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# Evasion of maternal antibody protection by an IBDV Argentine variant

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**ABSTRACT** Infectious bursal disease (**IBD**) is a viral disease that affects the ability of chickens to produce humoral immune responses. One way to prevent the disease is the passage of maternally derived antibodies (**MDA**) from dams to offsprings via the yolk. Despite sanitary measures, which include immunization with genogroup 1 (**G1**) vaccines, infections with IBDV genogroup 4 (**G4**) in young animals have been detected. The aim of this study was to determine whether a local IBDV isolate belonging to G4 could evade the immunity generated by MDAs. Twelve-day-old animals positive for MDA, were inoculated with G1 or G4 isolates or phosphate buffered saline (**PBS**) as a control. After 1 wk, the animals were sacrificed and the following parameters were evaluated: bursa-body (**BB**) ratio, viral load, and histologic damage in the bursa of Fabricius. Results showed that G4-infected animals had significant differences in the BB ratio compared to the PBS group. In addition, viral load was significantly higher in the G4 group than in the G1 group. Histologic damage in the bursa of Fabricius was detected only in G4-infected MDA chickens. Our results suggest that infection with G4 local isolate can circumvent the immunity generated by MDA and, furthermore, that G4 isolate does not differ in its pathogenicity from G1 isolate, which underlines the need to include variant strains in vaccine formulations to reduce potential losses caused by these viruses.

**Key words:** infectious bursal disease, maternally derived antibody, genogroup, local isolate

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## INTRODUCTION

Infectious bursal disease virus (**IBDV**) is the etiologic agent of a highly contagious and acute disease that affects young chickens, causing immunosuppression. It belongs to the Birnaviridae family and its genome is composed of 2 double-stranded RNA segments, encoding 5 viral proteins called VP1, VP2, VP3, VP4, and VP5 (Becht, 1980). VP2 is the main structural protein which constitutes the capsid of IBDV and induces protective immunity through the production of neutralizing antibodies (Fahey et al., 1989). Moreover, VP2 is responsible for antigenic variation, adaptation to cell culture and virulence (van Loon et al., 2002).

One of the most widely used strategies to prevent infections in the poultry industry is vaccination. Immunity generated by vaccination can be divided into 2 main types: passive and active immunity. Passive immunity consists in the passage of antibodies from hens to their offspring through the yolk (DuBourdieu, 2019). These antibodies, known as maternally derived antibodies (**MDA**), are the first line of defense against foreign pathogens in the first days of chickens live (van den Berg et al., 2000; Al-Natour et al., 2004; Kegne and Chanie, 2014). In the case of Infectious bursal disease (**IBD**), this is especially important, as it has been observed that the younger the infected animal, the higher the degree of immunosuppression (Faragher et al., 1974; Saif, 1991).

In Argentina, during the rearing period of breeding birds, attenuated vaccines are usually administered in the drinking water at different ages. Finally, an inactivated vaccine is applied before the laying stage of breeding birds (Lucero et al., 2019). This approach aims to achieve the necessary MDA levels to provide protection against IBDV during the first days of life (Lucero et al., 2019). All these vaccines belong to genogroup 1, classified as "classical vaccines," such as the S-706 or 228-E strain (Jackwood, 2013; Vera et al., 2015; Michel and Jackwood, 2017).



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In recent years, Gumboro was classified by the hypervariable region of VP2 protein into 7 genogroups (Michel and Jackwood, 2017). Different IBDV strains belonging to genogroup 4 (**G4**) are known to circulate in Argentina and other parts of the world in the presence of vaccination (Domanska et al., 2004; Ojkic et al., 2007; Hernández et al., 2015; Vera et al., 2015; Yamazaki et al., 2017; de Fraga et al., 2019). It has been proposed that variants are able to evade immunity induced by vaccines formulated from classical strains (van den Berg et al., 1991; Vera et al., 2015). Previous studies have demonstrated that a G4 isolate, named TY2, was able to cause disease in animals that had been previously vaccinated with a classical vaccine widely used in poultry. In addition, cross-neutralization assays in eggs have shown that anti-TY2 antibodies were able to neutralize the classical K and F539 strains; however, TY2 was only partially neutralized by antibodies against the K strain, suggesting differences in antigenicity (Yamazaki et al., 2017).

Considering that Argentine IBDV isolates belonging to G4 have been found in chickens of commercial farms, we investigated whether G4 may evade the protection afforded by classical vaccines.

## MATERIALS AND METHODS

### Experimental Chickens

In this study, 1-day-old males White Leghorn chicks were procured from Camila Farm (Suipacha, Buenos Aires, Argentina). The dams of these chicks had been vaccinated with classical attenuated vaccines at 1, 7, and 14 wk of age (strains S-706 Boehringer-Ingelheim, ST-12 UNIVAX-BD MSD and S-706 Boehringer-Ingelheim, respectively) and classical inactivated vaccine at 18 wk of age (Nobilis GUMBORO 228E MSD), which resulted in the chicks having MDA against IBDV. The level of maternal antibodies was determined using a commercial ELISA kit (IDEXX IBDV Ab Test-IDEXX, IDEXX Laboratories, Inc.) at 12 d posthatch.

Also, White Leghorn Specific pathogen-free (**SPF**) chickens were used. Embryonated eggs laid by SPF White Leghorn hens were purchased from Instituto Rosenbusch S.A. (Buenos Aires, Argentina) and hatched in an automatic incubator (Yonar, Buenos Aires, Argentina).

### Viruses

Infectious bursal disease virus, belonging to G4 (GenBank accession number MN313610.1) and to G1 (GenBank accession number ON464183.1), isolated from commercial farms, were amplified in embryonated White Leghorn SPF eggs (Instituto Rosenbusch S.A., Argentina).

### Experimental Procedure

Ninety-five chickens with maternal antibodies were divided into 3 groups at 12 d posthatch. G1 MDA group, consisting of 40 animals, was challenged orally with $1 \times 10^3$ $EID_{50}$ of the G1 isolate. G4 MDA group, also consisting of 40 animals, was orally challenged with $1 \times 10^3$ $EID_{50}$ of the G4 isolate. MDA group, the control group, consisting of 15 animals, received PBS by the same route. Additionally, to confirm the virulence of the viruses, seven 12-day-old SPF chickens were challenged orally with $1 \times 10^3$ $EID_{50}$ of the G1 or G4 isolate (G1 SPF and G4 SPF groups, respectively). Seven days postchallenge, animals were sacrificed to evaluate different parameters of protection.

All procedures involving the use of animals were performed in agreement with institutional guidelines and approved by the Institutional Committee for the Care and Use of Experimental Animals (CICUAE − CNIA − INTA, Approval no 17/23).

### Clinical Signs, Gross Analysis, and Sample Processing

Chickens were monitored daily for any anomalies. On d 7 postinfection (**pi**), postmortem examinations were conducted to evaluate pathologic changes, body and bursal weight. Bursae were harvested, weighed and cut into 2 pieces. One piece was submerged in TransZol solution (TransGen Biotech, Beijing, China) for RNA extraction, whereas the remaining piece was submerged in 10% formalin for histopathologic analysis.

### Bursa to Body Weight Ratio

Body and bursa weights were used to calculate the bursa to body (**BB**) weight ratio according to the following formula: BB ratio = [bursa weight (g)/body weight (g)] × 1,000.

### Histopathologic Analysis

Bursal samples were placed in 10% neutral buffered formalin and paraffin embedded. Sections of bursae of Fabricius (**BF**) were stained with hematoxylin and eosin following standard histologic procedures and microscopically examined for the presence of bursal lesions under light microscopy. The evaluated lesions were lymphoid depletion (**LD**) and inflammatory infiltration (**II**). The severity of each lesion was determined by evaluating each lesion in 5 fields at 100× and scoring them from 1 to 5, where 1 = normal BF, 2 = <25%, 3 = 25−50%, 4 = 50−75%, and 5 = 75−100% of affected tissue (Lucero et al., 2019).

### Serology

Anti-IBDV antibody titers were determined in serum samples using a commercial kit (IDEXX IBDV Ab Test-IDEXX, IDEXX Laboratories, Inc.), following the manufacturer's instructions.

### Viral Load

cDNA synthesis and qPCR were performed in a single step reaction using the Luna Universal OneStep RT-qPCR Kit (New England Biolabs, Ipswich, MA) according to the manufacturer's protocol. The primers used for retrotranscription and amplification were VP1f: 5′CCAACACACCTCATGATCTC3′ and VP1r: 5′GTCAATTGAGTACCACGTGTT3′, which amplify a product of 222 bp belonging to the VP1 gene of IBDV. The number of viral copies per microgram of RNA was calculated by extrapolation with a standard curve generated by qPCR from 10-fold serial dilutions of a plasmid containing the amplified VP1 fragment, ranging from $10^2$ to $10^9$ copies.

### Statistical Analysis

Statistical analyses were performed using 1-way ANOVA, and mean differences were analyzed with the Tukey test. The Shapiro−Wilk and Levene tests were applied to verify the assumptions. When assumptions were not fulfilled, the Kruskal−Wallis nonparametric test was applied followed by the Wilcoxon pairwise comparison. All the analyses were done using R 3.4.1 (R core team) and the agricolae package (De Mendiburu, 2014).

## RESULTS AND DISCUSSION

It is well known that MDA play a major role in early protection against various pathogens (Bar-Shira and Friedman, 2006). It has already been reported that chicks with detectable MDA are able to prevent IBDV infection when challenged before 14 d after hatch (Ahme and Akhter, 2003; Lucero et al., 2019). The aim of the present work was to evaluate the ability of MDA to protect chickens from G4 viruses. For this purpose, we determined MDA titers in experimental chickens using a commercial IDEXX kit. We found no significant differences among the 3 groups indicating that chicks received similar MDA levels from their mothers (Figure 1A). As expected, the SPF chickens that served as virus virulence control did not show detectable MDA titers (Data of anti-IBDV antibodies measured by IDEXX kit in SPF chicken sera). It was already demonstrated that Argentine IBDV strains belonging to G4 are able to infect young animals (Vera et al., 2015). Furthermore, in Japan, an isolate of this genogroup was able to infect chickens previously vaccinated with a classical vaccine (Yamazaki et al., 2017). Figure 1B shows the BB ratio results. Infected G1 group did not present significant differences with any of the other groups. In addition, there were significant differences between the control group (uninfected) and G4-infected group. This result indicates a partial protection of the antibodies against G4 at the time evaluated. It is well known that antibody titer correlates with protection in IBDV infections (Tsukamoto et al., 1995). Also, there is evidence showing a correlation between high MDA titers and protection against IBDV (Al-Natour et al., 2004). Unexpectedly, in our work, there was not a correlation between maternal antibodies level and resistance to infection in any of the groups (Pearson's test, $P < 0.05$), as measured by histologic damage and BB ratio (Data of anti-IBDV antibodies measured by IDEXX kit in SPF chicken sera). One explanation could be due to a lower neutralization power of the antibodies generated by the commercial vaccine strain, so that the MDAs could not bring complete protection enabling spread of the viral infection in chicks. When analyzing the groups of SPF-challenged chickens (G1 SPF and G4 SPF), no significant differences were found between them, indicating that the impact of these viruses on the BB ratio is not different (Figure 1B).

Figure 2A shows significant differences in viral load between G4 MDA chicks and G1 MDA chicks (being in G4 MDA group higher). Previous research by Lucero et al. (2019) has reported that the G1 isolate

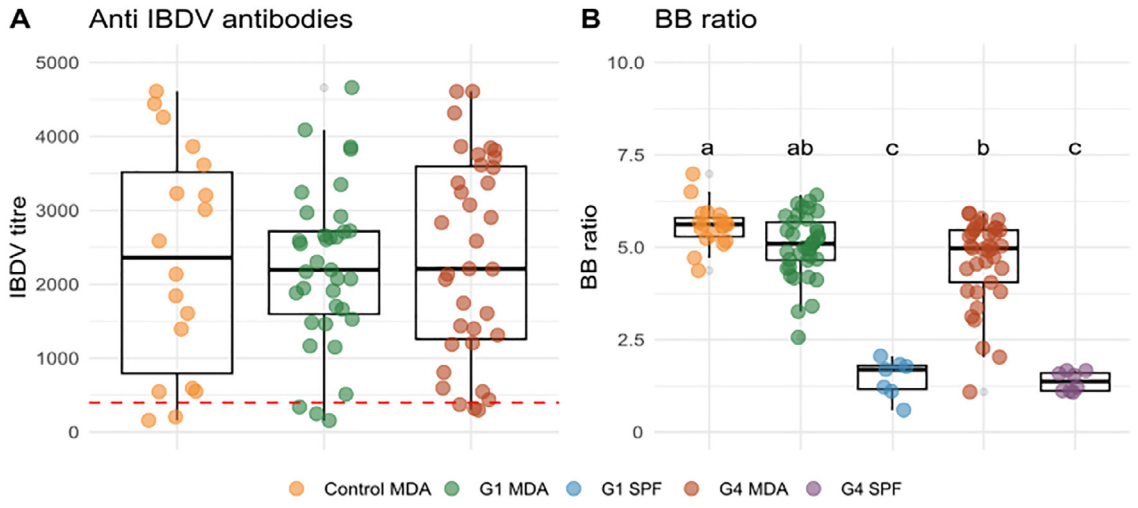


**Figure 1.** (A) Evaluation of anti-IBDV antibodies in chicken serum at 7 dpi by ELISA. Results are expressed as titer of each serum sample in a box plot graph, which shows data distribution. (B) Bursa/body weight (BB) ratio. Chickens were sacrificed and weighted at 7 dpi. Bursae were extracted and also weighted. Individual BB ratios were determined by the formula (bursa weight (g)/body weight (g)) × 1,000. Different letters indicate significant differences among groups (one-way ANOVA test and Tukey post hoc test, $P < 0.05$).

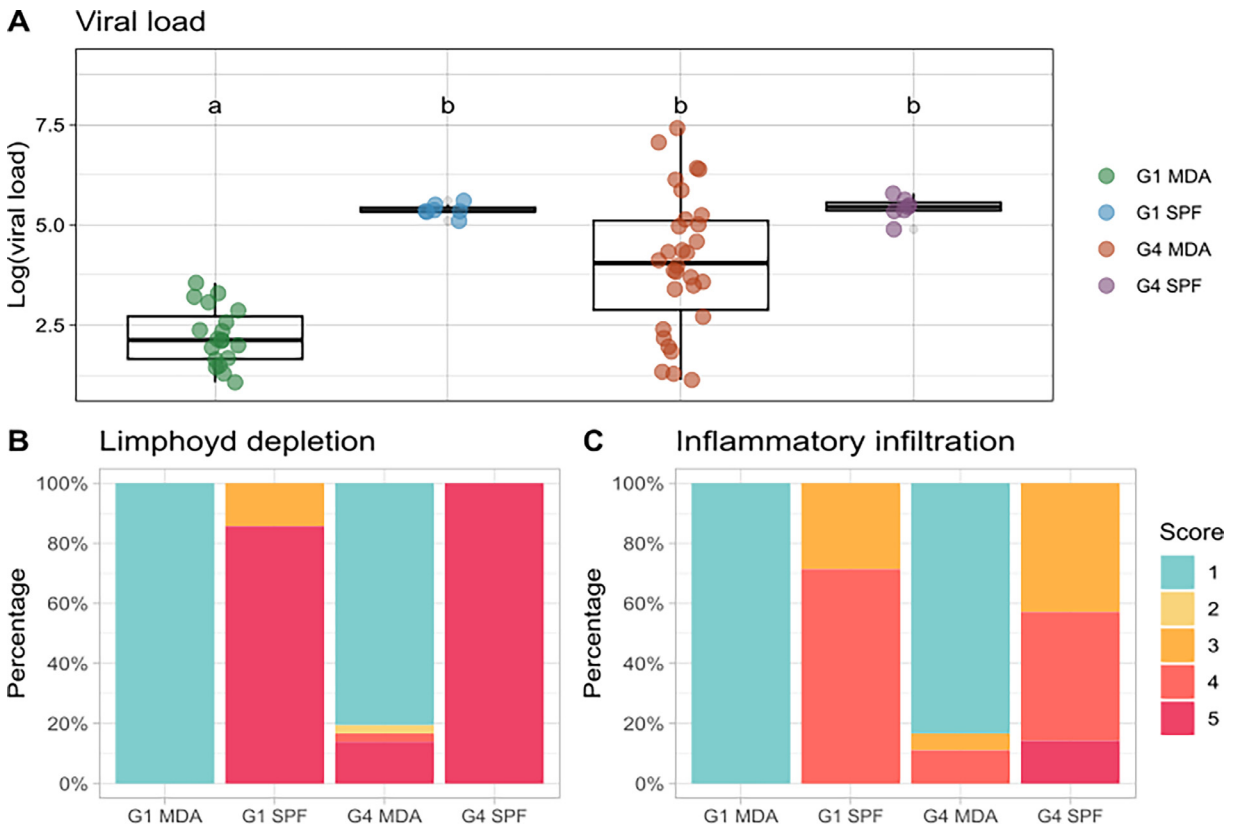


**Figure 2.** (A) Detection of IBDV viral load in the bursa of infected chickens. Individual values (dots), as well as box plots representing data distribution, are shown for each group. Different letters indicate significant differences among groups (Kruskal−Wallis test and Wilcoxon post hoc test, $P < 0.05$). Proportion of chickens with different degrees of lymphoid depletion (B) and inflammatory infiltrate (C) in the bursa. Colors indicate the severity of the lesion.

(ON464183.1) exhibits markedly limited replication capacity in the presence of MDA, generated in the hens by a vaccination scheme similar to the one used in our study. The ability of G4 isolates to evade protection generated by passive immunity had not yet been evaluated. Viral replication in the presence of subneutralizing concentration of specific antibodies has been shown to contribute to antigenic drift and the emergence of escape variants that could cause more severe disease in chickens (Asfor et al., 2022). On the other hand, there was no difference in viral load in the absence of antibodies (SPF chickens), at 7 dpi. These results indicate that the differences found in MDA chickens are due to the viral neutralizing activity against the isolates.

Figures 2B and 2C show the lesions resulting from infection with isolates G4 and G1. The histologic lesions observed included lymphoid depletion and inflammatory infiltrate, both notable features of IBDV infections (Jaton et al., 2022). Approximately 20% of the G4 MDA group infected with G4 had chickens with both types of lesions in the bursa, whereas the G1 MDA-challenged group had no animals with detectable lesions in this organ. On the other hand, as shown in Figures 2B and 2C, the SPF-challenged animals did not differ between the G1 SPF and G4 SPF groups. These results support the hypothesis that G4 may partially evade the protection afforded by classical vaccines. An in vitro antibody-mediated neutralization study using viruses exposing various capsid hypervariable regions, including genogroup 4, concluded that G4 is probably more antigenically related to available vaccine strains (Reddy et al., 2022). However, it is important to note that such strategies may not fully reflect what happens with in vivo isolates. Furthermore, it was demonstrated that the G4 isolate showed a higher replicative capacity in the presence of MDAs, suggesting that immune evasion of genogroup 4 may be more complex than previously thought.

This is the first study evaluating the efficacy of maternal antibodies in protecting chicks against a variant isolate belonging to G4 during the first days of their lives. IBDV is known to be especially problematic during early stages of life as it can compromise the immune status of the chickens for the rest of their lives (Faragher et al., 1974; Saif, 1991). The results of our study showed that approximately 20% of the animals infected with G4 showed typical IBDV lesions, whereas none of the animals infected with G1 showed those lesions. Given the narrow profit margins in intensive poultry farming, our results highlight the importance of taking measures capable of preventing infection by variant strains of IBDV, such as their inclusion in vaccine formulations.

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## DISCLOSURES

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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