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Original article

The influence of Ross 308 breeder flock age on serum Newcastle Disease Virus and *Infectious Bursal Disease Virus antibody* levels and their transfer to offspring

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Abstract

An effectively immunized parent flock forms the foundation for the proper development of healthy chicks, largely due to the mechanism of vertical transfer of maternal antibodies (MatAb), which provides passive immunity during the early life stages of the chicks. The aim of this study was to evaluate the influence of the age of the Ross 308 parent flock on antibody levels in the serum of the hens and their chicks during the same period with regard to *Infectious Bursal Disease* (IBD) and Newcastle Disease (ND), as well as to analyses the transfer of antibodies from the hens to their offspring. The study was conducted on a farm with 6500 hens. Blood samples for serological analysis were randomly collected from 84 hens of a single flock and 84 one-day-old chicks hatched from eggs laid by this flock at 30, 40, 50, and 60 weeks of age. In the laboratory, antibody levels in the hens' blood serum against Newcastle disease virus (NDV) and infectious bursal disease virus (IBDV) were determined using the ELISA test. At the same time, maternal antibody titers (MatAb) in chicks against the same pathogens were measured. The percentage (%) of maternal antibody transfer to the offspring was then assessed. The study demonstrated the impact of parent flock age on antibody levels against the tested pathogens, expressed as geometric mean titers (GMT). During the laying period, GMT values for antibodies against NDV increased with flock age. The highest values were observed at the end of the reproductive period (GMT_{60}=20456), and the lowest at the beginning (GMT_{30}=12631). An opposite trend was observed for IBDV antibodies, whose levels decreased with increasing hen age. The highest values (GMT_{30}=10759) occurred at the beginning of the laying period, and the lowest at its end (GMT_{60}=7421). The highest vertical antibody transfer to offspring was observed for IBDV antibodies (97.7%) at 40 weeks of age, while the lowest was for NDV antibodies (59.7%) at 60 weeks of age. The analysis of results showed significant variation in antibody levels in hen serum during the laying period, as well as in the amount of maternal antibodies transferred to offspring. The obtained data suggest that the effectiveness of passive immunity transfer to chicks is closely related to both the age of the parent flock and the nature of the pathogen targeted by vaccination.

Keywords: humoral immunity, maternal antibody transfer, broiler breeders hens, broiler, infection diseases

Introduction

The effectiveness of early immunological protection in broiler chicks is a key determinant of their health status and production performance during the initial stages of rearing (Panth et al. 2019, Wegner et al. 2025). In commercial poultry production systems, chicks are exposed to a variety of infectious agents shortly after hatch, including Newcastle disease virus (NDV) and infectious bursal disease virus (IBDV), both of which remain economically important pathogens worldwide (Alexander et al. 2000, Muller et al. 2012). Because the immune system of newly hatched chicks is not yet fully developed, protection during the first weeks of life largely depends on maternally derived antibodies (MatAb) transferred from the breeder hen via the egg (Kowalczyk et al. 1985, Chrząstek et al. 2014). The level of passive immunity in day-old chicks is influenced by multiple factors, including the immunological status of the breeder flock, vaccination program, environmental conditions, and hen age (Muller et al. 2012, Santos et al. 2022). Previous studies have shown that antibody titers in breeder hens may vary throughout the laying period, and that maternal antibody levels in chicks are associated with circulating immunoglobulin concentrations in hens (Hamal et al. 2006, Ali and Hasan 2018). However, published data are not fully consistent regarding the direction and magnitude of age-related changes, particularly under commercial production conditions. Moreover, relatively few field-based studies simultaneously assess antibody levels in breeder hens and their one-day-old progeny across successive stages of the reproductive cycle within the same flock (Kitaguchi et al. 2008, Murai 2013). This creates a practical and scientific gap: although the biological mechanisms of maternal antibody transfer are well described (Leslie and Clem 1969, Murai 2013), less attention has been given to the age-related dynamics of pathogen-specific antibodies in breeder hens and their offspring under real commercial conditions. Understanding age-related dynamics of antibodies against NDV and IBDV in both hens and chicks is of practical importance (van den Berg et al. 2000, Deka et al. 2020). Insufficient levels of maternal antibodies may increase susceptibility of chicks to early infection, whereas excessively high titers may interfere with early vaccination efficacy (Ahmed and Akhter 2003, Gharaibeh and Mahmound 2013). Knowledge of how antibody levels and their apparent transfer rates change during the laying period may therefore support optimization of vaccination schedules and hatch management strategies (Oviedo-Rondon et al. 2013, Ali and Hasan 2018).

The aim of the present study was to evaluate age-related changes in serum antibody levels against NDV and IBDV in a commercial Ross 308 breeder flock and to describe the corresponding levels of maternal antibodies in one-day-old chicks hatched at different stages of the laying period under field conditions. Additionally, the study assessed the proportion of antibody levels observed in chicks relative to those measured in hens, providing a descriptive analysis of antibody dynamics within a single commercial production system.

Materials and Methods

This study is observational and descriptive, focusing on age-related changes in NDV and IBDV antibody levels in the serum of Ross 308 breeder hens, as well as describing the corresponding levels of maternal antibodies in one-day-old chicks. The study was not designed as an experiment with control groups and does not allow for causal conclusions. The results should be interpreted as descriptive field data obtained under commercial production conditions. The blood samples from the breeder hens were collected as part of routine flock health monitoring throughout the reproductive period. No additional interventions or experimental procedures were performed specifically for this study; the samples were obtained during standard veterinary care. The laboratory analysis of antibody titers was conducted subsequently by the research team. As the sampling was part of routine veterinary procedures and did not involve any additional procedures affecting animal welfare, formal approval from an ethics committee was not required, in accordance with the Polish Animal Protection Act and in line with European Union Directive 2010/63/EU on the protection of animals used for scientific or educational purposes. The study followed the ARRIVE guidelines.

Animals and farm environment

For serological analysis, blood samples were collected from 84 randomly selected hens of a Ross 308 breeder flock and from 84 one-day-old broiler chicks hatched from eggs laid by hens during the same period (30, 40, 50, and 60 weeks of age). The hens were selected from a commercial flock consisting of 6500 birds, kept in a poultry house on a farm in Poland. The selected hens were marked on the neck and wing with a blue marker for identification in next sampling period. The same hens were sampled at each time point (30, 40, 50, and 60 weeks), allowing assessment of antibody level changes in the same individuals across the laying period (repeated-measures design). Rearing was conducted under controlled conditions until 18 weeks of

Table 1. Vaccination program of the broiler breeder flock Ross 308.

Age	Disease	Serotype/strain	Type of vaccine	Method of vaccination	Company
1 d	IBD+ND+HVT; ND	HVP360; VG/GA	live	injection/spray	MSD/BI
3 wk	IBD	Winterfield 2512	live	spray	Ceva
5 wk	ND	Ulster 2C	live	spray	Zoetis
10 wk	ND	VG/GA	live	spray	BI
18 wk	IBD+REO	VNJO+S1133	inactivated	intramuscular injection	BI
18 wk	ND+IB+EDS+ART	Ulster 2C+Mass 41+V127+VCO3	inactivated	intramuscular injection	BI

Note: MSD Animal Health, Boehringer-Ingelheim – BI, ND – Newcastle disease, IBD – Infectious Bursal disease, REO – Reovirus, IB – Infectious bronchitis, EDS – Egg Drop Syndrome, ART – Avian Rhinotracheitis

age, in accordance with Aviagen guidelines (Aviagen 2016). Environmental parameters, such as temperature (18-20°C), relative humidity (55-65%), and ventilation, were continuously monitored and adjusted based on daily observations. During the rearing period, a standard immunoprophylactic program was applied, as detailed in Table 1. After the rearing phase, the birds were transferred to a breeder farm. In the laying phase, males and females were housed together in the same building. Feeding was differentiated by sex, following the recommendations of Aviagen (2016). The birds had constant access to water and were kept under artificial lighting for 14.5 hours of light and 9.5 hours of darkness. The poultry houses were equipped with wood shavings litter and automatic nests.

The birds were then transported to a commercial farm. During the laying period, layers and turkey males were kept in a turkey house, where they received separate fodder as recommended (2016) and had unlimited access to water; exposure to light was 14.5 hours/day. The birds were kept on straw, and the turkey houses were equipped with manual nests – 4 layers per nest. The temperature inside the turkey houses was kept at 16-17°C, relative humidity in the laying period was 50% to 70%, and light intensity was 40 lux.

Serological analysis

At 30, 40, 50, and 60 weeks of age, during routine veterinary monitoring, blood samples were collected from 84 hens (21 birds at each of the four sampling periods). The samples were sent to an accredited veterinary laboratory for serological analysis. The obtained serum was used to determine antibody titers against Newcastle Disease Virus (NDV) and Infectious Bursal Disease Virus (IBDV), and to calculate the geometric mean titers (GMT) for both pathogens. In the same weeks, hatching eggs were collected from the breeder flock. After hatching, 84 randomly selected chicks (21 from each of the four sampling periods) were sampled for blood to determine the level of maternal antibodies (MatAb). These samples were delivered to the same laboratory, where ELISA tests were performed using commercial kits (NDV – IDEXX NDV, Netherlands; IBDV - IDEXX IBD Ab Test). The assays were conducted according to the manufacturer's instructions, and antibody titers (IgG/IgY) were quantitatively assessed using dedicated software. GMT values were also calculated for each group. All measurements were referenced against appropriate positive and negative controls, as well as standard antisera. The intra-assay and inter-assay coefficients of variation (CV) for the assays were below 10%, in accordance with the manufacturer's specifications. The percentage transfer of antibodies against ND (Newcastle disease) and IBD (Infectious Bursal Disease) was calculated using the following formula during the aforementioned study periods: Transfer % = (Maternal antibody level (MatAb) in chicks / Antibody titer level in hens) × 100%. Due to the group housing conditions and shared nesting systems, it was not possible to link individual hens with their offspring. Consequently, the percentage of antibody transfer was calculated at the age-group level, and the results should be interpreted as describing population level trends rather than individual transfer efficiency.

Statistical analyses

Antibody titers were expressed as geometric mean titers (GMT). Prior to statistical analysis, individual antibody titers were $\log_{10}$-transformed to improve normality and stabilize variance. Data from breeder hens and broiler chicks were analysed separately. For breeder hens, because repeated measurements were obtained from the same individuals at 30, 40, 50, and 60 weeks of age, a linear mixed-effects model was applied using the PROC MIXED procedure in SAS 9.4 (SAS Institute Inc., Cary, NC, USA). Week of age was included as a fixed effect, and bird identity was included as a random effect to account for within-subject correlation across repeated measurements. The covariance

Table 2. Geometric mean titers (mean ±SD) of the parent stock Ross 308 at different ages.

Trait	30 wk*	40 wk	50 wk	60 wk	P-Value
IBDV	$10759^a \pm 1218$	$7843^b \pm 2205$	$7914^b \pm 2085$	$7421^b \pm 2624$	<0.0001
NDV	$12631^c \pm 2\,456$	$13418^c \pm 1674$	$17772^b \pm 2540$	$20456^a \pm 4585$	<0.0001

IBDV – Infectious Bursal disease virus, NDV – Newcastle disease virus

^{a,b} mean values marked with different letters in columns differ significantly (p≤0.05).

* age of the par

Table 3. Geometric mean titers (mean ±SD) of broiler chicks on the first day of life.

Trait	30 wk*	40 wk	50 wk	60 wk	P-Value
IBDV	$10158^a \pm 1327$	$7675^b \pm 1918$	$7539^b \pm 2942$	$4731^c \pm 1853$	<0.0001
NDV	$8848^b \pm 2956$	$12119^a \pm 2474$	$13921^a \pm 2362$	$12223^a \pm 7212$	<0.0001

IBDV – *Infectious Bursal disease virus*, NDV – *Newcastle disease virus*

^{a,b} mean values marked with different letters in columns differ significantly (p≤0.05).

* age of the parent stock.

structure for repeated observations was modelled using compound symmetry. Model parameters were estimated using restricted maximum likelihood (REML), and denominator degrees of freedom were calculated using the Satterthwaite approximation. When a significant effect of age was detected, least squares means (LSMeans) were compared using Tukey-Kramer adjustment for multiple comparisons. For broiler chicks, antibody titers were analysed using one-way analysis of variance (ANOVA), with hen age (30, 40, 50, and 60 weeks) as the fixed factor. Post hoc comparisons were performed using Tukey's test. Significance of differences was verified at p≤0.05.

Results

Serological analysis

The analysis of the serological results from the Ross 308 breeder flock (Table 2) revealed significant differences (p≤0.05) in antibody titers (anti-IBD and anti-ND), expressed as geometric mean titers (GMT), throughout the laying period. In hens vaccinated against IBDV, antibody levels significantly decreased between 30 weeks of age (GMT=10759) and all subsequent time points (30 *vs.* 40: p=0.0026; 30 *vs.* 50: p=0.0045; 30 *vs.* 60: p<0.0001), whereas no significant differences were observed between weeks 40, 50 and 60.

In contrast, NDV antibody levels increased with the age of the birds. Significant differences were observed between 30 weeks of age (GMT_{30}=12631) and 50 and 60 weeks (GMT_{50}=17772 and GMT_{60}=20456) (p=0.0027, p<0.0001; respectively), as well as between 40 weeks of age (GMT_{40}=13418) and 50 and 60 weeks (p=0.0397, p=0.0005; respectively). No significant difference was observed between 50 and 60 weeks of age.

The serological results in chicks also showed significant differences in anti-IBD and anti-ND antibody levels depending on the age of the hens (Table 3). For maternal antibodies against IBDV, a significant decline was observed between 30 and 60 weeks of hen age (p<0.0001; GMT_{30}=1158 *vs.* GMT_{60}=4731), between 40 and 60 weeks (p<0.0001; GMT_{40}=7675 *vs.* GMT_{60}=4731), and between 50 and 60 weeks (p=0.0066), indicating a marked reduction in passive immunity toward the end of the laying period. For NDV, significant differences were observed mainly between 30 and 50 weeks (p=0.0019; GMT_{30}=8848 *vs.* GMT_{50}=13921), while the other comparisons were not statistically significant.

Table 4 presents the average transfer rate of antibodies from hens to chicks, which was 87.8% for IBDV-specific antibodies and 74.6% for NDV-specific antibodies. The highest anti-IBDV antibody transfer was observed from the beginning of the laying period up to the 50^{th} week of hen age (95.3%), while during the final phase of the reproductive cycle, the average transfer dropped to 63.7%. In the case of anti-NDV antibodies, the highest transfer to chicks occurred at 40 weeks of hen age (90.3%). However, at the beginning and end of the laying cycle, the transfer rate was lower, reaching 59.7% at 60 weeks.

Discussion

Prophylactic vaccination in poultry is a key element of immunoprophylaxis, aimed at eliciting a specific immune response against targeted antigens (Suardana et al. 2023). As this study is observational and descriptive, conclusions regarding causality between hen age and antibody transfer should be interpreted cautiously, reflecting trends at the flock level rather than individual hens. Antibody levels in breeder poultry flocks depend on age, genetic line, and genotypic predispositions

Table 4. The percentage (%) of antibodies transfer from broiler breeder hens to poults.

Trait	30 wk*	40 wk	50 wk	60 wk	mean
IBDV	94.4 %	97.7%	95.3%	63.7%	87.8%
NDV	70.0%	90.3%	78.3%	59.7%	74.6%

IBDV – Infectious Bursal disease virus, NDV – Newcastle disease virus
* age of the parent stock.

of the immune system (Leandro et al. 2011, Santos et al. 2022). In our observations, the age of the breeder flock influenced the levels of specific antibodies in serum against IBDV and NDV. The highest levels of anti-IBD antibodies were observed at 30 weeks of age, which is consistent with previous reports (Eidson et al. 1980, Murai et al. 2020), indicating that young hens, shortly after intensive immunization during the rearing phase, exhibit the strongest humoral response at the onset of laying. In the following weeks, there was a trend of stabilization or moderate decline in antibody levels, which may be related to the natural waning of the immune response or reduced exposure to booster antigen (Parker and de Wit 2014). In contrast, anti-NDV antibody levels in the present study showed an opposite trend, with GMT values increasing with the age of the hens, reaching the highest level at 60 weeks. The increase in antibody titers with age may be due to repeated exposure to various antigenic variants, which reinforces immunological memory and leads to higher serum antibody levels. These observations align with the findings of Ali and Hasan (2018), who also reported higher anti-NDV antibody titers in serum of breeder hens at the end of the laying period. Variations in antibody levels during laying may also be influenced by environmental and physiological factors (Oviedo-Rondon et al. 2013). Increased oxidative stress, intensified metabolism related to egg production, and the gradual aging of the immune system may affect the dynamics of the humoral response (Gharaibeh and Mahmound 2013). The antibodies produced by hens in response to vaccination are passed on to their offspring via vertical transfer, primarily through the egg yolk. The efficiency of this transfer, expressed as the percentage presence of maternal antibodies (MatAb) in chick serum, is variable and depends on the aetiology of the infectious agent (Ali and Hasan 2018). Maternal antibody transfer into the egg yolk is closely correlated with the circulating immunoglobulin levels in hens (Ali and Hasan 2018). The results obtained in the present observations clearly demonstrate the high efficiency of immunoglobulin transfer from hens to egg yolk, as confirmed by the average values: 87.8% for antibodies against IBDV and 74.6% for those against NDV. It should be noted that antibody transfer percentages were calculated at the group level, reflecting population trends rather than individual hen-offspring pairs. These findings are consistent with earlier research by Hamal et al. (2006), which showed that IgY is the main antibody class transferred to the yolk, and its level strongly correlates with the circulating immunoglobulin level in hens. The highest percentage of anti-IBDV antibody transfer was observed in the early and mid-phase of the laying cycle (up to 50 weeks of age), indicating optimal IgY transport activity during this period. The reduction in transfer efficiency later in life (averaging 63.7% at 60 weeks) may be related to physiological aging of the immune system, ovarian function changes, or a possible decline in the expression of immunoglobulin transport receptors-phenomena previously observed in studies by Kitaguchi et al. (2008) and Murai (2013). For anti-NDV antibodies, peak transfer (90.3%) occurred at 40 weeks of age. In both earlier (30 weeks) and later (60 weeks) phases of the reproductive period, lower passive immunity transfer efficiency was recorded (74.6% and 59.7%, respectively). This may indicate dynamic fluctuations in circulating antibody levels in hens, influenced by the reproductive cycle stage and the birds' physiological condition. Moreover, the observed differences in IgY transfer efficiency into the yolk may reflect antigen-dependent variation in the activity of antibody transport mechanisms. This phenomenon was also described by Kowalczyk et al. (1985), who highlighted the antigen-specific nature of passive immunity transfer, suggesting that not all antibodies are transmitted with equal efficiency – an aspect that may significantly impact chick immune protection during their first days of life. A high level of antibody transfer during the mid-laying period can provide chicks with effective immunological protection during their early life stages, when their own immune systems are not yet fully developed. Conversely, the decline in transfer efficiency in older hens may necessitate adjustments in vaccination schedules or the implementation of additional immunoprophylaxis strategies in older breeder flocks. From a poultry management and biosecurity perspective, monitoring antibody levels in breeder flocks throughout the entire laying period is essential. It not only allows for the evaluation of immunoprophylaxis program effectiveness but also for the optimization of hatch timing and chick vaccination strategies.

The findings of our observations emphasize the need to consider the age of breeder hens when planning vaccination schedules for chicks, as maternal antibody levels and transfer efficiency vary with hen age and pathogen. This may require adjustment of early-life immunization timing and monitoring of older breeder flocks to ensure effective passive immunity.

This study has several limitations. It is strictly observational, conducted on a single flock, with no independent biological replication and no possibility of individual hen offspring pairing. Antibody transfer percentages were calculated at the group level and should be interpreted as population trends rather than individual transfer efficiency. Additionally, measuring total IgY content in egg yolk or yolk sac could strengthen interpretation, as reduced transfer efficiency observed in older hens may partly reflect dilution due to larger egg size rather than a true decrease in antibody transfer.

Conclusions

The above observations demonstrated a significant influence of hen age on antibody levels in both the breeder flock and the chicks. For antibodies against infectious bursal disease virus (anti-IBDV), the highest GMT level in hens was observed at the beginning of the laying period (week 30), after which the values stabilized at similar levels. In contrast, the level of antibodies against Newcastle disease virus (anti-NDV) in hens increased with age, reaching its peak at 60 weeks of age. Significant differences were also noted in chick serum depending on the age of the breeder hens. The highest level of anti-IBDV antibodies in chicks was observed in those derived from 30-week-old hens, while the lowest level occurred at 60 weeks. In the case of anti-NDV antibodies, the opposite trend was noted where the antibody level increased with the age of the hens, stabilizing after week 40. The average maternal antibody transfer rate to chicks was 87.8% for anti-IBDV and 74.6% for anti-NDV. The highest transfer of anti-IBDV antibodies occurred up to week 50 of the hen's life, after which a significant decline was observed. Meanwhile, the peak transfer of anti-NDV antibodies was recorded at 40 weeks. It should be noted that antibody transfer percentages were calculated at the group level and reflect population trends rather than individual hen-offspring pairs.

As this study was strictly observational and descriptive, conclusions regarding causality between hen age and antibody levels or transfer should be interpreted cautiously. Nevertheless, these findings may inform vaccination planning for chicks, as maternal antibody levels and transfer efficiency vary with hen age and target pathogen.

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Author Declarations

Ethics approval

Ethical approval was not required, the blood samples were collected from the living animals during flock health monitoring throughout the laying period. There were no additional interventions on the animals and therefore ethics committee approval was not required, in accordance with the Polish Animal Protection Act and in line with European Union Directive 2010/63/EU on the protection of animals used for scientific or educational purposes.

Use of generative artificial intelligence

Generative artificial intelligence (AI) tools were not used in the preparation of the manuscript.

Conflict of interest

The authors declare no conflict of interest. The authors declare that although the first author is affiliated with a pharmaceutical company producing poultry vaccines, this study was conducted independently. The selection of samples, data collection, and analysis were carried out by the authors without influence from the manufacturer. All vaccines used in the studied flock are explicitly listed with their respective producers.

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