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PRODUCTION AND CHARACTERIZATION OF NDV IGY FROM TWO DIFFERENT CHICKEN BREEDS

Salwa Mohamed Hezema[1], Samy Kasem[2], Emad Eldin Elgendy[1], Madiha Salah Ibrahim[1*]

[1]Department of Microbiology, Faculty of Veterinary Medicine, Damanhour University, Damanhour, Egypt.
[2]Department of Virology, Faculty of Veterinary Medicine, Kafr ElShiek University, Kafr ElShiek, Egypt.

***Corresponding Author: Madiha Salah Ibrahim**

Department of Microbiology, Faculty of Veterinary Medicine Damanhour University, Damanhour, Egypt.

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ABSTRACT

This study aimed to prepare hyper-immune chicken IgY against Newcastle disease virus (NDV) and studying its immunogenicity against field isolates of NDV. Ten white Hy-line layers, and ten local house breeds were used. The birds received an initial immunization followed by two booster doses at time intervals of 2 weeks. Throughout the experiment (8-11) weeks, serum samples were collected weekly with daily egg collection. The purified IgY specificity and titers in sera and egg yolk were determined using HI and neutralization tests against NDV vaccinal strain and field isolates. Significant titers of IgY were detected against NDV with comparable neutralization efficacy for the two breeds. Thus, both hen breeds can be efficiently used for production of protective IgY against NDV fieldisolates.

KEYWORDS: NDV, IgY, HI, Neutralization test.

1. INTRODUCTION

IgY is an immunoglobulin in egg yolk that is transferred from the maternal circulation to confer passive immunity to embryos and neonates (Rose et al., 1974). The amount of IgY transported is known to be proportional to the maternal serum IgY concentration (Loeken and Roth, 1983). IgY technology was approved by animal welfare organizations and workshops such as the Veterinary Office of the Swiss Government (Office Vétérinaire Fédéral) and the European center for the validation of alternative methods (ECVAM) workshop to be used to support animal welfare (Schade, 1997). IgY has been widely used nowadays for immunodiagnostics and/or for treatment and prevention of infections in humans and animals (Miller, 2010.).

Recently, passive immunization using chicken egg yolk (IgY) has become an attractive approach with considerable attention as it possesses a variety of advantages over mammalian IgG as convenience, high yield, effectiveness and low cost (Carlander et al., 2000).). Therefore, the vast amount of available antibodies open the door for new fields of IgY application, such as immunotherapy and immune prophylaxis for several bacterial and viral infections in both human and veterinary fields (Losch et al., 1986; Schade and Hlinak, 1996; Muhammed, et al., 2001; Carlander, 2002).

Newcastle disease (ND) is one of the most important viral diseases (Orsi et al., 2010) affecting wild and domestic avian species (Seal et al., 2000 and Alexander, 2000) causing respiratory and nervous disorders in several species of poultry including chicken and turkeys (Lancaster, 1976). NDV is highly contagious, with high morbidity and mortality in susceptible birds (up to 100%) (OIE, 2012). Notification is required by OIE of any outbreak of ND (Cao et al., 2013). Vaccination for protecting chicken against ND is routinely practiced throughout the world, but the vaccination is ineffective with the high titer of maternal antibodies (Rahman, et al., 2002; Marangon, and Capua, 2006), thus, alternative protection early in chicks' life is of great importance. Therefore, our study aimed to prepare hyperimmunized eggs from two different chicken breeds and evaluate the different levels of IgY production among both breeds as well as assessing the neutralizing and detection capabilities of the produced IgY against NDV.

2. MATERIAL AND METHODS

2.1. Chicken

Ten local house breed (28 weeks) and ten white Hy-line (48 weeks) laying hens were used. Hens were purchased from Al-madina farm for layers, Cairo-Alexandria desert road and housed in animal facility joined with the Department of Microbiology, Faculty of Veterinary Medicine, Damanhour University. They were used for immunization and kept in separate cages. The hens received commercial rations (110g per hen) and water ad libitum.

2.2. Vaccine and adjuvant
Live attenuated laSota vaccine with HA titer 2^9 was used for vaccination **(KBNP, INC., Korea)**. Complete and incomplete Freund's adjuvants were obtained from Sigma-Aldrich US.

2.3. Experimental design and immunization schedule
Hens were subcutaneously immunized with an initial dose of 300µl of Lasota virus vaccine emulsified with equal volume of Freund's complete adjuvant, then subsequently followed by three subcutaneous booster doses using incomplete Freund's adjuvant **(Nahla et al., 2018)**.

2.4. Serum Samples
Blood samples were collected regularly every week. Serum samples were collected and stored at -20^{o}C for detection of specific antibodies. The eggs were daily collected throughout the entire period of the experiment (11 weeks) and stored at 4^{o}C.

2.5. Extraction of egg yolk and Purification of IgY
The egg shell was sterilized and broken then the yolk was separated. The egg yolk was washed with sterile double distilled water without puncturing of yolk membrane. Yolk contents were collected in a sterile container. Egg yolk was diluted 10 times in sterile PBS (Yokoyama et al., 1998). The IgY was purified from the egg yolk by direct freezing and thawing according to Jensenius and Koch, (1997). The supernatant containing IgY was filtered and stored at -20^{o}C till used.

2.6. Hemagglutination inhibition test (HI)
The HI test using the serum and the purified IgY was carried out according to OIE, (2015).

2.7. Neutralization test
Neutralization test was carried out according to the method adopted by Ohishi et al., (1994). Briefly, ten-fold serial dilutions of purified IgY were incubated in equal volumes with 100 EID_{50} virus (LaSota virus vaccine) and incubated for 60 minutes at 37°C then each dilution was inoculated into the allantoic cavity of five embryonated eggs. End- points of the IgY titers were determined according to Reed and Muench, (1938). They were expressed as reciprocals of log10 dilutions.

2.8. Field samples testing
Field samples from chickens (lungs) with NDV infection clinical and postmortem picture were collected from infected farms in Damanhour city, Beheria Government. Lung samples were ground, purified then inoculated in 9-11-day old chicken embryo eggs WHO, (2002). Allantoic fluid was collected and processed to HI test using the purified IgY. Two lung samples were ground, purified and were directly used in HI test using the purified IgY.

3. RESULTS
3.1. Evaluation of serum and yolk antibody titer from immunized chicken breeds against NDV
The antibody titer against NDV in serum and egg yolk were measured in white-Hy line breed (WHL) and local house breed (LB) by HI test before immunization and weekly thereafter. Serum antibody titer of the pre-immunized LB was 2^6, which increased after the initial immunization to 2^9 at the 2^{nd} and 3^{rd} weeks. Thereafter, the titer decreased to 2^6 and 2^4 at 4^{th} and 5^{th} week, respectively. After the 2^{nd} booster dose at the 6^{th} week the titer increased to 2^{12}, 2^{12} and 2^{11} at 7 th, 8^{th} and 9^{th} week, respectively (Table 1 and Fig. 1). In contrast, the yolk antibody titer of the pre-immunized LB was 2^6, which increased after immunization to 2^7 at 4^{th} week after the initial dose by 2 weeks and then decreased gradually to 2^6, 2^6, 2^5, 2^4 and 2^5 at 5^{th}, 6^{th}, 7^{th}, 8^{th} and 9^{th} week, respectively as shown in (Table 1 and Fig. 1).

On the other hand, the serum antibody titer of the pre-immunized WHL was 2^7, which increased to 2^9 at 2^{nd} week then 2^8 at the 3^{rd} week after the initial immunization. Then decreased to 2^6 at 4^{th} week and re-increased after 1^{st} booster dose and 2^{nd} booster dose to 2^{10}, 2^{11} and 2^{12} at 5^{th}, 6^{th} and 7^{th} week, respectively. Thereafter the titer decreased to 2^{11} at 8^{th} and 9^{th} week and to 2^{10} at 10^{10} and 11^{th} week **(Table 1 and Fig. 2)**. On the other hand, the pre-immunized yolk antibody titer of WHL was 2^2, which increased to 2^5 at 2^{nd} and 3^{rd} week after the initial immunization. Thereafter the titer increased to 2^8 at 4^{th} and 5^{th} week after the 1^{st} booster dose. The titer increased again from 2^6 at 6^{th} week to 2^8 at 7^{th} week after 2^{nd} booster then decreased to 2^4, 2^6, 2^5 and 2^5 at 8^{th}, 9^{th}, 10^{th} and 11^{th} week, respectively **(Table 1 and Fig. 2)**.

Table 1: Post-immunization serum and yolk antibody titers by HI test.

Week	White HY-line breed		Local house breed	
	Samples	HI Antibody titers	Sample	HI Antibody titer
Pre-immunization titer week 1	Yolk Serum	2^2 2^7	Yolk serum	2^6 2^6
Week 2 (Initial dose)	Yolk Serum	2^5 2^9	Yolk serum	2^6 2^9
Week 3	Yolk Serum	2^5 2^8	Yolk serum	2^6 2^9
Week 4 (1st booster dose)	Yolk Serum	2^8 2^6	Yolk serum	2^7 2^6
Week 5	Yolk Serum	2^8 2^{10}	Yolk serum	2^6 2^4
Week 6 (2nd booster dose)	Yolk Serum	2^6 2^{11}	Yolk serum	2^6 2^5
Week 7	Yolk Serum	2^8 2^{12}	Yolk serum	2^5 2^{12}
Week 8	Yolk Serum	2^4 2^{11}	Yolk serum	2^4 2^{12}
Week 9	Yolk serum	2^6 2^{11}	Yolk serum	2^5 2^{11}
Week 10	Yolk serum	2^5 2^{10}		
Week 11	Yolk Serum	2^5 2^{10}		

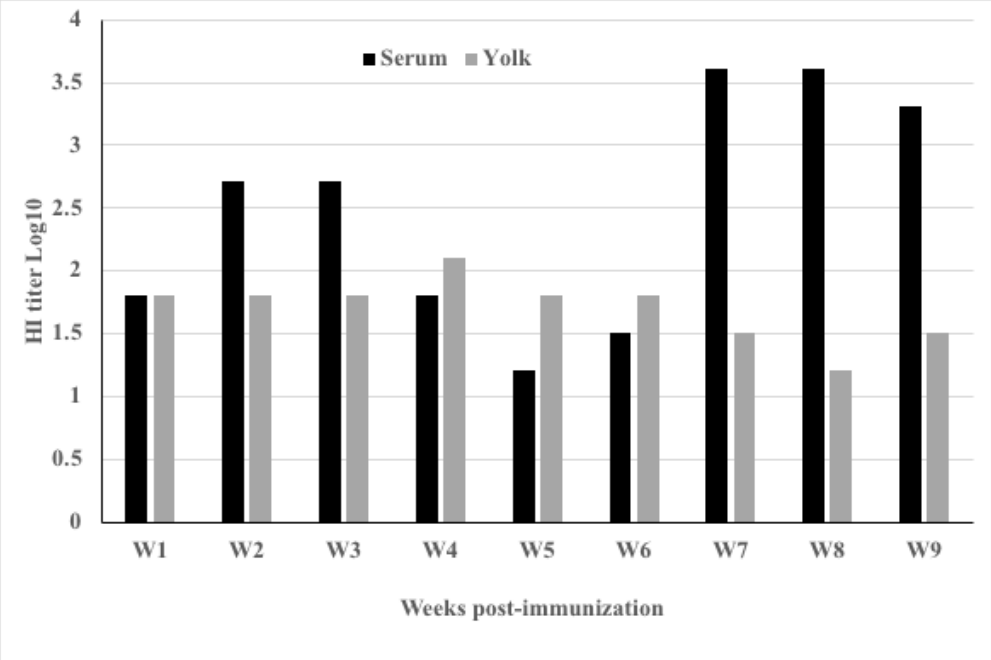

Figure 1: Serum and yolk antibody titers of LB following immunization.

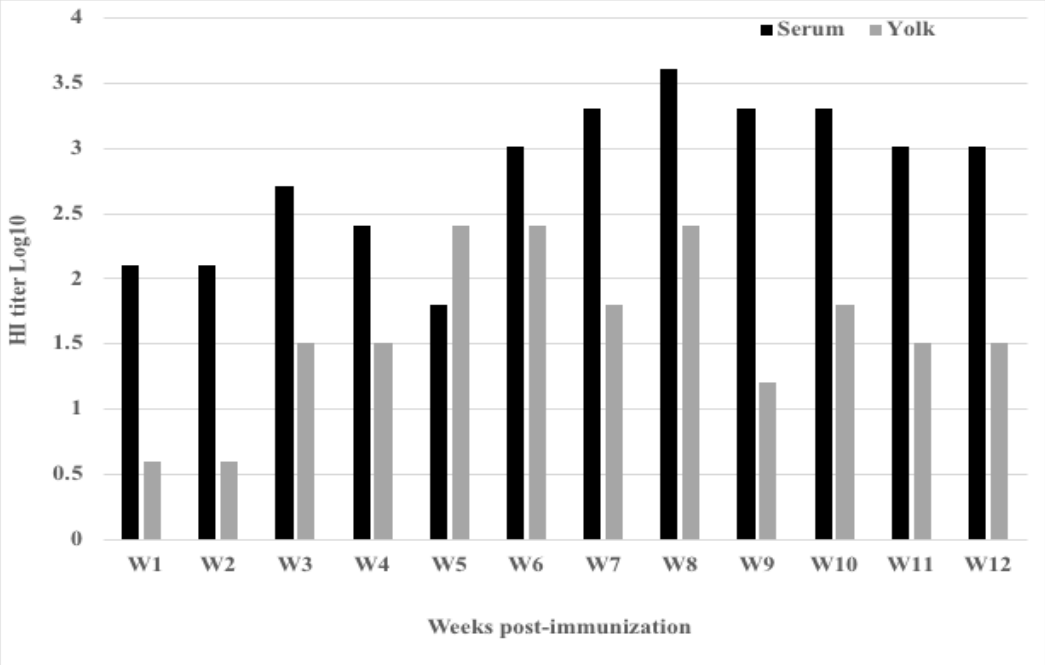

Figure 2: Serum and yolk antibody titer of WHL following immunization.

3.2. Antibody transfer from serum to yolk against NDV in LB and WHL

The antibody transfer percent ranged from 0.39% to 400% in LB, with the highest percentage on the 5th week after the first booster dose and lowest percent at 8th week (Table 2 and Fig. 3). While, the antibody transfer percent ranged from 0.78% to 400% in WHL, with the highest percentage on 4th week (two weeks after initial immunization) and lowest percent at 8th week as shown in Table (2) and Fig. (3). The results showed that the highest transfer in LB was during 4th, 5th and 6th week while, for WHL was during the 4th week only.

Table (2): Percentage of antibody transfer against NDV in LB and WHL breeds

Week	LB	WHL
Pre-immunization titer (w1)	100%	3.125%
Week 2 (Initial dose)	12.5%	6.25%
Week 3	12.5%	12.5%
Week 4 (1st booster dose)	200%	400%
Week 5	400%	25%
Week 6 (2nd booster dose)	200%	3.125%
Week 7	0.78125%	6.25%
Week 8	0.390625%	0.78125%
Week 9	1.5625%	3.125%
Week 10		3.125%
Week 11		3.125%

Percentage of transfer of antibodies from the serum to the egg yolk = yolk antibody titer/serum antibody titer X 100 (**Hamal et al., (2006), Kowalczyk et al., (2019).**

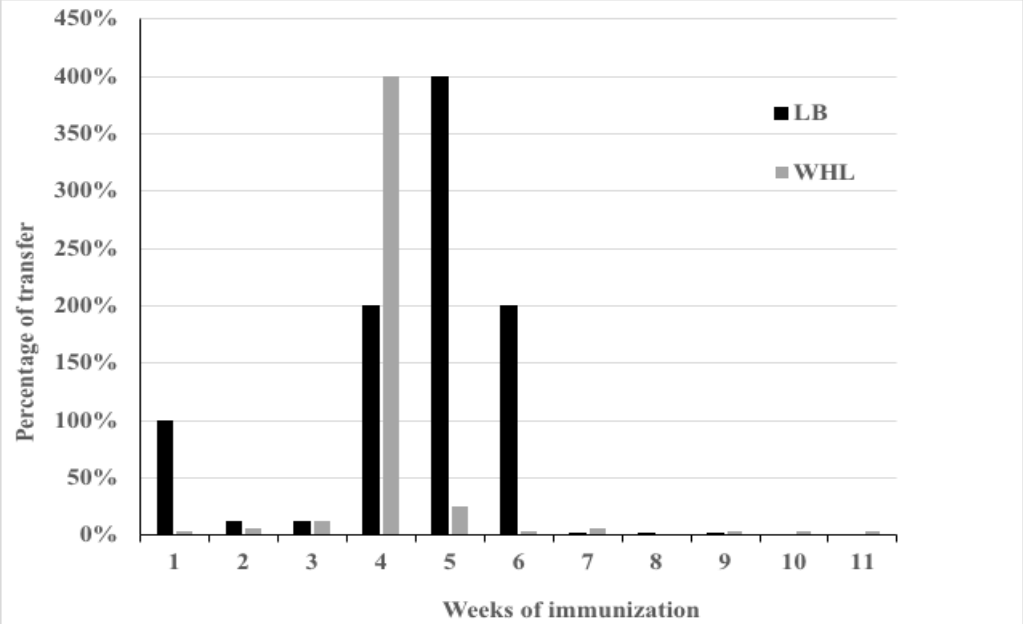

Figure (3): Percentages of antibody transfer from serum to yolk in LB and WHL.

3.3. Specificity and protective efficiency of egg yolk antibody from LB and WHL breeds against NDV

The highest egg yolk antibody titer from LB (2^7 at 4^{th} week) and from WHL (2^8 at 7^{th} week) were tenfold serially diluted. Then different dilutions of the yolk antibody were mixed with NDV ($10^{5.6}$ EID_{50}) then inoculated in ECE to calculate the neutralization index. The results showed that the protection with LB-derived IgY was 100% for 10^{-1} and 10^{-2}, 60% for 10^{-3} and 10^{-4} and 40% for 10^{-5} and 10^{-6}. While, the protection with WHL- derived IgY was 100% for 10^{-1}, 10^{-2} and 10^{-3}, 80% for 10^{-4} and 10^{-5} and 70% for 10^{-6} (**Table 3)**. The neutralization index value for LB-derived IgY was $10^{3.9}$/ml in contrast to $10^{4.2}$/ml for WHL-derived IgY.

Table (3): The percent of LB and WHL egg yolk antibody protection against NDV in ECE

IgY titer	Percent of egg protection in WHL	Percent of egg protection in LB
10^{-1}	100%	100%
10^{-2}	100%	100%
10^{-3}	100%	60%
10^{-4}	80%	60%
10^{-5}	80%	40%
10^{-6}	70%	40%

3.4. Neutralization efficiency of LB and WHL egg yolk antibodies against NDV field isolates

Seven NDV field samples were used to determine the efficiency of egg yolk antibodies produced from both LB and WHL breeds. Lungs were collected and homogenized. Five sample were propagated in embryonated chicken eggs and two isolates were used without previous egg inoculation. The HA titers of the seven samples ranged from 10^3 to 10^{11}. These viruses were neutralized by 2^7 and 2^8 egg yolk antibodies for LB and WHL, respectively. The HI titer of the egg yolk ranged from 10^4 to 10^{10} for LB and WHL, respectively as shown in **Table 4 and Fig. 4**. The LB IgY showed one log higher HI titers as compared to the WHL IgY.

Table 4: Egg yolk antibody titer of LB and WHL against NDV field isolates.

Sample number	Type of sample	Virus titer	Egg yolk antibody titer by HI	
			WHL (2^8)	LB (2^7)
1	Allantoic fluid	10^9	10^6	10^7
2	Allantoic fluid	10^6	10^7	10^8
3	Allantoic fluid	10^7	10^8	10^9
4	Allantoic fluid	10^4	10^4	10^5
5	Allantoic fluid	10^3	10^9	10^{10}
6	Lung	10^{11}	10^{10}	10^{11}
7	Lung	10^{11}	10^9	10^{10}

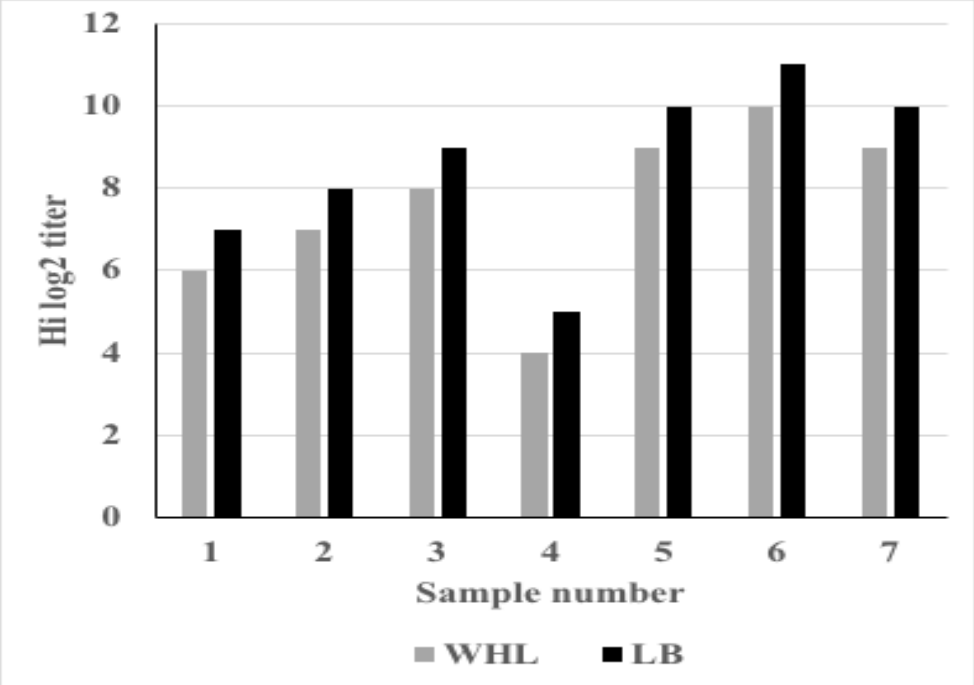

Figure (4): Egg yolk antibody titer of LB and WHL against NDV field isolates. 1-5; allantoic fluid samples, 6 and 7; lung samples.

4. DISCUSSION

laying hens are highly cost-effective as producers of antibodies compared to mammals (Larsson et al., 1993). Average volume of egg yolk (15 mL) contains 50–100 mg of IgY, of which 2%–10% can be of specific antibodies, this is a much higher amount of immunoglobulin that could be obtained by bleeding the animal (Sudjarwo, et al., 2012; Ferreira et al., 2012). The IgY has been applied successfully in diagnostics, prophylactic, therapeutic purposes, immunochemical reagents and in food formulation or supplements due to it is stability under food processing conditions (Raj et al., 2004 and Schade et al., 2006). In our study, IgY was produced from different chicken breeds. The neutralization efficiency by NT in ECE and HI test with laSota vaccine strain and NDV field isolates was tested.

Our results showed that egg yolk antibody titer reached the peak after the initial immunization to 2^7 and 2^8 at the 4th week in LB and WHL, respectively. Furthermore, the serum antibody titer reached a peak after the 2nd booster dose to 2^{12} at the 7th week in both LB and WHL chickens. These results indicate that IgY titer in egg yolk in LB is slightly lower by one log than WHL. It was reported that there are significant differences between breeding groups for antibody titer responses to Newcastle disease (Peleg et al., 1980; Gyles et al., 1986; Atta et al., 1990; Siam et al., 1994; El-Gendy et al., 1995; Li et al., 2001; Hassan et al., 2004; Chang et al., 2011), furthermore, the serum antibody titer decreased when the yolk titer increased, which means that there is a reverse relationship between the serum titer and yolk titer. The serum IgY titer was higher during all weeks of collection except at the 5th week for WHL, and the 4th to 6th week for the LB chicken where the titer was higher in the yolk. This indicates a longer maintenance of high yolk antibody titers in LB than WHL. This was also detected with the antibody transfer where it was much more efficient in the LB (5th and 6th week) as compared to the WHL (4th week) indicating a possible better fitting of LB chicks following hatching, possessing higher levels of protection. However, this may be due to differences in the age, genetic makeup or physiological status between both breeds. This was in contrast to Agrawal et al. (2016) who showed that there are no hen line differences in IgY transfer from serum to yolk.

The neutralization index for WHL ($10^{4.2}$) was slightly higher than that for LB ($10^{3.9}$). In contrast, the results obtained from the neutralization of IgY and field NDV isolates showed that even the IgY titer of LB (2^7) was one log lower than WHL (2^8), the LB IgY showed slightly higher neutralization efficiency than WHL IgY. Marked breed, strain and line differences have been reported for various immunological traits (Cheng and Lamont, 1988; Baelmans et al., 2005). Some investigations were done on the intensity of antibody production and wattle reaction to natural antigens showed that both humoral and cellular immune reactivity to sheep red blood cells were breed dependent in contrast to those against Brucella abortus crude antigen (Benda et al., 1990).

In conclusion, our results indicated that both LB and WHL breeds can produce IgY with high titer and good neutralization efficiency against both laSota virus vaccine and field ND viruses without significant differences. Both IgYs can be possibly used for protection and/or prophylaxis against NDV. Further studies are required to evaluate the protective potentials

of egg antibodies against other types of ND viruses with higher virulence that threat the poultry population in Egypt. Moreover, monitoring egg yolk antibodies can be a good tool for monitoring the maternal vaccination efficiency in a farm without hen interference.

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