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Re-evaluation the immune efficacy of Newcastle disease virus vaccine in commercial laying chickens

Qingsong Han [a,b,1], Xiaolong Gao [a,1], Pengpeng Wu [a,1], Sa Xiao [a], Xinglong Wang [a], Peng Liu [a], Lina Tong [a], Huafang Hao [a], Shuxia Zhang [a], Ruyi Dang [a], Zengqi Yang [a,*]

[a] College of Veterinary Medicine, Northwest A&F University, Yangling 712100, Shaanxi Province, PR China
[b] Department of Animal Science, Wenzhou Vocational College of Scienc & Technology, Wenzhou 325006, Zhejiang, PR China

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ABSTRACT

Newcastle disease virus (NDV) infection causes serious problems in laying chickens, like reducing egg production, increasing rate of abnormal eggs in spite of strict vaccination in layer farms program. A new evaluation system is needed to show complete protection of the immunization in laying chickens based on the egg-laying performance, rather than clinical signs of the disease. In this study, laying chickens with different anti-NDV HI (hemagglutination-inhibition) antibody titer after vaccination were divided into different groups. These chickens were then challenged with field isolated highly virulent NDV strains. Results showed that the chickens in low HI titers group (5 log2 to 8 log2) and medium HI titers group (9 log2 to 11 log2) had atypical symptoms, produced abnormal eggs, and shed virus. Whereas, with HI titers ≥ 12 log2, the chickens were completely protected, and did not show symptoms, or produce abnormal eggs or shed virus. Morbidity, positive viral shedding rate and abnormal egg-rate decreased with increase in pre-challenge HI antibody titer. Our result suggested that 12 log2 is the threshold of the HI antibody in providing complete protection to laying chickens under field condition, and protective efficacy is correlated with HI antibody titer. This study provides a valuable reference for the vaccination and control of ND in poultry.

Newcastle disease (ND) is an important poultry disease caused by Newcastle disease virus (NDV) (Alexander and Senne, 2008). Despite application of strict vaccination policies to control ND in the poultry industry, virulent NDV strains remain sporadically isolated from vaccinated chicken flocks (Lien et al., 2007; Cho et al., 2007; Diel et al., 2012; Rehmani et al., 2015). Although typical clinical signs of infected chickens are observed in vaccinated flocks, subclinical infection often causes a decrease in egg-production performance in layers leading to substantial economic losses. Therefore, a new evaluation system aiming to protect layers against egg drop, should be developed. Humoral antibody response has been proved to play a pivotal role in ND protection (Reynolds and Maraqa, 2000; Kapczynski and King, 2005; van Boven et al., 2008). Field results and European Pharmacopoeia suggested that birds required HI titers ≥8 as protective against mortality (Kapczynski and King, 2005). More commonly, HI levels of 32 or higher were typically thought to be protective (Allan et al., 1978). Whereas, field practice and research results demonstrated that chickens were protected post-NDV challenge against mortality, overt clinical signs, shedding of virus, and abnormal eggs-production could not be completely stopped (Miller et al., 2007). However, whether an HI antibody titer that could protect layers against ND completely exist remains unclear. Also, whether the antibody threshold was needed to stop complete viral shedding and abnormal egg-production under field conditions is uncertain.

One hundred twenty-five 18-week-old commercial hyline brown layer chickens were obtained from a commercially operated chicken farm. All chickens received several vaccines against infectious agents. NDV vaccinations were scheduled with one dose of live LaSota vaccine at 7 days of age via ocular-intranasal inoculation and booster with inactivated oil-emulsion LaSota vaccine at 8 weeks and 15 weeks of age via intramuscular injection. The NDV antibody form chicken sera were tested by HI and blocking ELISA (Shanghai Enzyme-linked Biotechnology Co., Ltd. The Clone30 was used as coating antigen). The chickens were divided into three experiment groups and one control group based on NDV-specific HI antibody titers (Tested using LaSota as 4 HAU), namely group L (5 log2 ≤ HI ≤ 8 log2, n = 40) (1231–3903 pg/ml by ELISA), group M (9 log2 ≤ HI ≤ 11 log2, n = 40) (4719–6807 pg/ml by ELISA), group H (12 log2 ≤ HI ≤ 15 log2, n = 40) (7779–13,380 pg/ml by ELISA), and group control (6 log2 ≤ HI ≤ 7log2, n = 5) (2361–2986 pg/ml by ELISA). To confirm the robust of HI antibody tier, twenty unimmunized chickens, which were obtained from the same farm and reared in isolators, were also

* Corresponding author.
E-mail address: yzq1106@nwsuaf.edu.cn (Z. Yang).
[1] These authors contributed equally to this work.

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used to test HI titers and the HI titer were between 0 and 2. The HI titers are highly correlated with ELISA antibody titer ($R^2 = 0.9828$) (Fig. 1A). Chickens in groups L, M and H, respectively, were further divided into two subgroups with 20 birds each, viz., LY and LC, MY and MC, HY and HC. Chickens in groups LY, MY, and HY were challenged with $10^{5.3}$ EID_{50}/100 μl of Yulin strain (Accession No. KP027403; ICPI 1.838; Genotype IX) via eye drop (50 μl) and intranasal (50 μl) routes, while chickens in groups LC, MC and HC were challenged with $10^{5.8}$ EID_{50}/100 μl of Chang'an strain (Accession No. KP027404; ICPI 1.60; Genotype IX). The control group were challenged with 100 μl of PBS. All chickens were monitored daily for clinical signs and mortality. Blood samples were collected at 0, 3, 6, 9, 12 and 15 days post-challenge (dpc). Oropharyngeal and cloacal swabs were collected at 0, 2, 4, 6, 8, 14, 20, and 30 dpc for virus isolation. Eggs were harvested every day for one month post-challenge. The rate of egg production and abnormal eggs were calculated every three days. All chickens were confirmed NDV, AIV, IBDV, MDV, ALV and CAV negative before challenge. The chickens were maintained in bio-security isolation units with feed and water administered ad libitum.

Statistically significant differences in serological analysis were evaluated using Student's *t*-test with the Prism 5.0. Frequencies of virus isolation, overall abnormal egg rate and morbidity were analyzed for significance by Chi-square test. All tests were performed with a 5% level of significance.

All chickens were alive post-challenge, none of the chicken exhibited overt clinical signs. Chickens in groups L and M only showed a transient depression. Group L exhibited a significant higher number of depressed chickens than group M (100% in group LY to 25% in group MY; 100% in group LC to 40% in group MC). Chickens in group H and control group showed no clinical signs. The result suggested that LaSota vaccines could protect chickens from death and apparent disease post challenge, which is in consistent with previous reports (Liu et al., 2003; Kapczynski and King, 2005). It was also found that the morbidity is highly correlated with pre-challenge HI titers ($R^2 = 0.9680$) (Fig. 1D). With HI titer ≥ 12 log2, chickens showed no clinical signs.

There are some reports about the level of HI antibody needed to provide protection against ND. Czifra reported that with the HI titers ≥ 3 log2, 95% of the chickens could protected from mortality, but birds still showed overt clinical symptoms (Czifra et al., 1998). Study from Michiel suggested that a high fraction of birds (85%) needed to have a high antibody titer (log2 titer ≥ 3) after vaccination to ensure no epidemic spread in vaccinated populations (van Boven et al., 2008). Both of the above published work did not show 100% protection, and also the virus shedding among birds were unknown. Darrell's results showed that broiler-breeders (66 weeks ages) with HI titers ≥ 9 could protect against overt clinical signs after virulent NDV challenge, but there was a period between which birds shed virus (Kapczynski and King, 2005). These reports showed that vaccination reduced mortality in ND-infected flocks, but failed to stop the spreading of disease, regardless of the vaccine, route or frequency of use. And which HI threshold could stop chicken shedding virus still unknown. Our results showed with HI titer ≥ 12 log2, laying chickens showed no clinical signs and shed no virus, and the productivity of laying eggs was also not affected. To our knowledge, this is the first report concerned the HI titer and chicken productivity.

Field NDV infection of layers may not show signs of ND, except for drop in egg production and quality, because vaccination during their production cycle (Cho et al., 2008; Bwala et al., 2012) can result in great economic losses. Thus, an ideal immune response, induced by vaccine, could protect chickens from clinical disease without affecting egg production when challenged with field-isolated virulent NDVs. In the present study, we found that abnormal eggs, including soft shell eggs, amorphous shell eggs, white shell eggs, and spotted shell eggs, were observed in groups L and M (Fig.1B and Fig. 1C). Production of abnormal eggs appeared initially in first 3 dpc, and could last until 27 dpc in group L, and 18 dpc in group M. Rate of abnormal eggs reached a peak at 9 dpc in groups L and M, but no abnormal egg was observed in group H and group control during the course of study. Egg-production amount was not affected in any groups. At the same time, results also demonstrated that the overall abnormal egg-ate was highly correlate

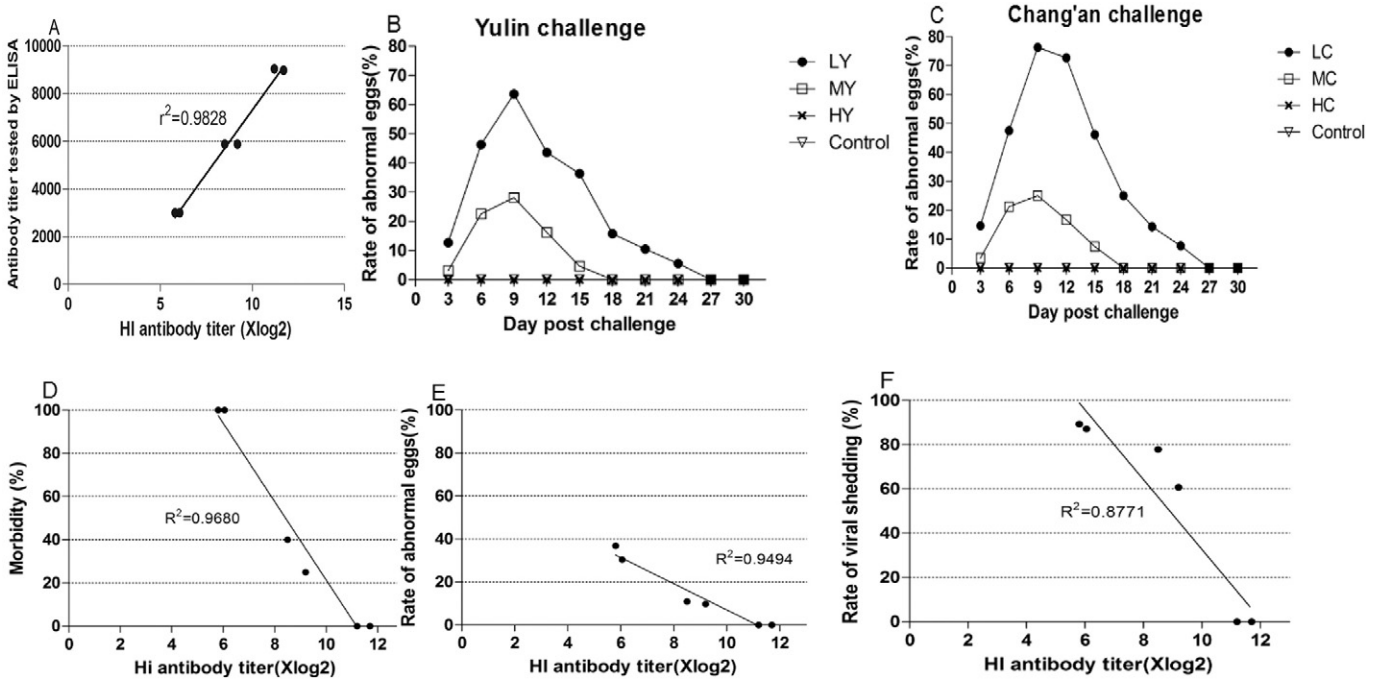

Fig. 1. The correlation between HI antibody titer and antibody titer tested by ELISA (A). Antibody titers were tested by HI assay and ELISA. The rate of abnormal eggs (B–C) and correlation between pre-challenge HI antibody titers and protective efficacy (D–F). Eggs showed soft shell, white shell, amorphous shell and spotted shell were thought to be abnormal eggs. The rate of abnormal eggs was calculated every three days as follows: Abnormal eggs rate = 100 × Abnormal eggs no./total eggs no. Yulin challenge (B). Chang'an challenge (C). The different level of HI antibody titers pre-challenge were plotted against corresponding morbidity (D), overall abnormal egg rate (E) and overall viral shedding rate (F). The relative correlation between the data point is given by R-value.