Short Communication

Comparison of Two Inactivated Vaccines Against Infectious Bronchitis Virus Evidences the Interference of Vaccine Formulations on Vaccine Performance

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Abstract | Inactivated water-in-oil vaccines are widely used to control viral diseases in poultry, however, the interference of physico-chemical properties of the emulsions are overlooked when compared to antigenic properties. This study was designed to assess the effect of two different commercial inactivated water-in-oil multivalent vaccines; vaccine A contains IBV (Massachusetts M41 and D274 strains), NDV (Clone 30), aMPV (BUT 8544) and IBDV (classic strain) whereas vaccine B contains IBV (Massachusetts M41 and a Brazil type strain), NDV (LaSota strain), aMPV (TRT strain) and IBDV (GP82 strain). Viscosity values for vaccines A and B where determined to be 40.2cP and 83.3cP, respectively, and a microscopic examination showed that vaccine A has a homogeneous emulsion, whereas vaccine B has a highly heterogeneous appearance. Birds vaccinated with vaccine A showed mean ELISA antibody S/P ratios from 0.099 to 0.995 between weeks 2 and 6 post-vaccination against IBV antigen, while for vaccine B group mean S/P ratio dropped to 0.550 at week 6. Virus neutralization test showed that birds vaccinated with vaccine A showed neutralizing antibodies against IBV Massachusetts, 4/91, D274 and Qx types, while titers for vaccine B were not detected, suggesting a role of the poor emulsion quality on the performance of vaccine B. These results demonstrate that effective quality control and emulsion preparation are essential benchmarks of immunization and vaccine potency.

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Introduction

Avian infectious bronchitis virus (IBV), avian metapneumovirus (aMPV), Newcastle disease virus (NDV) and infectious bursal disease virus (IBDV) are highly contagious and economically significant diseases in the poultry sectors around the world (Jones, 2010; Alexander et al., 2012; Mahgoub,

2012; Colvero et al., 2015). Vaccines are only means of immunization and occurring as a multitude of types with a diverse range of cross-protection (Cavanagh et al., 2007; Valastro et al., 2016). The control of these diseases is based mainly on the use of either live or inactivated virus vaccines with a range of valences and antigenic diversities, but evaluation trials of such vaccines has focused mainly on the antigenic point-of-view (de Wit et al., 2017; Zegpi et al., 2017) without taking into account physico-chemical properties of vaccine formulations.

Amongst several properties, the quality of the emulsion in an inactivated vaccine is the core benchmark of efficiency as water-in-oil emulsion-based vaccines allow a long-term immunity based on the recruitment of cell immunity (Aucouturier et al., 2001). Therefore, for any vaccine to induce effective immunity must comply with essential quality control standards. The aim of this study was to assess effects of two different multivalent inactivated virus vaccines on layers birds based on antibody response, production performance and physico-chemical properties of the vaccines focusing on the anti-IBV responses.

Materials and Methods

Vaccines

Two commercially available multivalent inactivated water-in-oil emulsion vaccines were used for the trials. Vaccine A contains IBV (Massachusetts M41 and D274 strains), NDV (Clone 30), aMPV (BUT 8544) and IBDV (classic strain); vaccine B contains IBV (Massachusetts M41 and a Brazil type strain), NDV (Lasota strain), aMPV (TRT strain) and IBDV (GP82 strain). IBV antigens for all strains in both vaccines A and B allow a minimum of HI titers> 20 as per the Brazilian Ministry of Agriculture. All vaccines were diluted and used as per manufacturers' instructions for the trials.

Physico-chemical evaluation

Vaccines A and B were tested for viscosity, density and pH as per the Brazilian Pharmacopoeia 5th edition and observed for particles size and homogeneity under a light microscope.

Vaccination trial in isolators

Forty 6-week old specific pathogen free (SPF) chickens were divided into two groups (A and B) and 10 birds served as negative controls (group C), 15 birds were used per vaccine groups A (vaccinated with Vaccine A) and B (Vaccinated with vaccine B), respectively. All birds were sampled prior to vaccination and at two, four and six weeks after vaccination. Sera were tested for total anti-IBV antibodies using IDEXX IBV Ab Test ELISA™ and for anti-IBV neutralizing antibodies using IBV strains M41 (Massachusetts type), 4/91, D274 and Qx using virus neutralizations test (VN). In short, serial two-fold serum dilutions were prepared in microtitre plates and mixed with an equal volume containing each of the IBV strains in separate assays. After pre-incubation, chicken embryo kidney (CEK) cells where added and for at least 3 days the cells were examined for the presence or absence of a typical cytopathic effects (CPE) of IBV.

Results

Physico-chemical evaluation

Vaccines A and B showed comparable results for density (0.9283 and 0.9188g/cm3, respectively) and pH (6.48 and 6.87, respectively). The most striking difference between vaccines A and B was the viscosity, with 40.2cP (centiPoise) for vaccine A and 83.3cP for vaccine B. A microscopic examination of both vaccines revealed that vaccine A is a homogeneous emulsion, while for vaccine B a highly heterogeneous emulsion was observed (Figure 1).

Vaccination trial in isolators

Prior to vaccination, mean S/P ratios for anti-IBV antibodies for groups A, B and C were tested to be 0.005, -0.003 and -0.001, respectively. For vaccine A group, mean S/P ratios raised from 0.099 to 0.995 between weeks 2 and 6 post-vaccination, while for vaccine B group mean S/P ratio at these same time points were 0.236 and 0.550 (Figure 2). Only at week 4 mean S/P values were significantly different between vaccines A and B (T-Test $p < 0.05$). VN antibodies against IBV strains M41 (Massachusetts type), 4/91, D274 and Qx were not detected for both vaccine A and B groups' prior to vaccination and for groups C (control) during all sampling points.

For the vaccine A group, rising VN antibodies titres were detected from weeks 2 to 6 after vaccination for all four IBV strains, reaching average 2log Ab titres of 8.8 (M41), 9.3 (D274), 4.9 (4/91) and 4.6 (Qx) at 6 weeks. On the other hand, for the vaccine B group, VN titres were detected against any of the four IBV strains.

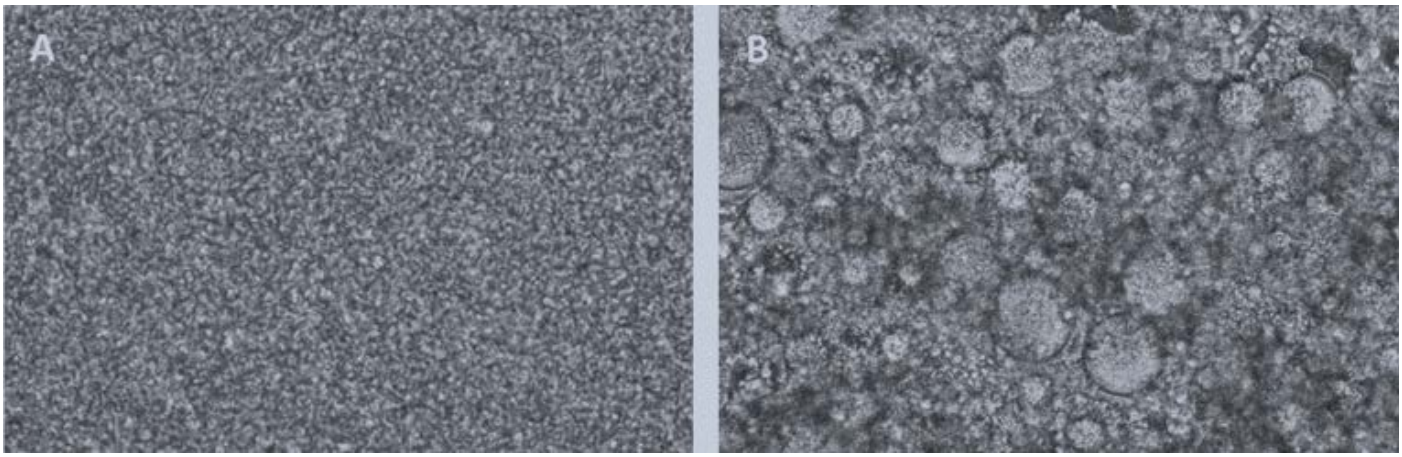

Figure 1: *Light microscopic view (400x) of vaccine A and B evidencing differences in droplet sizes and in emulsion homogeneity.*

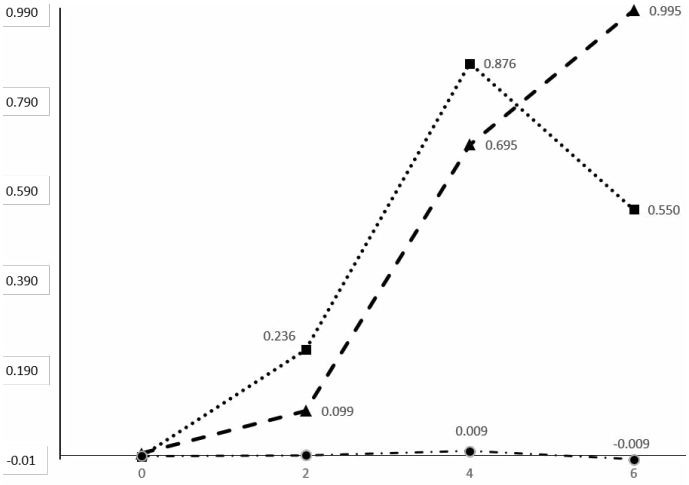

Figure 2: *Mean ELISA S/P ratios for anti-IBV antibodies (vertical axis) for SPF chickens vaccinated with vaccine A (triangles), B (squares) and control birds (circles) at weeks 2, 4 and 6 pots-vaccination (horizontal axis). Numbers close to each point are mean S/P values; values at week 0 (prior to vaccination) were 0.005, -0.003 and -0.001 for vaccines A, B and C, respectively, and are not shown on the graph due to their proximity.*

Discussion

After comparing two multivalent inactivated vaccines focusing on the anti-IBV responses, not only vaccination trial in isolators showed that birds vaccinated with vaccine A had a higher total anti-IBV antibody titer when compared to those vaccinated with vaccine B in ELISA, but, more strikingly, for vaccine B no VN antibodies were detected for IBV strains/serotypes 4/91, QX, D274 and M41 (Massachusetts), while 2log Ab titres VN titers for vaccine A ranged from 4.6 to 9.3.

Its known that homologous IBV types are able to cross-neutralize the virus neutralization test (Shimazaki et al., 2009), the absence of VN antibodies against the Massachusetts serotype in birds vaccinated with vaccine B was not expected, as the very same M41 strain is included in the formulation of this vaccine. The quality of a water-in-oil emulsion for a vaccine with inactivated viruses is of utmost importance as low quality emulsions might result in poor or uneven antigen presentation (Aucouturier et al., 2001).

Results of vaccine B showed a marked difference in size and distribution of droplets when compared to vaccine A, with a viscosity of almost twice as that of vaccine A. This lower emulsion quality might be one of the basis of the poor antigen presentation that lead to the absence of VN titers for vaccine B, though titres have been detected using ELISA. Yet ELISA shows a high sensitivity for anti-IBV antibodies detection, it neither allows for a distinction among IBV types of antibodies nor is IBV type-specific, in opposition to the association between high sensitivity and specificity of VN (de Wit et al., 1997; Pradhan et al., 2014).

The most plausible explanation for a combination of these results would be that a low-quality emulsion led to a poor antigen presentation culminating in a low neutralizing antibody level against IBV. However, due to the lack of similar studies, the results presented herein cannot be compared to data generated elsewhere.

In conclusion, the vaccine-based control of IB must take into account an association between antigen diversity and efficacy and emulsion quality for the assurance of a better protection.

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Authors Contributions

All the authors contributed equally

Conflict of interest

The authors declare that they have no conflict of interest

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