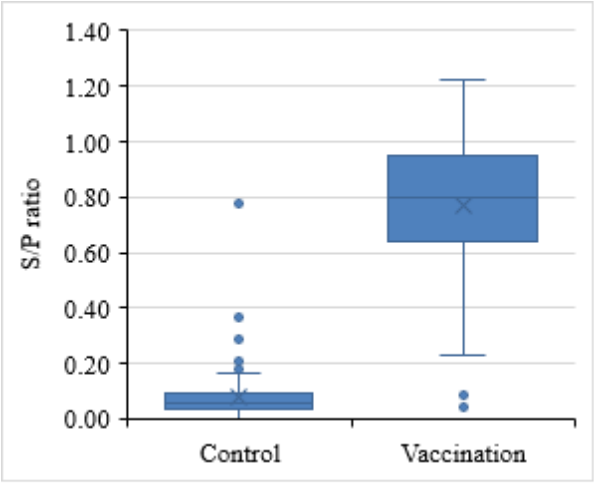

Fig. 1 S/P ratio at 2 weeks after first vaccination (21 days old).

Table 1 The distribution of S/P ratio at 2 weeks after first vaccination (21 days old).

S/P ratio	Control		Vaccination	
	#	%	#	%
< 0.20	84	93.33	3	3.33
0.20-0.30	4	4.44	2	2.22
0.301-0.40	1	1.11	3	3.33
0.401-0.50	-	-	5	5.56
0.501-0.06	-	-	4	4.44
0.601-0.07	-	-	10	11.11
0.701-0.08	1	1.11	18	20.00
0.801-0.09	-	-	19	21.11
0.901-1.00	-	-	9	10.00
> 1.00	-	-	17	18.89
Total	90	100	90	100

3.2 S/P Ratio at 2 Weeks after Second Vaccination (35 Days Old)

For S/P ratio at 35 days, the control group was still very low, even in 3rd quartile, 0.157, and it was a bit higher than at 21 days, but still below the threshold, 0.2. However, the vaccination group was relatively high, even in 1st quartile, 1.166, which was higher than the threshold and two times higher than at 21 days (Fig. 2).

At 35 days old, the control group seemed to develop more antibody, accounting for 21.11%, which was higher than the threshold. But vaccination group seemed to increase the number of sample developed lower antibody than threshold, accounting for 12.22%. However, for the positive sample were extremely high level of S/P ratio, accounting for 83.33% had S/P ratio higher than 1 (Table 2).

3.3 S/P Ratio at 4 Weeks after Second Vaccination (49 Days Old)

For S/P ratio at 49 days, the control group was still very low, even in 3rd quartile, 0.189, and it was a bit higher than at 21 days and 35 days, and was close to the threshold, 0.2. The vaccination group was still relatively high, even in 1st quartile, 0.459, which was higher than the threshold and three times lower than at 35 days (Fig. 3).

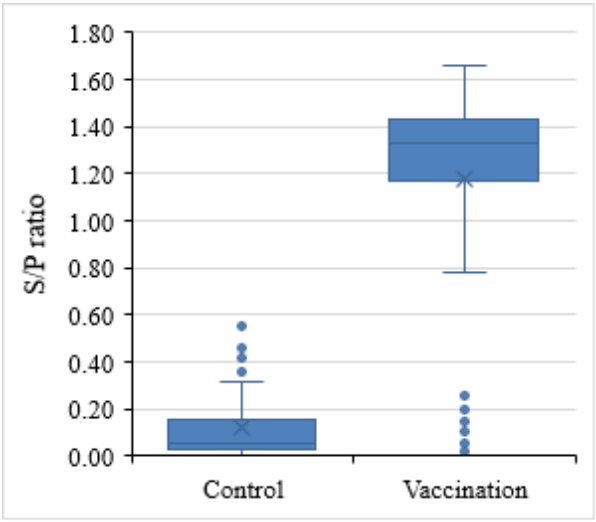

Fig. 2 S/P ratio at 2 weeks after second vaccination (35 days old).

Table 2 The distribution of S/P ratio at 2 weeks after second vaccination (35 days old).

S/P ratio	Control		Vaccination	
	#	%	#	%
< 0.20	71	78.89	11	12.22
0.20-0.30	6	6.67	1	1.11
0.301-0.40	8	8.89	0	0.00
0.401-0.50	3	3.33	0	0.00
0.501-0.06	2	2.22	0	0.00
0.601-0.07	-	-	0	0.00
0.701-0.08	-	-	1	1.11
0.801-0.09	-	-	0	0.00
0.901-1.00	-	-	2	2.22
> 1.00	-	-	75	83.33
Total	90	100	90	100

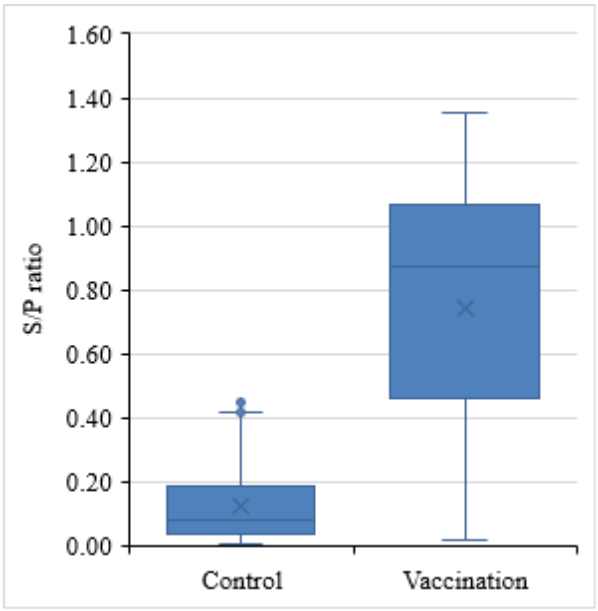

Fig. 3 S/P ratio at 4 weeks after second vaccination (49 days old).

At 49 days old, the control group seemed to be more and more developing the antibody, accounting for 21.11%, which was higher than the threshold. But vaccination group seemed to increase the number of sample and developed less antibody than threshold, accounting for 22.22% (Table 3).

3.4 Comparison of the Average of S/P Ratio

The average of S/P ratio in control and vaccination group was significantly different in all those 3 age-categories ($p < 0.001$), and the vaccination group had higher S/P ratio than control (Table 4).

Table 3 The distribution of S/P ratio at 4 weeks after second vaccination (49 days old).

S/P ratio	Control		Vaccination	
	#	%	#	%
< 0.20	69	76.67	20	22.22
0.20-0.30	14	15.56	2	2.22
0.301-0.40	4	4.44	0	0.00
0.401-0.50	3	3.33	0	0.00
0.501-0.06	-	-	3	3.33
0.601-0.07	-	-	5	5.56
0.701-0.08	-	-	10	11.11
0.801-0.09	-	-	10	11.11
0.901-1.00	-	-	11	12.22
> 1.00	-	-	29	32.22
Total	90	100	90	100

Table 4 The mean of S/P ratio in different age.

	Control	Vacci-nation	SE mean	*p* value
21 days old	0.082	0.772	0.035	< 0.001
35 days old	0.117	1.176	0.036	< 0.001
49 days old	0.123	0.739	0.043	< 0.001

3.5 Comparison the Average Log-Titer

There was similarities with S/P ration, yet, the average of log-titer of control and vaccination group was significantly different in all those 3 age-categories ($p < 0.001$), and the vaccination group had higher S/P ratio than control (Table 5).

3.6 Risk for Control and Vaccination Group

According to Table 6 it showed that at 2 weeks after first vaccination (21 days old), the control group seemed to have higher percentage of getting risky when disease is introduced into the flock, 93.33%, 28 times higher than vaccination group. In addition, if the control group was eliminated or vaccinated then we can protect this group up to the level of AF (Attributable Fraction), 96.43% (Table 6).

Table 5 The mean of log-titer in different age.

	Control	Vacci-nation	SE Mean	*p* value
21 days old	1.31	3.28	0.083	< 0.001
35 days old	1.40	3.49	0.155	< 0.001
49 days old	1.58	3.01	0.175	< 0.001

Table 6 The risk of ND at 2 weeks after first vaccination (21 days old).

Treatment	Positive	Negative	Total	Risk (%)
Control	6	84	90	93.33
Vaccination	87	3	90	3.33
RR	28.00			
CI 95%	9.19-85.30			
AF (%)	96.43			
p value	<0.0001			

RR: Risk Ratio, AF: Attributable Fraction, CI: Confident Interval.

Table 7 The risk of ND at 2 weeks after second vaccination (35 days old).

Treatment	Positive	Negative	Total	Risk (%)
Control	19	71	90	78.89
Vaccination	79	11	90	12.22
RR	6.46			
CI 95%	3.67-11.34			
AF (%)	84.51			
p value	< 0.0001			

RR: Risk Ratio, AF: Attributable Fraction, CI: Confident Interval.

Table 8 The risk of ND at 4 weeks after second vaccination (49 days old).

Treatment	Positive	Negative	Total	Risk (%)
Control	21	69	90	76.67
Vaccination	70	20	90	22.22
RR	3.450			
CI 95%	2.31-5.16			
AF (%)	71.01			
p value	< 0.0001			

RR: Risk Ratio, AF: Attributable Fraction, CI: Confident Interval.

At 2 weeks after second vaccination (35 days old), the control group seemed to have higher percentage of getting risky when disease is introduced into the flock, 78.89%, which seemed to be lower than at 21 days, 6.46 times higher than vaccination group. In addition, if the control group was eliminated or vaccinated then we can protect this group up to the level of AF, 84.51% (Table 7).

At 4 weeks after second vaccination (49 days old), the control group seemed to have higher percentage of getting risky when disease is introduced into the flock, 76.67%, and seemed to be lower than at 21 days and 35 days, 3.45 times higher than vaccination group. In addition, if the control group was eliminated or vaccinated then we can protect this group up to the level of AF, 71.01% (Table 8). This value was lower than chicken at 21 days and 35 days.

4. Discussion

In our finding, even the birds were vaccinated 2 times, but there were around 3.33% to 22.22% of birds that could not detect the antibody, and it increased the risk when the birds became older. In reality, the vaccines cannot be expected to provide 100% protection for birds/flocks vaccinated under field conditions [9]. This challenge may be related with fact that the virulent ND virus exists as endemic in many countries around the world despite the application of billions of doses of vaccination due to numerous factors that may affect the effectiveness of vaccination [10].

Herd immunity is another a successful vaccination program since it provided some protection to infection of a population with vaccinated individuals as well as unvaccinated individuals [11]. However, this efficiency is only achieved when greater than 85% of the flock have HI (Hemagglutination Inhibition) antibody titers greater than 8 after two vaccinations [5]. The field result suggests that the protection from disease correlated with the presence of antibody titers [12].

5. Conclusion

In conclusion, the vaccination 2 times, at 7 days and 21 days had higher development of antibody against ND, but it was decreased when chicken get old, especially when reaching 49 days old. Through this finding, for practicing with local chicken with long cycle production, the third vaccination should be considered to increase flock immunity.

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