

# ASSESSMENT OF *Ornithobacterium rhinotracheale* (ORT) ANTIBODY TITER FLUCTUATION ON SOME CHICKEN BREEDS AFTER VACCINATION WITH ORNITIN TRIPE

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## Abstract

This study aimed to investigate *Ornithobacterium rhinotracheale* antibody titres (ORT Ab) in 4 different broiler breeds (Noi, Ac, Tre, and Luong Phuong) and different types of chickens (layers and broilers) after vaccination with Ornitin Triple vaccine from the data of field trials from the year 2019 to 2021. Four different broiler breeds were taken 10 – 20 blood sample at 3 time points: 0 week (before vaccinating), 2 weeks and 4 weeks (after vaccinating). Isa Brown layer were monitored 10 blood samples for ORT Ab titer after vaccinating at 8, 17, 27, 37, 47 and 57 weeks after the first dose. The results showed that the ORT Ab in 4 different broiler breeds post-vaccination was significantly higher than pre-vaccination ( $P < 0.001$ ). After 2 weeks of vaccination, the ORT Ab of Ac breed was significantly lower than the other breeds with 14,106 titers. However, 4 weeks after vaccination, the ORT Ab of Luong Phuong (24,576) was the lowest; The ORT Ab of Noi (44,574) and Ac (41,175) chicken breeds had higher than Tre (31,701 titers) and Luong Phuong chickens ( $P < 0.001$ ). In addition, ORT Ab in vaccinated Isa Brown groups after 8 weeks was significantly higher than in the control group (1736 titers), reached 18,078 in vaccinated at 8 weeks old group, and average titers of 28199 in vaccinated at 8 and 12 weeks old. At 65 weeks of age, ORT Ab remained high at 15,891 and there was no difference when using a single shot versus with booster shots Ornitin Triple vaccine. In summary, ORT Ab was a different across chicken breeds and it has been last longer more than 65 weeks of age in Isa Brown layer.

**Keywords:** ORT; ORT vaccine; *Ornithobacterium rhinotracheale* antibody titer; Isa Brown layer; local broiler

## 1. Introduction

Respiratory infections in chickens have been the most serious group of diseases and were accompanied by increasing mortality and treatment costs, drop in egg production, and decreasing in eggshell quality. *Ornithobacterium rhinotracheale* (ORT) is a Gram-negative, discovered in the 1990s, contributed to the exacerbation of respiratory diseases in poultry (Hafez et al. 2014; Vandamme et al., 1994) and caused heavy economic losses in the poultry industry (Van Empel & Hafez, 1999). To control the disease, it is necessary to use the ORT vaccine appropriately. Vaccination was to induce high levels of immunity to protect birds in the face of disease challenges (Bermudez, & Stewart- Brown, 2008). Nowadays, in Vietnam Ornitin triple vaccine (Phibro Animal Health, USA) with an oil emulsion adjuvant was the first and only vaccine used to prevent disease caused by ORT. Various epidemiological methods are available for the investigation of diseases caused by ORT. In particular, the ELISA technique has been proven effective in monitoring the immune status and quantifying the antibody titer (Ab titer) against ORT in chicken (Van, & Hafez, 1999). The commercial kit, IDEXX ORT Ab Test, USA was used to diagnose ORT Ab titer in serum samples. According to the manufacturer's instructions, samples with Ab titer  $\leq 844$  (group 0) were negative, and samples with Ab titer  $> 844$  (groups 1 to 18) were positive (Table 1). However, the manufacturer had

not given the level of ORT Ab titer that could protect chickens after vaccination and depended on breeds or lifetime. The level of ORT Ab is one of the methods to monitor chickens infected with ORT naturally infected or ORT vaccination. When chickens were infected with ORT bacteria naturally, the Ab titer changed sharply from group 1 to group 18 and depended on the age of chickens (Idexx, USA) Chickens with Ab titer average from group 6 showed signs of respiratory disease, dropped in egg production and reduced of eggshell quality. In contrast, chickens with an Ab titer average around group 3 did not show respiratory disease (Le et al., 2019). However, in Vietnam from the year 2019 until now only some research on local broiler breeds to determine the appropriate injection age, dose, and route. Moreover, injection site reaction and broiler performance were investigated. However, study of active immune response (antibody titer) post-vaccination in chickens remains limited. Therefore, it is necessary to investigate Ab titers after being vaccinated with Ornitin Triple on different chicken breeds and at different ages to assess the immune response of different chickens.

## 2. Materials and Methods

### 2.1. Experiment design

The survey was carried out on 4 different local chicken breeds including Noi, Tre, Ac, Luong Phuong, and Isa Brown laying hens. The ORT vaccine used in this study was “Ornitin Triple” consisting of inactivated ORT 10<sup>9</sup> CFU serotype A, 10<sup>9</sup> CFU serotype B, and 10<sup>9</sup> CFU serotype C in 0.25 mL in a water-in-oil emulsion. It was stored in the dark at 4°C and brought to room temperature 4 to 6 h before use and administered to chickens by subcutaneous injection, dose 0.25 mL. Chickens were monitored for antibody titer after being vaccinated with the Ornitin Triple vaccine.

**Table 1.** Grouping of ORT antibody titer (IDEXX, USA)

Group	0	1	2	3	4	5	6	7	8	9
Ab titer	0-844	845 -999	1000 -1999	2000 -2999	3000 -3999	4000 -4999	5000 -5999	6000 -7999	8000 -9999	10000 -
										11999
Group	<b>10</b>	<b>11</b>	<b>12</b>	<b>13</b>	<b>14</b>	<b>15</b>	<b>16</b>	<b>17</b>	<b>18</b>	
Ab titer	12000 -13999	14000 -	16000 -	18000 -	20000 -	22000 -23999	24000 -	28000 -	≥32000	
		15999	17999	19999	21999		27999	31999		

### 2.2. ORT antibody in different broiler breeds

This survey monitored 4 common slowly growing broiler breeds raised in Vietnam including Noi, Tre, Ac, and Luong Phuong. Chickens were vaccinated with the Ornitin Triple vaccine as recommended by the manufacturer. Broiler lifetime was much shorter than a layer, therefore, the serum of these breeds was taken at 3-time points: 0 week (before vaccinating), 2 weeks, and 4 weeks (after vaccinating) to monitor Ab titer anti ORT. The experiment design for broiler breeds was presented in Table 2.

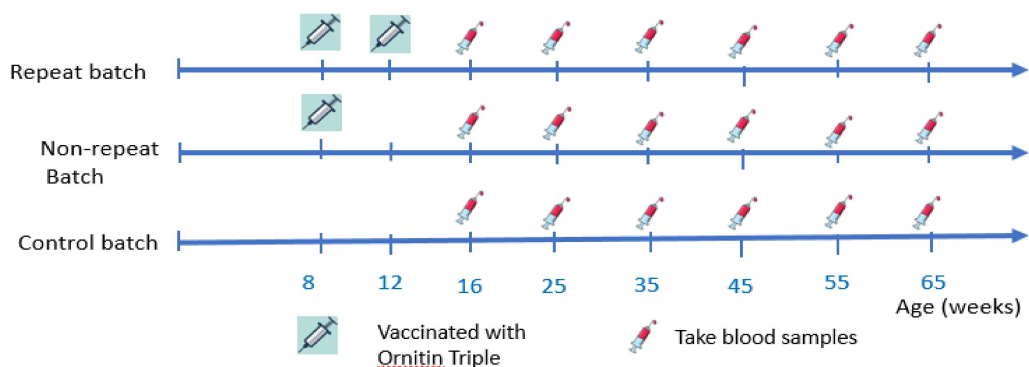
**Table 2.** The ORT antibody titer of Noi, Tre, Ac and Luong Phuong broiler breeds.

Breeds	Noi	Tre	Ac	Luong Phuong
Number of chickens	20	39	32	20
Number of blood samples	10	20	10	10
Number of sampling times	3	3	3	3

### 2.3. ORT antibody in Isa-Brown Layer

The ORT Ab titer on Isa Brown laying hens was monitored up to 65 weeks of age with 6 sampling times. In addition, this experiment also aimed to compare the Ab titer between repeated and non-repeated vaccination groups in order to evaluate the necessity of booster vaccination in laying hens. The experiment was arranged in 1 factor completely randomized

with 3 batches (Figure 1). The control batch was not vaccinated with Ornitin Triple. Batch 1 was immunized once at 8 weeks of age. Batch 2 was vaccinated with Ornitin Triple twice (repeat) at 8 and 12 weeks of age. Birds were monitored for ORT Ab titer after vaccinating during the laying period at 8, 17, 27, 37, 47, and 57 weeks after the first dose. The experimental design of laying hens was shown in Table 3.



**Figure 1.** The experimental design on laying hens.

**Table 3.** The ORT antibody titer of Isa Brown laying chicken.

Experimental batch	Control batch	Non-repeated vaccination (Batch 1)	Repeated vaccination (Batch 2)
Ages when Ornitin Triple was vaccinated	None	8 weeks age	8 and 12 weeks age
Number of layer chickens	50	50	50
Number of blood samples	10	10	10
Number of sampling times	6	6	6

### 3. Results and discussion

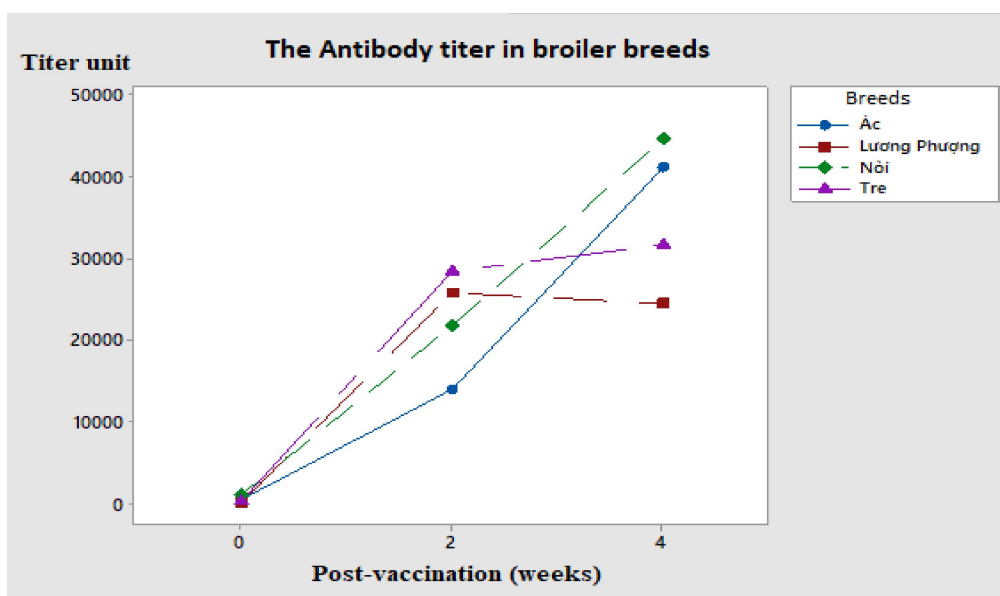
#### 3.1. ORT antibody in different broiler breeds

Before being vaccinated with the Ornitin Triple vaccine, the antibody titer of all 4 broiler breeds was low and almost negative with ORT ( $< 844$ ). The results of Ab titer in the post-vaccination on the broiler were presented in Table 4. Two weeks after being vaccinated with Ornitin Triple, Ab titer of all chicken breeds reached high levels. The Ab titer of Tre, Luong Phuong, Noi, and Ac breeds were 28478, 25745, 21908, and 14106, respectively (from group 11 to group 17). In which, the Ab titer of the Tre breed was the highest and the Ab titer of the Ac breed was the lowest. The Ab titer of the Ac breed (14106, group 11) was lower than other breeds significantly ( $P < 0,05$ ). At this stage, the Ab titer of Tre, Luong Phuong, and Noi breeds was not different ( $P > 0,05$ ). Four weeks after vaccinating Ornitin Triple, the Ab titer of these breeds was increased continually. The Ab titer of Noi and Ac were higher than Tre and Luong Phuong breeds significantly ( $P < 0,001$ ). The Ab titer of Noi and Ac breeds were 44574 and 41175 (group 18) respectively. Meanwhile, the Ab titer of Tre and Luong Phuong breeds increased slowly and reached 31701, and 24576 (groups 17 and 16). In brief, the Ab titers of these breeds got peak titers 4 weeks after vaccination.

**Table 4.** The Antibody titer in different broiler breeds after vaccinated with Ornitin Triple

Post-vaccination (weeks)	Statistical parameters						P-value
	Breeds	N	Mean	SE	SD	CV(%)	
0	Noi	10	1089 <sup>a</sup>	228	722	66.30	0.010
	Tre	20	377 <sup>b</sup>	168	751	199.19	
	Ac	10	543 <sup>ab</sup>	148	467	85.91	
	Luong Phuong	10	177.0 <sup>b</sup>	41.8	132.3	74.73	
2	Noi	10	21908 <sup>ab</sup>	3257	10301	47.02	0.002
	Tre	20	28478 <sup>a</sup>	2255	9829	34.51	
	Ac	10	14106 <sup>b</sup>	2030	6089	43.16	
	Luong Phuong	10	25745 <sup>a</sup>	1884	5957	23.14	
4	Noi	10	44574 <sup>a</sup>	3228	10209	22.90	0.000
	Tre	20	31701 <sup>b</sup>	1579	7062	22.28	
	Ac	10	41175 <sup>a</sup>	2562	8103	19.68	
	Luong Phuong	10	24576 <sup>b</sup>	791	2501	10.18	

Note: <sup>ab</sup>Means within a column with different superscripts differ ( $P < 0.05$ )



**Figure 2.** The Antibody titer in broiler breeds after vaccinating with Ornitin Triple.

Two weeks after vaccination, the weight gain of Tre and Luong Phuong breeds were reduced significantly. Meanwhile, the weights of Noi and Ac breeds were not affected until slaughter (Nguyen et al., 2020; Le, 2020). The immune response of Tre and Luong Phuong breeds were rapid, strong and almost peaking at 2 weeks after vaccination. Consequently, it may affect their weight gain negatively. In contrast, the weights of Noi and Ac breeds were not affected as the immune response increased more slowly and increased about 4 weeks after vaccination. Besides, when ORT vaccine had not been used in Vietnam, at Ninh Thuan province, 75% of broiler chickens were infected ORT bacteria naturally. The ORT Ab titer in broiler chickens infected from the environment was only 2671 (group 3) (Le et al., 2019). Therefore, it could be seen that the Ab titer of vaccinated broiler chickens were much higher than naturally infected chickens ( $P < 0.001$ ).

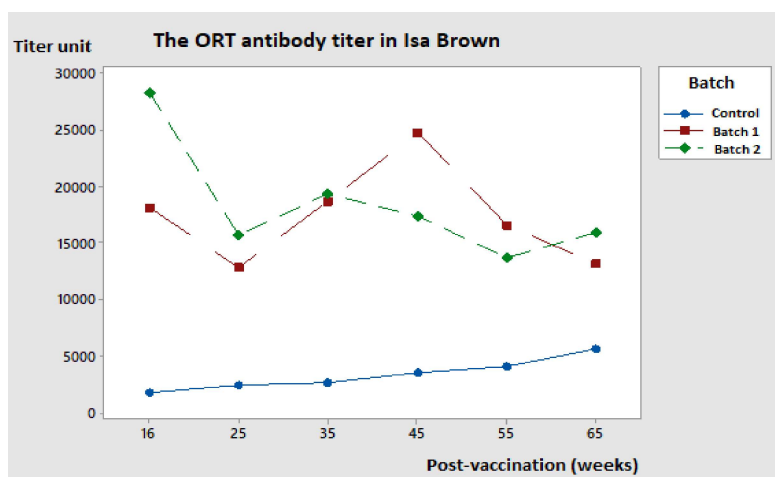
### 3.2 ORT antibody in Isa Brown Layer

The lifetime of layers extended to after 65 weeks of age. The ORT Ab titer was monitored until the end of the laying period. The Ab titer in layer was shown in Table 5. At 8 weeks after the first dose Ornitin Triple vaccine, the Ab titer batch 1 and batch 2 were 18078 and 28199 (groups 13 and 17) respectively. The Ab titer of repeated vaccination batch was higher than non-repeated one significantly ( $P < 0.001$ ). Around this same time, 6 weeks after the first dose of ORT vaccine, (Murthy et al., 2007) showed that the layer chickens would have good immune response with repeated vaccination. Furthermore, repeated vaccination against ORT is recommended in laying hens to maintain Ab titer until the end of laying period (Van, & Van, 1998)

**Table 5.** The ORT antibody titer in Isa Brown laying hens after vaccinated with Ornitin Triple.

Post-vaccination (weeks)	Statistical parameters						
	Batch	N	Mean of Ab titer	SE	SD	CV(%)	P-value
8	Control	15	1736 <sup>c</sup>	147	568	32.69	0.000
	Batch 1	15	18078 <sup>b</sup>	2863	11090	61.34	
	Batch 2	15	28199 <sup>a</sup>	1456	5641	20	
17	Control	10	2499 <sup>b</sup>	332	1049	41.96	0.000
	Batch 1	10	12760 <sup>a</sup>	2052	6490	50.86	
	Batch 2	10	15622 <sup>a</sup>	1814	5738	36.73	
27	Control	10	2620 <sup>b</sup>	510	1612	61.51	0.000
	Batch 1	10	18590 <sup>a</sup>	2746	8682	46.7	
	Batch 2	10	19254 <sup>a</sup>	2728	8627	44.8	
37	Control	10	3596 <sup>b</sup>	834	2637	73.34	0.000
	Batch 1	10	24709 <sup>a</sup>	2580	8157	33.01	
	Batch 2	10	17333 <sup>a</sup>	2551	8066	46.54	
47	Control	10	4063 <sup>b</sup>	644	2035	50.1	0.001
	Batch 1	10	16512 <sup>a</sup>	2981	9427	57.1	
	Batch 2	10	13714 <sup>a</sup>	2217	7012	51.13	
57	Control	10	5684 <sup>b</sup>	947	2996	52.71	0.008
	Batch 1	10	13105 <sup>ab</sup>	2405	7606	58.04	
	Batch 2	10	15891 <sup>b</sup>	2792	8830	55.57	





**Figure 3.** The ORT antibody titer in Isa Brown laying hens after vaccination with Ornitin Triple.

However, at subsequent sampling times until chickens reached 65 weeks of age, there was no difference between vaccination in the 2 experimental batches ( $P > 0.05$ ). In batch 1, the Ab titer changed at different sampling times and reached the highest at 37 weeks post-vaccination (24709, group 16). Besides, the Ab titer in batch 2 was highest at 8 weeks post-vaccination, then gradually decreased and maintained until the end of the laying period. The Ab titer at 17, 27, 37, 47 and 57 weeks post-vaccination of batch 1 and batch 2 were 12760, 18590, 16512, 13105. and 15622, 19254, 17333, 13714, 15891, respectively ( $P > 0.05$ ). On the other hand, a field survey showed that 100% of layer chickens in Ninh Thuan were infected with ORT bacteria and their Ab titer was 6,097 titer units. Layers infected with ORT naturally showed signs of dropped in egg production and reduced of egg shell quality. (Le et al, 2019). After vaccinated with Ornitin Triple, the Ab titer of control batch increased significantly during the experimental period. Also, the control layers were infected with ORT from the environment. The Ab titer of vaccinated layer were higher than non-vaccinated one significantly. In brief, the Ab titer of repeated vaccination laying was significantly higher than single injection at 8 weeks after the first vaccination but the rest of the time of layer, there were no difference between two-batch experiment.

#### 4. Conclusion

The antibody titer on vaccinated chickens with Ornitin Triple was higher than non-vaccinated one significantly ( $P < 0.001$ ). For broilers, 2 weeks after vaccination, the Ab titer of Ac breed was lower than other breeds significantly. However, 4 weeks after vaccination, the Ab titer of Luong Phuong breed was the lowest. The Ab titer of Noi and Ac breeds were higher than Tre and Luong Phuong ( $P < 0.001$ ). Besides, Ab titer of Isa Brown layer was maintained high until 65 weeks of age. At 8 weeks after the first vaccination, the Ab titer of two dose – treatment was higher significantly than the one dose - treatment ( $P < 0.05$ ). However, at subsequent stages until the end of the laying period, the difference disappeared. In brief, The ORT Ab titer was variable in different chicken breeds and could last up to 65 weeks of age.

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