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Reciprocal Antibody and Complement Responses of Two Chicken Breeds to Vaccine Strains of Newcastle Disease Virus, Infectious Bursal Disease Virus and Infectious Bronchitis Virus

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ABSTRACT

Serum antibody responses and haemolytic complement activity were evaluated in White Leghorn (WLH) and Rhode Island Red (RIR) chickens that were vaccinated with live-attenuated vaccines of Newcastle disease virus, or infectious bronchitis virus, or infectious bursal disease virus by means of ocular challenge at 10 times the normal vaccination dose. Complement titres in non-vaccinated birds were significantly higher in WLH birds compared to RIR birds. The lentogenic viral infection resulted in an immediate stimulation of complement activity, followed by a decrease to initial complement levels within 2 weeks post vaccination, when the antibody response took over immune defence. As compared to WLH chickens, RIR birds mounted a faster and significantly higher antibody response to the vaccine viruses used. In WLH hens, significantly higher haemolytic complement activity post vaccination was found as compared to RIR hens. Possible consequences of the observed differences in immune responsiveness of the two breeds to viral vaccines are discussed.

Keywords: complement, antibodies, chicken, viruses

Abbreviations: APW, alternative (calcium-independent) complement pathway; CPW, classical (calcium-dependent) complement pathway; dpv, days post vaccination; EID_{50}, median embryo infective dose 50%; ELISA, enzyme-linked immunosorbent assay; HB1, Hitchner B1; IBDV, infectious bursal disease virus; IBV, infectious bronchitis virus; MBL, mannan-binding lectin activation pathway; NDV, Newcastle disease virus; RIR, Rhode Island Red; WLH, White Leghorn

INTRODUCTION

The avian immune system has a variety of tools at its disposal to combat virus infections, including the complement system that, as an innate immune component, is immediately ready to target and eliminate virus particles and to interact with the surface of virus-infected cells (Koch *et al*., 1982; Lachmann and Davies, 1997). Complement activation is a crucial component of both innate immunity, in the forms of alternative (APW) and mannan-binding lectin (MBL) activation pathways (Laursen and Nielsen, 2000; Thiel *et al*., 2002; Juul-Madsen *et al*., 2003), and adaptive immunity (classical, antibody-dependent complement activation, CPW) (Favoreel *et al*., 2003) acquired over time following virus

infection or vaccination (Vossen *et al*., 2002). The purpose of vaccination of chickens, as of other species, is usually to induce enhanced specific and protective immune responses such as specific antibodies or memory T cells (Pei *et al*., 2003) against important viral diseases at an appropriate time.

The effects of the complement system on the course of infections and diseases have received increased interest, especially since the discovery of the MBL pathway. Controlling the activation of complement might become more relevant in the future, seeking a balance between immune enhancement and involvement in pathogenesis. In the present study, two chicken breeds, White Leghorn and Rhode Island Red, were used to study complement activity and serum virus-specific antibody responses to three lentogenic viral infections. Vaccination with lentogenic viral strains might affect innate and/or specific immunity differently in chicken breeds. Birds were challenged with 10 times the vaccination dose to study whether a change of either complement or antibody levels might affect (disease) sensitivity to lentogenic strains. The data obtained suggest an important role of complement in the early immune defence of a chicken breed to the three viruses, whereas the other breed mounted higher humoral responses to the same viruses. The importance for disease resistance of vaccination at the right moment of the right breed is discussed.

MATERIALS AND METHODS

Chickens

Day-old White Leghorn (WLH) (Hisex White), and Brown Rhode Island Red (RIR) (Derco) female pullets were purchased. Birds were fed commercial diets *ad libitum* and held under normal husbandry (cage) conditions. At 5 weeks of age, 144 birds were allocated into four groups (one WLH + RIR control, and three WLH + RIR vaccine groups) of 12 individuals in three separate trials: Newcastle disease virus (NDV), infectious bursal disease virus (IBDV), and infectious bronchitis virus (IBV) vaccination, respectively.

Experimental design

Antibody and complement responses in blood were assessed prior to and at regular intervals during 2 weeks following vaccination. Blood was collected via vein puncture, and quickly put on ice for about 1 h to impair spontaneous complement activation between the first and last collections of blood. Serum samples were kept at -80°C until testing for complement and antibody responses over a 2-week period.

Vaccine strains

Live-attenuated commercial (Intervet Vaccine Nobilis[1]) lentogenic vaccine strains of Newcastle disease virus (NDV) type Hitchner B1 (HB1), infectious bursal disease virus (IBDV) strain D78 and infectious bronchitis virus (IBV) type H120, all from Intervet, Boxmeer, The Netherlands, were used. The vaccines were dissolved in sterile normal saline solution

and administered by ocular inoculation by means of a standardized 50 µl dropper, containing 7.3 $\log_{10}$ EID_{50} (median embryo infective dose 50%) (NDV); 5.0 $\log_{10}$ plaque-forming units (pfu) (IBDV), or 5.5 $\log_{10}$ EID_{50} (IBV), respectively. The control group received a 50 µl sterile normal saline solution. No adverse reactions were observed following vaccination.

Complement

Both classical calcium-dependent (CPW) and alternative calcium-independent (APW) complement activity were determined with a haemolytic technique (Demey *et al*., 1993; Parmentier *et al*., 2002) using an adapted light-scattering method. Activity was expressed as Δ CH_{50} U/ml, this being the difference between initial complement levels and the complement levels at given days post vaccination (dpv).

Antibodies

Quantification of serum antibody responses to NDV, IBV and to IBDV were monitored by commercial NDV, IBV, and IBDV antibody ELISA test kits, respectively, all from IDEXX FlockChek, Westbrook, Maine, USA. Titres were calculated with the aid of positive and negative reference sera, according to manufacturer's protocols. Virus-specific antibodies were recognized by conjugates binding both chicken IgM and IgG.

Statistical analysis

Statistical analyses were carried out in Stata 8 (StataCorp, 2003). The means of the (Δ) antibody titers, and classical and alternative complement consumption were compared by a population-averaged negative binomial regression using Generalized Estimating Equations and an unstructured within-group correlation structure. A Spearman ρ was used to assess the correlation between the three immune parameters. Different days were analysed separately with the use of a Bonferroni correction and as such only values of ρ with a p-value of 0.002 or lower are considered significant.

RESULTS

During 2 weeks post vaccination, control (non-vaccinated) groups showed marginal antibody titers, and no differences in time of haemolytic CPW and APW complement levels. Complement levels in birds prior to vaccination were significantly higher in WLH birds compared to RIR birds both for the CPW (706 ± 60 (SD) CH_{50} U/ml (WLH) vs 557 ± 49 CH_{50} U/ml (RIR), APW (377 ± 32 CH_{50} U/ml (WLH) vs 296 ± 34 CH_{50} U/ml (RIR)).

Antibody responses to NDV-HB1, IBDV-D78, and IBV-H120 vaccination

All three vaccinations induced an increase in NDV-, IBDV- and IBV-specific antibody levels, from marginal levels before vaccination to significantly higher levels 10 days after exposure (Figure 1). Peak antibody levels were found at 10 dpv, followed by a decrease in both lines. In both breeds, vaccination with the NDV strain Hitchner B1 caused an antibody response that was up to 10 times higher than the antibody responses after vaccination with the IBD-D78, and IBV-H120 strains. WLH birds had significantly lower antibody levels to all three vaccines than did RIR birds.

Haemolytic complement activity after NDV-HB1, IBDV-D78 and IBV-H120 vaccination

All vaccinated birds responded to the vaccines by a significant increase in activity of both complement pathways. While the peak of antibody response occurred at 10 dpv, complement activities showed an earlier peak at 7 dpv, both in the CPW (Figure 2) and APW (Figure 3) pathways. After a peak at 7 dpv, complement levels decreased to levels similar to initial levels (or slightly lower in the case of RIR with an IBV infection) at 14 dpv. Vaccination with IBDV induced significantly higher CPW and APW activities in WLH birds than in RIR birds. No differences in CPW and APW activities were observed between NDV-, IBDV- and IBV-vaccinated groups.

Correlation between antibody and haemolytic complement responses

No correlation was found between the CPW and APW responses to the three vaccines, or between the APW response and antibody response during 14 dpv. In the case of the CPW and antibody response, a significant ($p = 0.002$) negative correlation ($\rho - 0.599$) was estimated at 14 dpv for IBV-H120. In the NDV-HB1 trial, a significant ($p < 0.001$) negative correlation was demonstrated at 7 dpv ($\rho - 0.690$) and 10 dpv ($\rho - 0.902$) between the classical complement and antibody responses.

DISCUSSION

In the present study, the vaccine viruses that were inoculated via the normal route, but in high doses in two different unrelated chicken breeds, activated both CPW and APW pathways in the same non-antigen/virus-specific manner. The high vaccination doses had no visible adverse effects on health status of either both breeds. During the first week post vaccination, viral antigens may have stimulated haemolytic complement activity as a result of the CPW in virus neutralization by proposed mechanisms earlier, such as (1) deposition of complement proteins to the virus, blocking virus attachment and entry; (2) aggregation of virion–lectin complexes, causing increased phagocytosis of C3b-coated viral particles and destruction by macrophages; (3) activation of the entire complement pathway, resulting in lysis of the virus; or (4) generation of inflammatory and specific immune responses (Cooper and Nemerow, 1984; Friedman *et al*., 2000; Bernet *et al*., 2003). Complement and antibodies

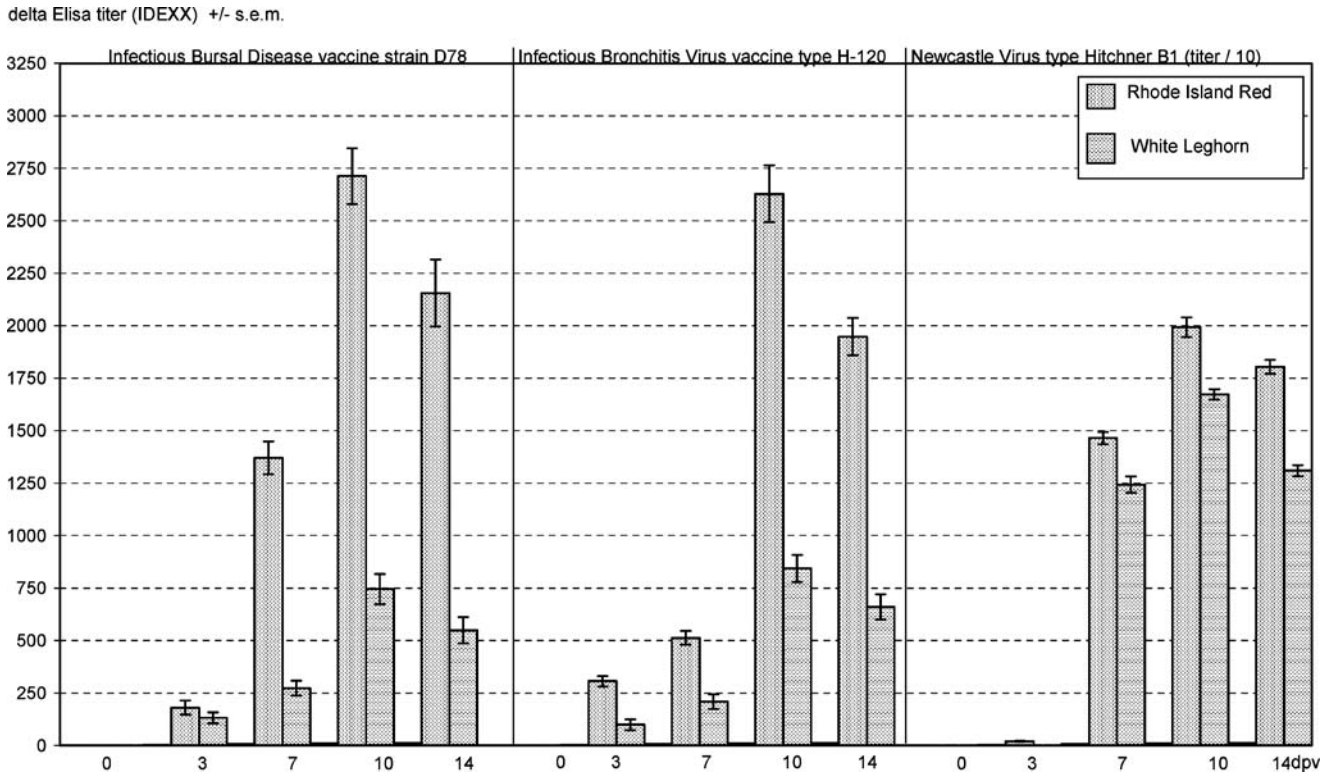

Figure 1. Antibody response (Δ-ELISA titer (IDEXX) ± SEM) of 12 RIR and 12 WLH chickens to ocular vaccination with IBDV-D78, IBV-H120 and NDV-HB1 established in three separate trials

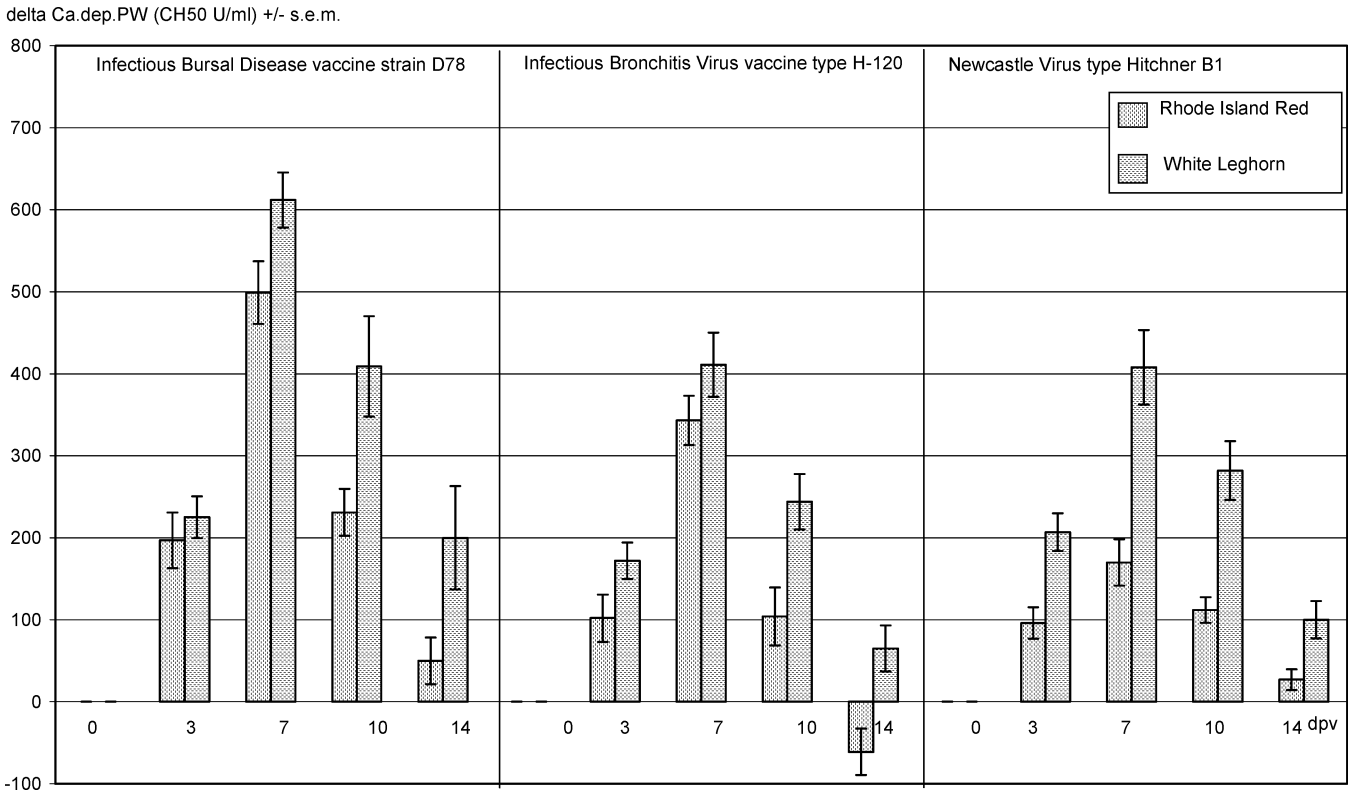

Figure 2. Calcium-dependent (classical) complement response (CH_{50} U/ml ± SEM) of 12 RIR and 12 WLH chickens to ocular vaccination with IBDV-D78, IBV-H120 and NDV-HB1 established in three separate trials

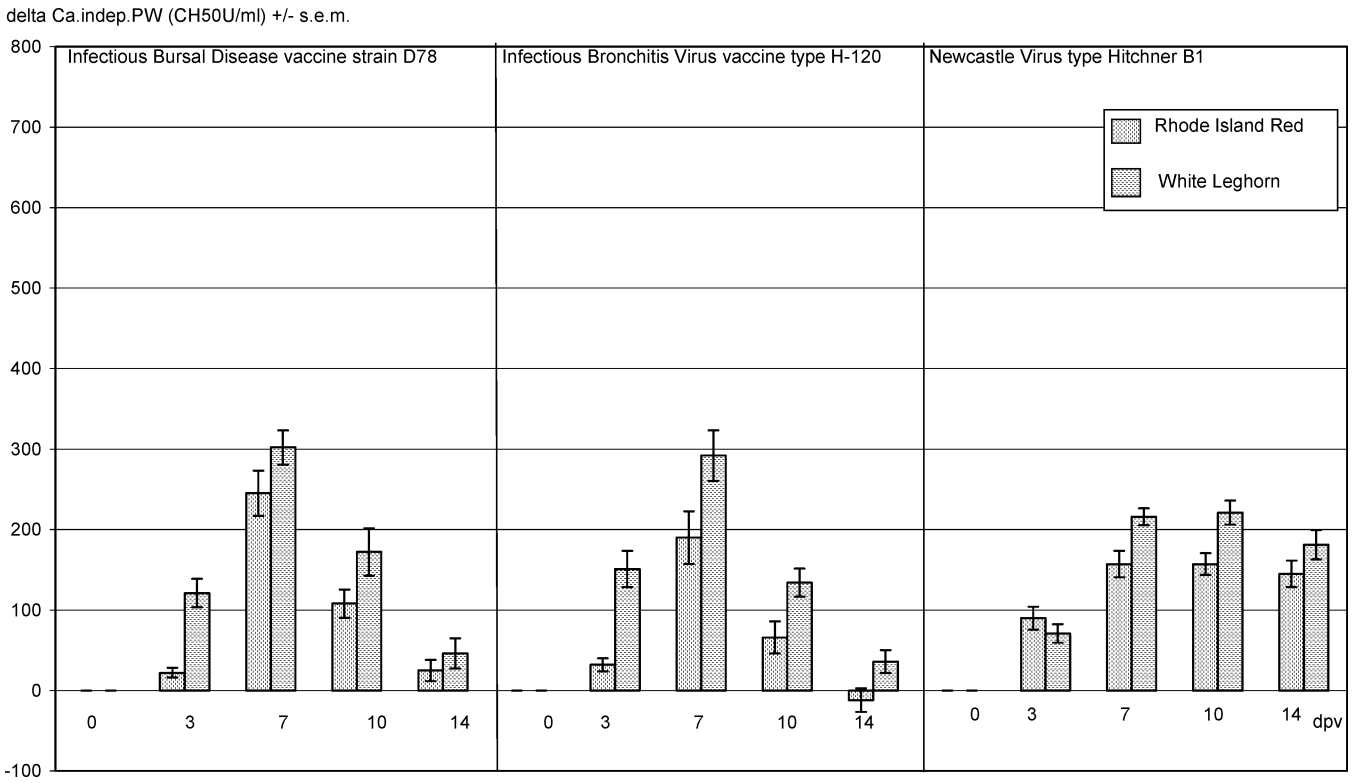

Figure 3. Calcium-independent (alternative) complement response (CH_{50} U/ml ± SEM) of 12 RIR and 12 WLH chickens to ocular vaccination with IBDV-D78, IBV-H120 and NDV-HB1 established in three separate trials

are important mechanisms in combating viral infections, but whether they operate alone or in concerted action depends on the type of virus (Friedman *et al*., 2000). The CPW may interact with the virus in the absence of antiviral antibodies by binding C1q directly to the virus, or with the interaction of natural IgM antibodies (Ravetch and Clynes, 1998) playing a crucial role in the process of opsonization of the viruses.

Although both complement pathways share the lytic pathway, we found no correlation between CPW and APW interaction with the viruses. As well as CPW, enhanced APW activity was also found, albeit the APW activity was pronounced only at 7 dpv with IBDV and IBV, and somewhat enhanced at days 10 and 14 after NDV vaccination. The kinetics of APW were, however, similar in both breeds. This suggests a non-specific maximized APW response that is not associated with CPW and antibody responses.

The natural complement status of WLH birds was significantly higher than that of the RIR birds. This difference of innate immune competence between the two breeds was also exemplified by the significantly higher haemolytic complement activities in response to the vaccine virus infections in WLH birds (and significantly less antibodies) than in the RIR birds. Although the present study included only two breeds, it is tempting to speculate on two different strategies for combating viral infections: early high innate responses versus late specific responses. In this respect, it is noteworthy that, although RIR birds mounted much higher antibody responses to the viral vaccines, and CPW activities of RIR birds were less than in WLH birds, the kinetics of CPW was similar in both breeds, as was true for the APW. Earlier we found high levels of complement (activity) in WLH birds (Parmentier *et al*., 2004) that far exceeded those of various indigenous breeds (Baelmans *et al*., 2004). In addition, we found various combinations of high antibody and high complement responders, low antibody and high complement responders, and vice versa (Parmentier *et al*., 2002; Baelmans *et al*., 2005) in various chicken breeds, which suggested genetically based as well as environmental predispositions in response to immunization.

During the second week post vaccination, a decrease of complement levels to the initial levels occurred in both chicken breeds, which may be due to either hypercatabolism or hyperactivation associated with circulating immune complexes (CPW). During this period, high titres of serum antibody directed against the vaccine viruses appeared. The adaptive humoral defence against viral infections takes time to develop. In this respect it is noteworthy that the broiler RIR hens mounted earlier and significantly higher antibody titres than to the layer WLH hens. This finding and the negative correlation between antibody titres to the vaccine viruses and the CPW response suggest that these differences in antibody–complement interactions result in a faster elimination of viruses and infected cells in the RIR birds. This correlation seems to be based on complement response and not the initial innate complement status: RIR chickens showed significantly lower initial complement levels but significantly higher antibody responses than WLH birds.

We have addressed CPW and APW activity in response to viral infections. Nielsen and colleagues (1999) investigated the concentration of serum MBL in IBV-infected chickens. The concentration of serum MBL increased in the first week after infection and returned to normal values thereafter. Another study associated the complement activation directly with the concentration of MBL in avian serum, indicating neutralization of the IBV virus before the antibody response took over (Juul-Madsen *et al*., 2003). The same inverse relation was found in the present study between complement activity and antibody responses. Our

data suggest that an early high innate (non-specific) immune response may provide initial protection until specific immunity is operational, as exemplified by WLH birds, whereas in RIR birds protection may rest on a high specific immune reaction that is accompanied by exhaustion of the preceding innate (complement) immune response. Pathogenic phenotypes of viruses were associated with increased resistance of the virus to the attack of complement. Mortality of chickens infected with velogenic IBDV-was associated both with a severe drop in complement titres and with depletion in circulating levels of haemolytic complement due to formation of immune complexes at sites of viral replication (Skeeles *et al*., 1979). We found a decrease of complement activity at the time at which specific antibodies to the virus appeared after an initial non-antigen-specific stimulation of complement by lentogenic viruses. A similar increase of complement titres after vaccination was found by others (Srinivasa Rao *et al*., 1994), who proposed that the subsequent decrease of complement activity is necessary to mount a better humoral defence (stimulator effect). Even at a 10-fold overdose, the use of commercial lentogenic viral vaccines does not implicate a possibly dangerous complement decrease. The complement response in chickens can even be used as a moderate acute-phase reactant, as shown previously for avian MBL (Nielsen *et al*., 1999). Vaccination strategies should rest on the knowledge of relevant immune defence mechanisms to prevent undesirable immune responses (in this case, antibody responses) that may counteract relevant defence mechanisms (in this case, complement activation) in a specific breed. But it can also be argued that the relative deficiency of complement in the RIR birds after vaccination might help to propagate the vaccine virus in the birds, resulting in better activation of specific immunity. In this respect, it remains to be established whether the differences between the two breeds in complement and antibody titres have consequences for resistance to velogenic virus infections. Our results suggest that greater knowledge of the possible three-way interactions between virus, complement and specific antibody in poultry is required. It is hypothesized that efficient priming of specific humoral immunity might lead to a decrease in complement that could allow spread of the vaccine virus. However, immunization at the wrong time in specific breeds might interfere with relevant immune defences to virulent strains owing to a relative deficiency of complement components.

We did not measure antibody and complement components after day 14 post vaccination. In future studies, the time after vaccination when both antibodies and complement have returned to base levels should be used to challenge these birds and other chicken breeds with virulent viral strains in order to establish the relevant immune protective mechanisms.

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