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Immunogenicity of a Novel Inactivated Trivalent Avian Reovirus Vaccine in Egyptian Broiler Breeders and their Progeny

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Abstract | The global poultry industry continues to encounter a significant challenge, experiencing severe production losses in broiler, breeder, and layer flocks due to the inability of classical commercial avian reovirus (ARV) vaccines to control circulating variant strains. Currently, customized vaccines tailored to match ARV variants are not yet available. This study evaluates the first novel inactivated trivalent vaccine produced by MEVAC company in Egypt, which includes the classical strain "S1133" along with the most prevalent circulating variant strains in Egypt (GC4 and GC5). The evaluation focuses on the humoral immune response in three breeder flocks and their progeny, specifically assessing the duration of immunity during different ages, aiming to assess both active and passive immunity through common commercial ARV ELISA and serum neutralization test (SNT) for the homologous ARV strain. The commercial breeders were vaccinated with a classical live attenuated vaccine containing S1133, followed by immunization with the trivalent vaccine using a prime-boost strategy. The results indicated that effective seroconversion began at 5 weeks post-prime from the inactivated vaccine, as measured by both common ELISA and SNT against each homologous strain. Antibody levels increased in most flocks, peaking up to 25 weeks post-booster (40 weeks of age). A significant transfer of maternal antibodies was observed, providing robust passive immunity to broiler progeny across multiple hatches, from the 9th to 25th week following booster vaccination (24-40 weeks of breeders age). In conclusion, this study demonstrates that the newly developed inactivated avian reovirus vaccine, when administered to breeder flocks, is highly immunogenic eliciting a robust and specific immune response. The successful vertical transfer of these maternal antibodies to progeny grants them substantial passive immunity during the critical early stages of life. We recommend conducting a challenge study in progeny to assess the vaccine's effectiveness in conferring protection against variant field avian reovirus challenges at the different critical life stages.

Keywords | Inactivated vaccine, Avian reovirus, Broiler breeders, Immunity, S1133

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INTRODUCTION

Avian reovirus (ARV) is a globally widespread pathogen that affects chickens, turkeys, and other birds (Pitcovski and Goyal, 2020). ARV was first isolated from chickens in 1954 (Fahey and Crawley, 1954), since that time, it has continued to cause severe production losses in broiler, breeder, and layer flocks. Infected chickens

often develop different pathological problems such as viral arthritis (VA), immunosuppression, pericarditis (Schat and Skinner, 2022), as well as digestive system diseases such as runting stunting syndrome (RSS) and respiratory complications (Jones, 2000; Pitcovski and Goyal, 2020).

ARVs are non-enveloped viruses belonging to the genus *Orthoreovirus*, subfamily *Spinareovirinae*, and family *Reoviridae*, the genome consists of segmented double-stranded RNA, which makes it prone to mutation, reassortment and recombination, leading to the continuous emergence of new variant strains. The ARV genome is divided into 10 distinct segments (L1–L3, M1–M3, and S1–S4), S1 segment encoding three viral proteins, including the minor capsid protein sigma c "σC" (Jones, 2013). This protein is particularly important because it enables the virus to attach to the host cells during infection and triggers the neutralizing antibodies production (Wickramasinghe *et al*., 1993; Kant *et al*., 2003; Vasserman *et al*., 2004).

Recently, based on the σC gene phylogeny "genetic marker", ARVs were classified into seven genogroup clusters (GC1-GC7) (De la Torre *et al*., 2021; Egana-Labrin and Broadbent, 2023; Sallam *et al*., 2025). The existence of subclusters within the same ARV cluster may be attributed to low amino acid identity among samples, which can range from 40% to 100% make the virus classification more complicated (Lu *et al*., 2015; Palomino-Tapia *et al*., 2018; Zhang *et al*., 2019; De Carli *et al*., 2020). Sallam *et al*. (2025), confirmed that GC1, GC2, and GC4 each contain four sub-lineages, while GC3 and GC5 have three sub-lineages. In contrast, GC6 and GC7 display only two and one sub-lineage, respectively. Current studies revealed that all vaccine seeds such as S1733, S1133, and 2408 belong to classical lineage of GC1, whereas most field isolates fall into variant clusters and subclusters of GC1 to GC7 (Kant *et al*., 2003; Lu *et al*., 2015; Arafa *et al*., 2024a, b; Sallam *et al*., 2025). These variants are associated with several disease conditions as pericarditis, VA, RSS, tenosynovitis and subclinical infections in vaccinated breeder flocks and their progeny (Davis *et al*., 2013; Zhong *et al*., 2016; Ayalew *et al*., 2023; Arafa *et al*., 2024b).

In Egypt, ARV was first reported in 1984 (Tantawi *et al*., 1984). The circulating ARVs in Egypt are GC1,2,3,4,5 and GC6 based on σC gene classification with predominance of GC5 and GC4 respectively (Kovács *et al*., 2023; Mahmoud Sabra, 2023; Mosad *et al*., 2023; Zanaty *et al*., 2023; Arafa *et al*., 2024a, b; Safwat *et al*., 2024; Sallam *et al*., 2025). We have previously isolated several ARVs from broiler chickens, breeders, and commercial grandparent chickens showing clinical signs of ARV infection and performed phylogenetic analysis of these strains which demonstrated that the circulating ARVs were genetically distant from the vaccine and vaccine strains (Arafa *et al*., 2024a, b). Numerous reports have confirmed that ARV variants can undermine maternal immunity induced by existing classical vaccines (Goldenberg *et al*., 2010; Ayalew *et al*., 2017; Palomino-Tapia *et al*., 2018; Ayalew *et al*., 2023). Despite the urgent need for effective countermeasures against emerging ARVs variants in Egypt, the development of customized vaccines has received little attention.

The global literatures provide limited studies detailing the efficacy and immunogenicity evaluation of customized vaccines against variant ARV strains (Lublin *et al*., 2011; Ayalew *et al*., 2023; Liu *et al*., 2023a, b). Consequently, this study aimed to evaluate immunogenicity of the first inactivated ARV vaccine in Egypt which includes the classical strain S1133 along with the most prevalent circulating variant strains in Egypt (GC4 and GC5), focusing on breeders and their offspring, as well as the duration of immunity.

MATERIALS AND METHODS

Avian reoviruses used

ARVs representing GC1 S1133 (MEVAC/Avian_Reovirus_GC1/1133/2021, Accession No. OQ675704), GC4 (MEVAC/Avian_Reovirus_GC4/2022, Accession No. OQ675707), and GC5 (MEVAC/Avian_Reovirus_GC5/2021, Accession No. OQ675709) were developed and purified by Middle East for Veterinary Vaccines (MEVAC, El-Sharkia, 44671, Egypt) and were used for preparation of a novel trivalent inactivated vaccine as well as antigens for serum neutralization test.

Novel inactivated trivalent vaccine

A newly developed trivalent inactivated vaccine "MEVAC™ MULTI REO" was manufactured by MEVAC company incorporating the classical strain S1133 along with the most predominant circulating variant strains in Egypt (GC4 and GC5) formulated as a water-in-oil emulsion inactivated vaccine using Montanide ISA 70 (Seppic, France) with antigen at ratio of 30/70 (v/v) according to the manufacturer's instructions (means 30% water phase "antigen and saline" and 70% "adjuvant"). Each bird received 0.5 ml vaccine by intramuscular (IM) route containing $TCID_{50} \geq 6$ log 10 for GC4 and GC5 and $TCID_{50} \geq 7$ log 10 for S1133 per bird.

ARV commercial classical live vaccine

Nobilis® Reo 1133 (MSD, batch no. A061AJ01) live attenuated vaccine (strain S1133) with dose ≥ 3.1 log 10 $TCID_{50}$ per bird. The vaccine represented classical sub-lineage of genogroup cluster I (GCI) and was administrated at 3 weeks of age by injection 0.2 ml per bird subcutaneously (S.C) as a prime live vaccination.

Birds and experimental design

Three commercial breeder flocks (Farms 1, 2, and 3; n=60,000 each) were raised in Egypt, originating from different grandparent flocks under field conditions. They received vaccinations with a commercial classical live attenuated vaccine containing S1133 at 3 weeks of age. All flocks were immunized using a prepared trivalent inactivated vaccine by IM route through prime-boost strategy at 7 and 15 weeks of age. Fifteen serum samples were collected from each flock at 5-7 different time points/flocks to monitor antibody titers using ELISA and SNT. This included samples taken at 5 weeks of age (prior to the administration of the inactivated vaccine), at 12 weeks of age (5 weeks following the first dose of the inactivated vaccine) and at 24, 30, 33, and 37 weeks of age in Flock 1. The same samples time points were obtained from Flock 2, with an additional collection at week 40 of age (corresponding to 9, 15, 18, 22, and 25 weeks after the booster dose of the inactivated vaccine, respectively). In contrast, samples collected from Flock 3 at 5, 12, 24, 30 and 33 weeks of age (Figure 1).

Evaluation of the efficacy of vaccination in broilers progeny

Fifteen broiler progeny, each four days old, were selected from vaccinated breeder flocks at different weeks of egg production, ranging from 24 to 40 weeks of the breeders' age (9 to 25 weeks post-booster vaccination), to evaluate the level of passive maternal antibodies transferred and their persistence using both ELISA and SNT. Progeny chicks from Flock 1 were gathered at 24, 30, 33, and 37 weeks breeders' age, while the same samples were obtained from Flock 2, with an additional collection at week 40 of breeders' age. In contrast, Flock 3 collected samples solely at 24 and 30 weeks of breeders' age.

Enzyme linked immunosorbent assay

Antibody titers of ARVs were determined using the ELISA assay in accordance with the manufacturer's guidelines (IDEXX Laboratories, REF 99-09264, Lot EZ017, USA). A cut-off value of 396 (S/P ratio > 0.2) was established, and results above this threshold were considered positive. All serum samples from the collected time points were evaluated for the presence of common ARV antibodies.

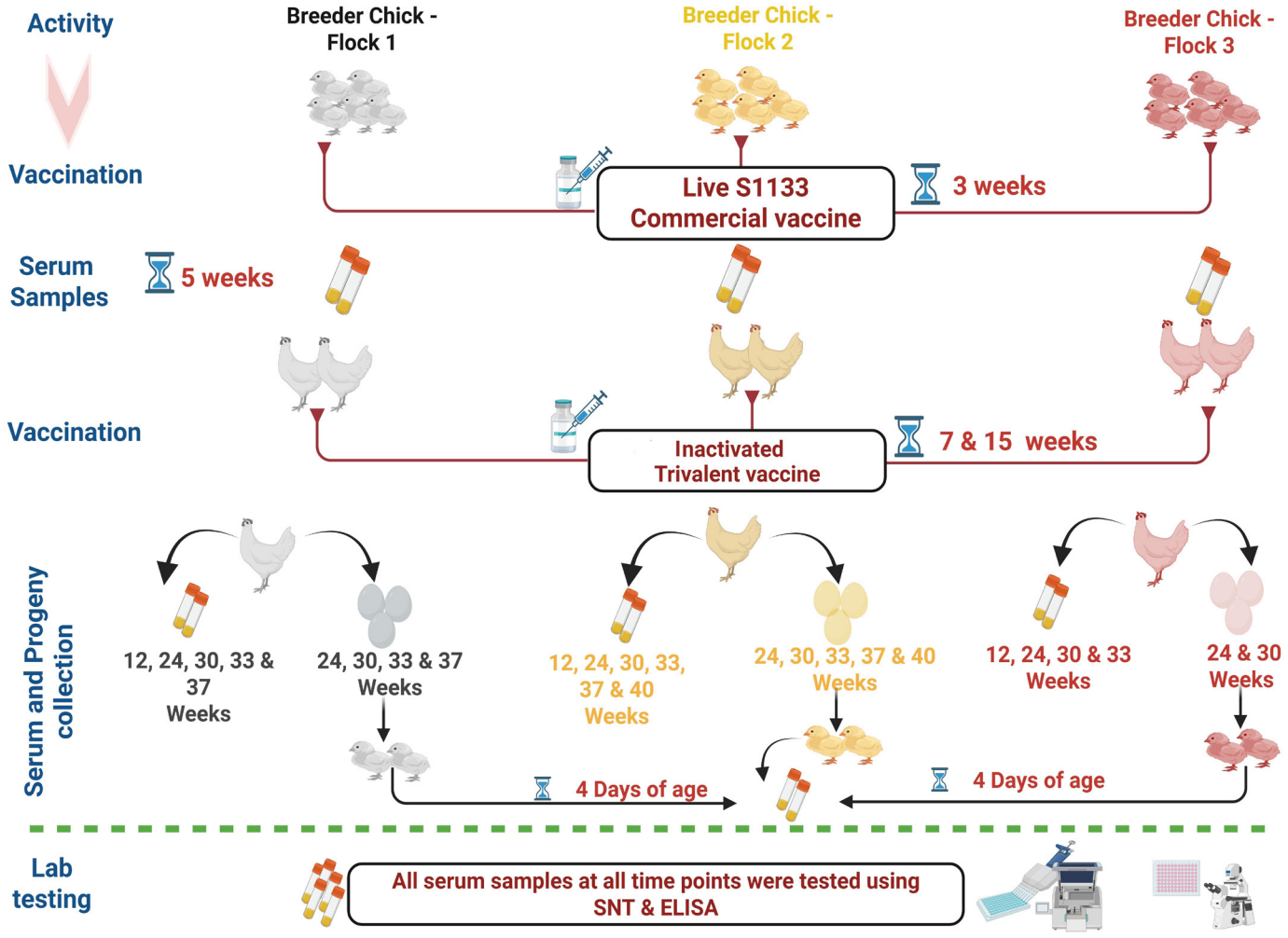

Figure 1: Detailed study design for immunogenicity evaluation in breeder and progeny flocks.

Serum neutralization test

All collected serum samples were evaluated for each homologous strain of ARVs (S1133, GC4 and GC5) using beta method of SNT (3 replicate for each sample). The serum samples underwent heat inactivation at 56◦C for 30 minutes and were subsequently filtered through a 0.22 µm filter. After performing a two-fold serial dilution of the serum samples (50 micron/well) in 96-well TC plates, the inactivated serum was mixed with an equal volume of the homologous ARV (100 TCID50/well) which was determined by (Reed and Muench, 1938) and confirmed by back titration on chicken embryo liver cells (primary culture – passage 2). The plates were incubated for 1 hour at 37˚C in a 5% CO_2 environment. Chicken embryo liver cells were cultured in Dulbecco's Modified Eagle medium (DMEM) (Capricorn scientific, Cat No. DMEM-HPA, lot NO. CP25-8046) with 10% fetal bovine serum were added to the mixtures (seeded at 40,000 cells per well). The plates were incubated at 37˚C in a 5% CO_2 atmosphere and examined up to 48 hours or until 100% CPE is observed in virus control wells following (Markis and Rosenberger, 2016). A serum dilution was considered neutralizing when all three replicate wells showed no formation of giant cells. A positive result is indicated by an SNT titer exceeding 6 log2, while a titer of 6 log2 is considered suspect, and a titer below 6 log2 is classified as negative according to (Markis and Rosenberger, 2016). The detailed study design was illustrated in Figure 1.

Statistical analysis

Data were analysed using GraphPad Prism 9 (Graph Pad Inc., San Diego, CA), one-way analysis of variance (ANOVA) with statistical significance level of $p < 0.05$.

RESULTS

ELISA results

The mean ELISA titers for the three breeder flocks at 5 weeks of age ranged from 365 to 1315. In contrast, at 12 weeks of age (5 weeks after the first dose of inactivated vaccine), the titers increased to 2454, 1766, and 4401 for Farms 1, 2, and 3, respectively. Elevated antibody titers were maintained after the booster dose across all subsequent time points (3-5 time points) for the three breeder farms, achieving a peak level of 13480 in breeder farm 1 at 30 weeks of age (15 weeks post booster dose). Subsequently, titers declined, but the mean titer remained at 2959 at 37 weeks of age. In farms 2 and 3, the titers continued to increase reaching 9417 and 15468 at age 40 weeks and 33 weeks of age, respectively (Figure 2A).

The maternal antibody levels in broiler progeny, as measured by ELISA, corresponded to the antibody levels in their parents at all time points (24-40 weeks of breeder age). The ELISA results for progeny hatched from breeders in Farm 1 were 2662, 2612, 1730 and 1634 at 24, 30, 33, and 37 weeks of breeder age, respectively (after 9-, 15-, 18- and 22-weeks post-booster vaccination). In comparison, they were 4413, 3925, 3725, 5901, 5933 from egg batches in Farm 2 for breeder ages 24, 30, 33, 37 and 40 weeks respectively. Additionally, the progeny from Farm 3 had ELISA titers of 10,432 and 7,056 from breeders aged 24 and 30 weeks, respectively (Figure 3A). Comprehensive ELISA results are detailed in Tables 1 and 2.

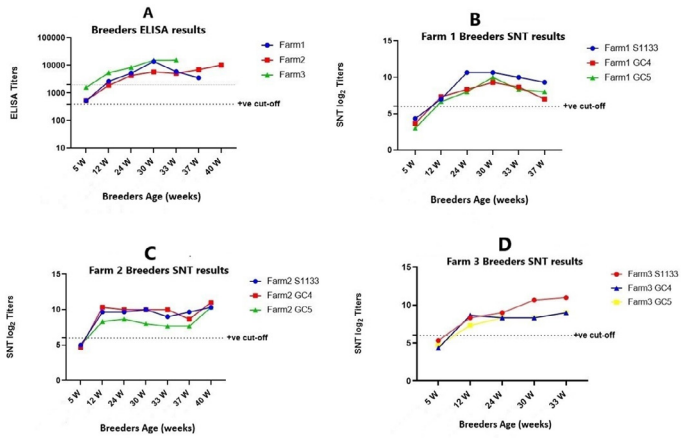

Figure 2: ELISA and SNT results for breeders. (A) breeders ELISA results, (B) farm 1 breeders SNT results, (C) farm 2 breeders SNT results, (D) farm 3 breeders SNT results.

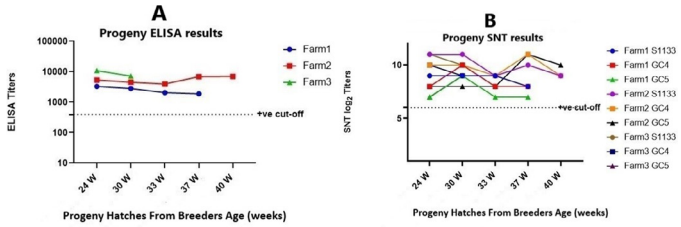

Figure 3: ELISA and SNT results for progeny. (A) Progeny ELISA results. (B) Progeny SNT results.

Serum neutralization test results

The average SNT results for S1133, GC4, and GC5 across the three breeder flocks at 5 weeks of age varied from 3 log2 to 5.3 log2 and continued to rise by 12 weeks of age, reaching values between 6.7 log2 and 10.3 log2 against the homologs classical S1133, variant GC4, variant GC5 strains.

The results indicated that high immunized titers were maintained after the second inactivated dose at all measured time points (3-5 time points) across the three breeder farms, achieving titers of 9.3 log2 for S1133, 7 log2 for GC4, and 8 log2 for GC5 at 37 weeks of age in Farm 1. In Farm 2, SNT readings reached between 10.3 and 11 log2 for the three homologous genotypes at 40 weeks of age. In breeder Farms 3, SNT readings reached between

Table 1: ELISA and SNT detailed data for breeder farms (n=15 for each time point).

Farm	Breeder age (wks.)	Time Point	ELISA GMT	SNT S1133 (log2)	SNT GC4 (log2)	SNT GC5 (log2)	Standard deviation "average"
Farm 1 Breeder	5w	Before vaccination	365.3	4.3	3.7	3	0.7
	12w	5 w post prime	2453.8	7	7.3	6.7	0.7
	24w	9 w post booster	4720.9	10.7	8.3	8	1.5
	30w	15 w post booster	13480.4	10.7	9.3	10	0.7
	33w	18 w post booster	5541.2	10	8.7	8.3	1.4
	37w	22 w post booster	2958.5	9.3	7	8	1.5
Farm 2 Breeder	5w	Before vaccination	453.9	5	4.7	5	0.3
	12w	5 w post prime	1765.8	9.7	10.3	8.3	1.3
	24w	9 w post booster	3992.8	9.7	10	8.7	1
	30w	15 w post booster	4890.4	10	10	8	1.2
	33w	18 w post booster	4598.2	9	10	7.7	1.2
	37w	22 w post booster	6509	9.7	8.7	7.7	1.3
	40w	25 w post booster	9417.4	10.3	11	10.3	0.7
Farm 3 Breeder	5w	before vaccination	1315.1	5.3	4.3	4.7	1
	12w	5 w post prime	4400.5	8.3	8.7	7.3	1.1
	24w	9 W post booster	6472.8	9	8.3	8.3	1.1
	30w	15 W post booster	15223.7	10.7	8.3	8.3	1.8
	33w	18 W post booster	15468.2	11	9	9	1.3

Table 2: ELISA and SNT detailed data for progeny farms aged 4 days old (n=15 for each time point).

Farm	From breeders age	Time Point	ELISA GMT	SNT S1133 (log2)	SNT GC4 (log2)	SNT GC5 (log2)	Standard deviation "average"
Farm 1 Progeny	24w	Egg from 9 w post booster	2661.9	9	8	7	1
	30w	Egg from 15 w post booster	2611.7	9	10	9	0.6
	33w	Egg from 18 w post booster	1729.7	9	8	7	1
	37w	Egg from 22 w post booster	1634	8	8	7	0.6
Farm 2 Progeny	24w	Egg from 9 w post booster	4413.3	11	10	8	1.5
	30w	Egg from 15 w post booster	3925.3	11	10	8	1.5
	33w	Egg from 18 w post booster	3725.1	9	9	8	0.6
	37w	Egg from 22 w post booster	5901.2	10	11	11	0.6
	40w	Egg from 25 w post booster	5933.3	9	9	10	0.6
Farm 3 progeny	24w	Egg from 9 w post booster	10431.6	11	10	7	2.1
	30w	Egg from 15 w post booster	7056.1	10	9	9	0.6

9 and 11 log2 in the three homologous genotypes at 33 weeks of age (Figure 2B, C, D). The SNT results for the progeny hatched from farms 1, 2 and 3 ranged from 7 to 11 log2 across the three homologous genotypes consistent with the ELISA results points mentioned (Figure 3B). The detailed SNT results are provided in Tables 1 and 2.

DISCUSSION

Avian reovirus (ARV) has a significant economic impact on the poultry industry worldwide (Sellers, 2017; Palomino-Tapia *et al*., 2018; Gallardo, 2022; Arafa *et al*., 2024b; Sallam *et al*., 2025), including RSS, VA, immunosuppression and respiratory distress. To date, all traditional commercial vaccines used in Egypt and worldwide contain vaccine seed strains such as S1133 strain (attenuated by serial passaging in chicken embryo fibroblast cells and embryonated chicken eggs) which protects against tenosynovitis/arthritis (Van der Heide *et al*., 1983; Gallardo, 2022) and other strains such as 2408, 1733, and 2177 (Van der Heide *et al*., 1983; Gallardo, 2022) despite its inability to control challenges from variant field strains (Kant *et al*., 2003; Kort *et al*., 2015; Palomino-Tapia *et al*., 2018; Ayalew *et al*., 2023; Arafa *et al*., 2024b). All of these vaccine seeds belonged to the classical lineage of GC1, whereas most of the field isolates belonged to variant

clusters and subclusters of GC1 to GC7 (Kant *et al*., 2003; Lu *et al*., 2015; Arafa *et al*., 2024a, b; Sallam *et al*., 2025).

In Egypt, the circulating ARVs according to σC gene classification are GC1, 2, 3, 4, 5, and GC6, with GC5 (52%) and GC4 (30%) being the predominant ones, which have very low amino acid identity with vaccine strains reaching 38% (Kovács *et al*., 2023; Mahmoud-Sabra, 2023; Mosad *et al*., 2023; Zanaty *et al*., 2023; Arafa *et al*., 2024a, b; Safwat *et al*., 2024; Sallam *et al*., 2025). This high antigenic and genetic diversity explains why classic vaccines often fail in the field control of ARV infection (Palomino-Tapia *et al*., 2018). The development of autologous vaccines is urgently needed as an alternative approach to controlling ARV infections (Sellers, 2017; Gallardo, 2022), while efforts to develop new customized vaccines against these variants remain limited (Lublin *et al*., 2011; Ayalew *et al*., 2023; Liu *et al*., 2023a, b). Therefore, this study aimed to evaluate the immunogenicity of the first developed trivalent ARV inactivated vaccine in Egypt and worldwide which contain both classical S1133 strain and variant ARVs (GC4 and GC5) in three commercial breeder and three of their progeny flocks with assessment of the duration of immunity in both.

Breeders in this study were first vaccinated with a traditional S1133 live vaccine and then boosted with the trivalent customized preparation, aiming to match the circulating ARV variants. To evaluate seroconversion, we employed ELISA and the serum neutralization test (SNT) as common tests in ARV serology. ELISA provides a high-throughput, rapid measure of ARV-specific IgY, while SNT measures the neutralizing antibodies of different ARV genotypes (Caeiro, 2017; Achhal-Elkadmiri *et al*., 2023; Ayalew *et al*., 2023). The commercial ELISA achieved ~89% titers positivity compared to σC/σB-handmade ELISAs which achieved 100% concordance with SNT according to (Liu *et al*., 2002). But according to our results, positivity in both commercial ELISA and SNT is the same. Cases have been noted where ELISA results are highly positive but very low with SNT reported by (Caeiro, 2017), but our serological results are compatible in both tests (with exception in 5 weeks breeder age time point). Our results showed mean ELISA titers after 2 weeks post live S1133 vaccine in farm 1 and farm 2 (365-454 respectively) whereas ELISA cut off (396) this can be attributed to the mean results; however, the individual data demonstrated good positivity while in farm 3 it was 1315. Positive ELISA titers in this time point not reflect SNT against S1133 as the SNT titers were (4.3-5-5.3 log2) in Farm1 , 2 and 3 respectively and these results complying with (Caeiro, 2017). After post priming by 5 weeks (12 w age), the ELISA titers duplicated by 3-6 folds in the three farms showing a highly immunogenic titers which comply with the titers reported by (Ayalew *et al*., 2023) after 3 weeks post priming. In this point, ELISA results were complying with SNT titers which ranged from 6.7 to 10.3 log 2, indicating a highly immunogenic results for each homologous strain.

ELISA and SNT results showed high antibody titers in different breeders ages (24 w to 40 w age) up to 25 w post booster and their progeny from different hatches of breeders ages (up to 40 w age) indicating a highly efficient passive and active immunity elicited by the inactivated vaccine and this is, to our knowledge, the first report to discuss the immunogenic response and duration of immunity in both breeders and their progeny for an inactivated trivalent ARV vaccine containing classical and two variant strains. In Farm 1, a marked increase in ELISA titers was observed at 30 weeks of age, followed by a subsequent decline at the next two sampling points. We propose that a homologous subclinical field infection may have occurred, resulting in a transient rise in antibody levels that subsequently declined over time. Alternatively, the observed pattern could be attributed to exposure to a different genotype not covered by the vaccine, as the SNT results before and after this time point were aligned and showed no significant differences, while a subclinical field infection or exposure to a heterologous strain could explain the observed ELISA serological fluctuation, this cannot be confirmed without molecular detection (e.g., PCR) or neutralization assays. Importantly, ARVs are known to transmit vertically via the egg (Woernle *et al*., 1974), so breeder vaccination provides optimum passive immunity to progenies at the critical life stage (Meanger *et al*., 1997; Ayalew *et al*., 2023). Meanger *et al*. (1997) demonstrated that progenies from immunized breeders with ARV achieved neutralizing antibodies that protected 80% of chicks from homologous tenosynovitis homologues strain. In this study, each hatch of broilers (9-25 weeks after the breeder boost vaccination) had robust SNT and ELISA titers at 4 day-olds, confirming effective maternal immunity transfer. This agrees with a previous report showing that immunizing breeders with a trivalent prime-boost vaccination can achieve optimum passive immunity protecting progenies from homologues strain challenge with milder symptoms (Ayalew *et al*., 2023) but our serological findings still need experimental challenge to prove the vaccine efficacy.

The persistence of high maternal antibodies titers suggests that the current inactivated trivalent vaccine could protect early-life progenies during the critical first weeks of age. However, ARV immunity waning and coverage period should be monitored because the passive antibodies decline with age. We agree with previous papers (Meanger *et al*., 1997; Ayalew *et al*., 2023) that challenge studies are the definitive method of vaccine efficacy. The strong maternal immunity in the breeders was clearly reflected in their progeny, as demonstrated in our farm-level evaluation.

Notably, Farm 3 exhibited the highest maternal immune responses, which were consistently mirrored in their progeny. Our recommendation, challenge trials in progeny should be implemented using both homologous and heterologous variant strains. Previous work has shown that such challenges are informative maternal antibodies markedly reduced disease from homologous ARV, whereas protection against heterologous genotypes was minimal (Ayalew *et al*., 2023). Vaccination of customized vaccines and challenge of the same birds has shown high efficacy either by prime vaccination only or by two doses (Lublin *et al*., 2011; Liu *et al*., 2023a). Thus, experimental challenges using the circulating ARV GC4/GC5 strains will directly confirm whether the trivalent vaccine's induced immunity (as measured in our study by ELISA and SNT) translates into challenge protection.

CONCLUSIONS AND RECOMMENDATIONS

In conclusion, our study demonstrates that the novel inactivated trivalent vaccine induces a strong and durable humoral immune response in breeders and successfully transfers maternal antibodies to their progeny. These serological results are promising and justify further investigation through a challenge study to confirm clinical protection against variant field viruses.

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NOVELTY STATEMENT

Evaluation of the first novel inactivated trivalent vaccine in Egypt containing classical strain "S1133" and most predominant circulating variant strains in Egypt (GC4 and GC5) for humoral immune response in three breeder flocks and three flocks of their progeny and assess both active and passive immunities by common commercial ARV ELISA and serum neutralization test (SNT) for homologous ARV strain and duration of immunity at different ages.

AUTHOR'S CONTRIBUTION

Each author has made an equal contribution in offering their technical expertise and insights to develop this article.

Ethics approval

The ethical and research committee at the Faculty of Veterinary Medicine, Suez Canal University, approved the study protocol under reference number (SCU-VET-REC-2024063).

Generative AI and AI-assisted technology statement

All authors of this work declare that generative AI technol-ogiesss including large language models (e.g., ChatGPT, Copilot) and text-to-image generators were not utilized in any capacity during the preparation, writing, or editing of this manuscript

Conflict of interest

The authors have declared no conflict of interest.

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