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Preliminary Evidence on Passage Attenuation of *Orthoavulavirus javaense* Genotype VII.1.1 and Its Potency as a Living Vaccine Candidate

Wahid Hussein El-Dabae*, Eman Shafeek Ibrahim, Eslam Gaber Sadek, Mai Mohamed Kandil

Department of Microbiology and Immunology, Veterinary Research Institute, National Research Centre, Dokki 12622 Giza, Egypt.

Abstract | Newcastle disease (ND) is one of the most significant poultry viral diseases threatening the poultry industry worldwide. Despite mass vaccination against ND, genotype VII.1.1 epidemics in vaccinated and non-vaccinated flocks are still recorded, indicating an urgent need to generate a new vaccine candidate. In this study, the live *Orthoavulavirus javaense* (AOAV-1) genotype VII.1.1 strain (CH-EGY-GIZA-VVTNRC-2021) (Genbank accession number MW603772) was attenuated by combining passaging with limiting 10-fold dilutions in the allantoic cavity of embryonated hens eggs for 20 passages. The attenuated virus was used to vaccinate 20 SPF chicks and its protective efficacy was checked. Intracerebral pathogenicity index (ICPI) of the attenuated strain was 0.2, the mean death time (MDT) was 120 hours and infectvity titer was 7 log10 EID50/ml compared to 1.97 ICPI, 36 hours MDT, and 9.5 log10 EID50/ml of the original strain. The attenuated AOAV-1 was protective in vaccinated chicks by means of HI antibody response and protection from heterologus virulent challenge while showing little to no residual pathogencity. Histopathological inspection showed less severe pathological lesions in vaccinated challenged chickens than in non-vaccinated challenged chickens. Quantitation of viral loads following challenge indicated reduction of viral loads shed from birds vaccinated with the attenuated AOAV-1 virus. These preliminary insights collectively underline a new hope for the attenuation of live AOAV-1 genotype VII.1.1 as a vaccine candidate that can be used to control ND outbreaks in Egyptian poultry.

Keywords | *Orthoavulavirus javaense*, Attenuated vaccine, Genotype VII.1.1, Vaccination, Protection, Challenge

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***Correspondence** | Wahid Hussein El-Dabae, Department of Microbiology and Immunology, Veterinary Research Institute, National Research Centre, Dokki 12622 Giza, Egypt; **Email:** dr_wahidhussein@yahoo.com
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INTRODUCTION

Newcastle disease (ND) is a fatal, contagious, and notifiable avian disease with significant economic damage to the global poultry industry (Afonso, 2016). The disease is caused by *Orthoavulavirus javaense* formerly known as Newcastle Disease Virus (NDV), which belongs to the *Paramyxoviridae* family, subfamily *Avulavirinae*, genus *Orthoavulavirus* (ICTV, 2022). *Orthoavulavirus javaense* is enveloped with a non-segmented single-stranded negative sense RNA genome that measures about 15,200 nt in length. The genome encodes six structural proteins; nucleocapsid protein (N), phosphoprotein (P), matrix protein (M), fusion protein (F), hemagglutinin-

neuraminidase (HN) and RNA-dependent polymerase protein (L) (Paldurai *et al*., 2014). The two major glycoproteins F and HN are responsible for the virulence and antigenicity of NDV (De Leeuw *et al*., 2005). During P protein mRNA editing, two minor nonstructural proteins; V and W were also induced (Czeglédi *et al*., 2006). Based on sequence analysis and the phylogenetic tree of the F gene, *Orthoavulavirus javaense* is classified into two distinct classes within a single serotype; those two classes were further categorized into genotypes. Class I viruses comprise a single genotype, usually avirulent, and recovered from live and wild poultry sectors (Zhu *et al*., 2014), while class II viruses include 21 genotypes (I-XXI), which are implicated in the spread of NDV panzootics (Dimitrov *et al*., 2019).

Genotype VII is one of the most dominant genotypes distributed worldwide as it has been related to recent epidemics in Africa, the Middle East and Asia (Miller *et al*., 2010). Moreover, in the recent classification of AOAV-1, genotype VII was categorized into two sub-genotypes VII.1.1 and VII.1.2. The sub-genotype VII.1.1 includes the earlier sub-genotypes VIIb, VIId, VIIe, VIIj, and VIIl. The sub-genotype VIIf was renamed as sub-genotype VII.1.2. The sub-genotype VII.2 includes the former sub-genotypes VIIa, VIIh, VIIi, and VIIk (Dimitrov *et al*., 2019).

Orthoavulavirus javaense was first identified in Egypt in 1948, and since then, it has been responsible for causing destructive epidemics (Daubney and Mansy, 1948). The VIId genotype (VII.1.1) was first identified in 2011. It likely resulted from the poultry trade with China (Radwan *et al*., 2013). Numerous waves of NDV outbreaks have been identified in Egypt in both vaccinated and unvaccinated poultry flocks (Osman *et al*., 2014; Awad *et al*., 2015; AbdEl-Aziz *et al*., 2016; Ahmed *et al*., 2017; Megahed *et al*., 2018; Sedeik *et al*., 2019; Shakal *et al*., 2020; Ali *et al*., 2022). The sub-genotype VII.1.1 was the predominant strain that caused repeated outbreaks in Egypt in both acute and sub-acute forms despite the widespread application of various vaccine programs in poultry populations, particularly in recent years (Ahmed *et al*., 2022; Amer *et al*., 2022; Moharam *et al*., 2019).

Effective Vaccination regimens and strict biosecurity procedures are considered the most effective ways to prevent and control NDV epidemics worldwide. Traditional ND vaccines comprise attenuated and inactivated vaccines available in many countries (Zhao *et al*., 2013). The classical NDV vaccinal strains are related phylogenetically to genotypes I and II and have been used for over fifty years. However, their divergence from current circulating field strains contributed to recent outbreaks (Dortmans *et al*., 2012; Mahmud *et al*., 2022).

Inactivated ND vaccines induce protection against clinical symptoms and have a high antibody immune response that is transmitted to progeny but induce lower cellular immunity (Dimitrov *et al*., 2017). In contrast, inactivated vaccines have multiple shortcomings, including requiring individual injection via intramuscular or sub-cut routes, which increases the cost of immunization, so they are not suitable for use mainly in large poultry sectors and possibly the presence of residual virus during the manufacturing process (Mansur *et al*., 2007). Attenuated NDV vaccines are more likely to provide early onset of both cellular and mucosal immune responses due to their capability to replicate in chickens. Furthermore, live NDV vaccines are more appropriate in large poultry populations as they are commonly used via aerosol spray and drinking water, hence reducing vaccination costs (Martinez *et al*., 2018).

Using live ND vaccines from genotype II, including Lasota and B1 strains, protects against clinical signs but does not inhibit virus shedding and mortality. To minimize the risk of virulent strain infection, attenuated NDV vaccines could be used at earlier weeks of age in breeders, layers, and broilers, either by respiratory or systemic route. Additionally, eye drop instillation with live vaccine provided protective mucosal immunity by IgA and induced strong herd systemic protection and high immunogenic status (Miller and Koch, 2013).

The application procedures of live ND vaccines may cause extensive differences in the immunity of immunized birds and initiate disease occurrence followed by shedding of virus that exaggerates the risk of predisposed birds, decreases the rate of growth in immunized birds and reverts back to virulence due to genome mutations of live vaccines (Kapczynski and King, 2005).

Attenuation of virlent AOAV-1 has attracted the attention of many scientists. Mesogenic virulent K strain of NDV was passaged for eighteen passages in chicken embryo fibroblasts to reduce vaccine virulence for safer immunization; MDT has increased from 71 hours (fifth passage) to 92 hours at the final passage, ICPI decreased from 0.4 at the 5th passage to be 0.1 on the final passage, the tissue culture infective dose fifty increased from $10^{3.4}$/ml (4th passage) to $10^{5.3}$/ml (final passage) indicating the efficacy of attenuation process (Visnuvinayagam *et al*., 2015).

On the other hand, the adaptation of velogenic neurotropic NDV on Vero cells failed after ten passages, and the Vero-adapted virus has retained its virulence. The MDT of the adapted virus was 58 hours, the intravenous pathogenicity index was 2.12, and the Vero-adapted virus developed nervous manifestations and haemorrhagic proventriculus followed by death on the 5th day post-inoculation (Ravindra *et al*., 2017). Furthermore, attenuation of AOAV-1 genotype

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VIId failed after 35 passages in SPF eggs as the harvested allantoic fluid from the 35th passage was lethal to embryos of inoculated eggs on the second day of inoculation (El-Dabae *et al*., 2016). Moreover, using nitrous acid for chemical attenuation of NDV genotype VIId harvested from the 35th passage did not achieve attenuation, and the virus was still causing mortalities upon inoculation in three weeks old SPF chicks (El-Dabae *et al*., 2016).

Updating currently used vaccines to combat existing circulating NDV strains is necessary to overcome the mismatch between traditional vaccines and circulating field strains. hence, this research aimed to generate an attenuated AOAV-1 genotype VII.1.1 vaccine candidate to control epidemic waves of currently circulating AOAV-1 in Egypt.

MATERIALS AND METHODS

Virus seed and SPF eggs

The recent Egyptian *Orthoavulavirus javaense* genotype VII.1.1 strains (CH-EGY-GIZA-VVTNRC-2021) (Genbank accession number MW603772) and (CH-EGY-ALEX-NRC-2020) (Genbank accession number MW580389) were kindly provided by Poultry Diseases department, Veterinary Research Institute, National Research Center for attenuation and challenge, respectively. Specific Pathogen-Free (SPF) fertile eggs were obtained from Koum-Oshiem SPF-farm, Fayoum, Egypt, and were used for virus propagation.

Propagation and evaluatation of the original AOAV-1

The original AOAV-1 (CH-EGY-GIZA-VVTNRC-2021) (Genbank accession number MW603772) was diluted 1:1 in phosphate buffer and inoculated into the allantoic cavity of 9-11 day-old SPF eggs (0.2ml/each) (Burleson *et al*., 1994). After inoculation, the eggs were incubated at 37°C and a humidity level of 40-60% for 3-5 days. Embryos that died were collected and chilled overnight at 4°C. The collected allantoic fluid was clarified for 30 minutes at 13,000 rpm, supernatant was concentrated for 2.5 hours at 30,000 rpm. The purified virus was then resuspended in phosphate buffer and stored at -80°C until needed. Endpoint egg infective dose 50 (EID50) of the harvetsted AOAV-1 was titrated in 9-11 day SPF eggs, as previously reported (Villegas and Purchase, 1989; Reed and Muench, 1938). MDT and ICPI were also calculated in 9-11 day-old SPF eggs and one-day-old SPF chicks, respectively, as previously described (OIE, 2012).

Attenuation of the original NDV isolate

Attenuation of the seed virus was carried out according to Reeve *et al*. (1974). Briefly, tenfold serial dilutions of allantoic fluid between 10^{-3} to 10^{-10} from the propagated virus were prepared and inoculated in 9-11 days old SPF eggs (6 eggs/ dilution). The inoculated eggs were incubated at 37°C with 40-60% relative humidity for 6-7 days with daily candeling. Dead eggs were collected at 4°C. After six to seven days of incubation, surviving eggs of all dilutions were tested for HA activity rapid slide haemagglutination (HA) test using 10% chicken RBCs. Repeated passaging and dilution of the virus harvested from the eggs with still embryos alive for seven days was selected for further attenuation and pathogencity evaluation.

Titration and pathogenicity of the attenuated AOAV-1

After 20 passages, the attenuated AOAV-1 was titerated in 9-11 days SPF eggs, and the endpoint egg infective dose 50 (EID50) was calculated (Reed and Muench, 1938; Villegas and Purchase, 1989). The MDT and ICPI were calculated for the attenuated AOAV-1 as done with the original (OIE, 2012). Sterile lactaalbumin hydrolysate-sucrose 4% was added as a stabilizer to the attenuated AOAV-1 (for keeping antigenic content).

Testing the efficacy of the attenuated AOAV-1 as a vaccine candidate

One-week-old SPF chicks (N=60) were divided into three equal groups; a test group that were inoculated with the attenuated AOAV-1 virus via the ocular route with a dose of 100µl containing 10^{7}EID50/ml. A positive control group and a negative control group (Figure 1). On the 21th day post-immunization, the test and the positive control groups were challenged with 10^{6} EID50/chick of the virulent AOAV-1 (CH-EGY-ALEX-NRC-2020) (Accession number MW580389) via intramuscular injection route (OIE, 2012). The protection percent was detected ten days post-challenge (Tizard, 1996). Serum samples were collected weekly from test group chicks and at 7 and 14 days post-challenge to determine antibody titer by HI test. Tracheal and cloacal swabs were collected

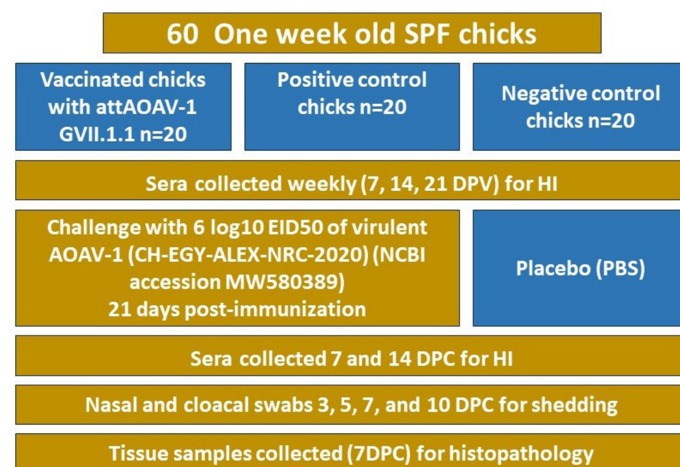

Figure 1: Diagram illustrating the details of *in vivo* experiment.

Table 1: Summary of the virus-shedding data of positive control and vaccinated challenged groups.

Days post challenge	Trachea of positive control groups C.t values and quantity as EID50	Cloacal swabs of positive control groups C.t values and quantity as EID50	Tracheal swabs of vaccinated challenged group C.t values and quantity as EID50	Cloacal swabs of vaccinated challenged group C.t values and quantity as EID50
3	16.68 (540.4×10^{6})	28.20 (113×10^{7})	30.66 (8.8×10^{2})	33.71(1.6×10^{2})
5	15.02(45.6×10^{8})	13.78(104.5×10^{7})	27.34(1.4×10^{5})	29.23(2.9×10^{4})
7	N/A	N/A	23.19(2.3×10^{2})	33.80(5.2×10^{1})
10	N/A	N/A	25.62(6.1×10^{5})	28.02(4.1×10^{3})

N/A: Not assessed due to the death of positive control groups these days.

from all groups to estimate virus shedding using qRT-PCR on the 3rd, 5th, 7th, and 10th days post-challenge (Wise *et al*., 2004). Lung, liver, cecal tonsils, kidney, brain and spleen samples were collected from all groups on the 7th day post-challenge (Eze *et al*., 2014), and kept on formol saline 10%. Thin sections were prapered and then stained with H & E stain and 20X magnification power was used to identify pathological lesions (Banchroft *et al*., 1996) for histopathological lesions examination as shown in (Figure 1).

Statistical analysis

To analyze the data significance and standard deviation (SD) among test group and control groups; SPSS 21 software with one-way ANOVA was used. Differences were identified significant when the probability (p) value was less than 0.05.

RESULTS AND DISCUSSION

Atteunation parameters of the virulent AOAV-1 virus

After twenty passages, the MDT of inoculated eggs increased from 36 hours for the original strain to 120 hours for the attenuated one while the ICPI has decreased from 1.97 to 0.2 indicating the shift from velogenic to lentogenic phenotype. The infectivity titer of the original strain was 9.5 log10 EID50/ml while that of the attenuated strain was 7log10 EID50/ml.

Potency of the attenuated AOAV-1 genotype VII.1.1

Upon immunizing chicks with the attenuated AOAV-1 and for 21 days observation there were no adverse effects or disease signs. Following virulent challenge and during the ten DPC observation period, immunized chicks (test group) showed no adverse effects. However, the positive control group, which was challenged with the virulent AOAV-1 (CH-EGY-ALEX-NRC-2020) virus, developed clinical signs on the third-day post-inoculation, such as depression, sneezing, coughing, torticollis, and paresis. Mortalities began on the fifth-day post-challenge, and by the 10th day post challenge, all chicks in the positive control groups had died. The attenuated AOAV-1 provided complete protection to the immunized chicks upon challenge with virulent NDV, indicating its potency.

HI was used to detect the serological immune response inimmunized chicks. HI antibody titers detected were 7, 8.5, and 9 log2 on the 1st, 2nd, and 3rd weeks post-vaccination (DPV), respectively. Furthermore, the antibody titers were 11 and 10 log2 on the 7th and 14th days post-challenge (DPC), demonstrating a robust immune response and high titers of antibodies induced by the prepared vaccine.

Reduction of virus shedding, and histopathological lesions

Quantitation of virus shedding of the infected groups in tracheal and cloacal swabs using qRT-PCR revealed a positive amplification curve from the positive control group and the test group., as shown in Table 1. lower amounts of virus shedding were observed among the vaccinated challenged group in thetracheal as well as cloacal swabs compared to those of positive control group chicks, as shown in Table 1. No virus shedding was detected among the negative control group.

Moreover, histopathological examination of organs collected from test group chicks on the 7th day post-challenge showed lower lesion severity compared to the positive control group Figure 2. The lungs of test group chicks showed hyperplasia of the epithelial lining of secondary bronchioles, their liver illustrated thrombus formation, the spleen of showed mild capillary sheath proliferation, cecal tonsils also showed moderate lymphocyte proliferation, mild intertubular congestion and mononuclear aggregation was observed in kidneys, of , and mild blood vessels congestion in the Brains was also evident. On the other hand, positive control group chicks showed lung thrombi and congestion of blood vessels. The liver showed severe necrosis and sinusoidal thrombus formation, the spleen showed severe lymphocyte depletion and coagulative necrosis. Cecal tonsils showed lining epithelium necrosis and fibrinohemorrhagic exudates, the kidney showed congestion of blood vessels and vacuolar

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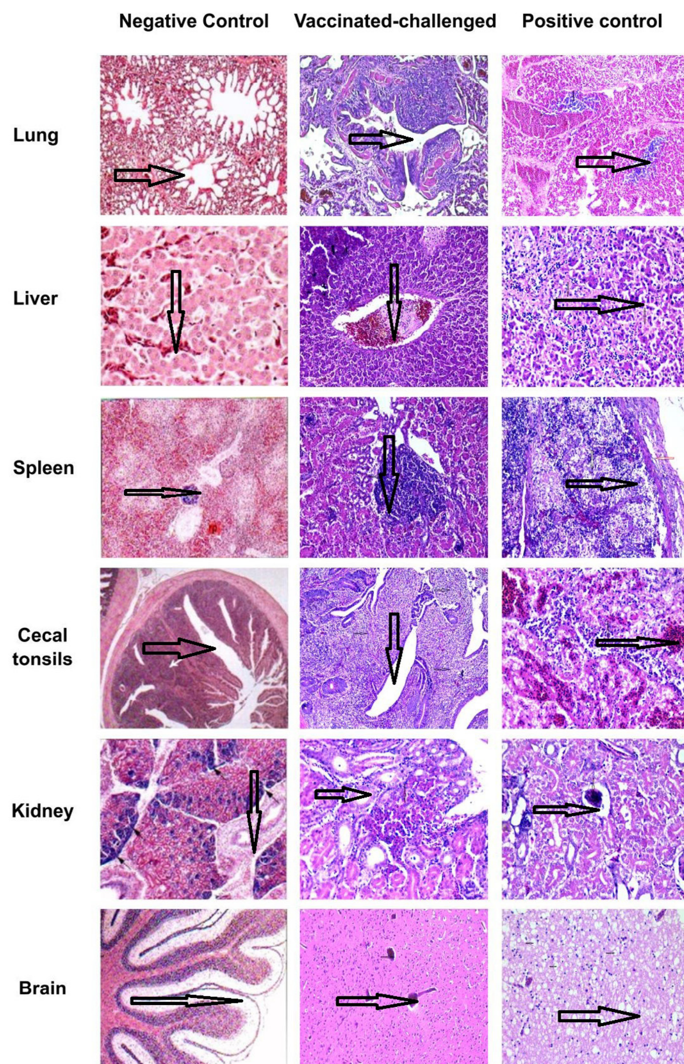

Figure 2: Histological examination of organs collected from different chick groups 7 DPC sections were stained with H & E stain with 20X magnification to identify different pathological lesions. Test group and positive control group showed a great difference in the severity of histological lesions compared with the normal histological appearance of the negative control group. The lung of the test group showed epithelial lining hyperplasia of secondary bronchioles, whereas the positive control group shows thrombus formation and blood vessel congestion. The livers of test group chicks showed thrombus formation, while the livers of the positive control group showed severe necrosis and sinusoidal thrombus formation. The spleen of test group chicks showed mild capillary sheath proliferation, while the spleen of the positive control group showed severe lymphocyte depletion and coagulative necrosis. The cecal tonsils of the test group showed lining epithelial necrosis and fibrinohemorrhagic exudates but those of the positive control group showed lining epithelial necrosis and fibrinohemorrhagic exudates. The kidneys showed congestion of blood vessels and vacuolar degeneration in both groups where milder lesions were evident in the test group. The brain showed demyelination and neuron degeneration with central chromatolysis which were milder in the test group.

degeneration, and the brain showed demyelination and neuron degeneration with central chromatolysis. The negative control group showed no histopathological lesions (Figure 2).

Vaccination is a fundamental tool for controlling ND infections, especially in developing countries. The classical commercial live vaccinal AOAV-1 strains of genotype II or I, and III produce protection against morbidity and mortality in susceptible chicks. However, they did not inhibit the infection with velogenic AOAV-1 genotype VII.1.1 and subsequent dissemination the virus in poultry firms as they are not harmonized genetically and antigenically with predominant field strains (Bwala *et al*., 2012; Kapczynski *et al*., 2013). Thus, novel matching AOAV-1 vaccines are highly recommended for active immunization and primary usage in epidemics to protect chicks from infection. Therefore, the present study was conducted to attenuate virulent AOAV-1 subgenotype VII.1.1 circulating in Egyptian poultry flocks since 2011.

The main principle in preparing live ND vaccines is the attenuation of the original virus isolate to decrease its virulence by using empirical steps like cultivation under suboptimal circumstances (passaging) in fertilized eggs or chick embryo tissue (Allan *et al*., 1978). In this aspect, the original (CH-EGY-GIZA-VVTNRC-2021) virus strain was propagated in 9-11 day SPF eggs via the allantoic cavity. The harvested allantoic fluid was assessed for pathogenicity, including MDT, ICPI and titration. Based on the attenuation method used by (Reeve *et al*., 1974) that utilized tenfold serial dilution after each passage, the pathogenicity of the original virus has gradually reduced, indicating the success of the attenuation process.

A recent approach for effectively controlling AOAV-1 epidemics is the generation of homologous vaccines (genotype-matched vaccines) against recent circulating field viruses. These vaccines induce higher protective efficiency and minimize replication of virulent viruses after exposure to infection (Bello *et al*., 2020). Similarly, formulated vaccines from phylogenetically closer genotypes to the circulating virus reduced shedding upon challenge trial (Hu *et al*., 2011). Genotype-matched attenuated AOAV-1 mRNA vaccine of genotype VII.1.1 induced higher antibody titers compared with Lasota (genotype II) and complete protection against challenges with virulent virus and reduction of shedding amount (Xu *et al*., 2020). Comparative efficacy of the conventional Lasota vaccine against virulent genotype VII.1.1 of NDV revealed insufficient protection and dissemination of the virus post-challenge with virulent NDV. Nevertheless, both Lasota and live attenuated Genotype VII induce protective immunogenicity (Dewidar *et al*., 2022). Contrary to this study, antigenically adapted live vaccine of matched NDV

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genotype VII.1.1 did not induce augmented protection in comparison with commercial live NDV genotype II vaccine, and chicks were protected entirely from either clinical disease or mortalities as well as virus replication when challenged with genotype VII.1.1 of AOAV-1 even with a suboptimal dose of vaccine (Dortmans *et al*., 2014).

Likewise, vaccinal NDV strains Lasota and QV4 afford adequate protection for birds from mortalities and morbidity against AOAV-1 genotype VII andgenotype XVII with no substantial variations in the quantity of virus shedding (Susta *et al*., 2015). Vaccination using heterologous attenuated NDV genotypes II and XI in chicks and subsequent challenge with genotypes II, VII and XI showed antibody titer of more than 6.5 log_2 with full protection and complete blocking of viral shedding and replication from different genotypes (Liu *et al*., 2018). Two wild types of genotype III induced the same efficient protection provided by the Lasota vaccine against NDV genotype VII with a decrease in virus shedding in vaccinated challenged birds (Wu *et al*., 2019). Lasota and PT3 vaccinal strains confer 100% protection post-challenge with genotypes VIId and VIb, diminishing virus load in the trachea and cloaca (Li *et al*., 2020).

In this study, attenuation of the virulent AOAV-1 was achieved by combining tenfold serial dilution with repreated passaging in embryonated hen's eggs. The attenuation was assured by the changes in the pathogenicity of the attenuated strain where MDT increased to 120 hours and ICPI decreased to 0.2 which resemble those of lentogenic AOAV-1. Attenuated virus titer declined from $10^{9.5}$ EID50/ml to 10^7 EID50/ml after twenty passages using this attenuation method may be due to changes in the virus growth kinetics or the selection of slowly replicating form the quasispecies pool of the original virus.

Immunization of one week old SPF chicks with the novel attenuated AOAV-1 subgenotype VII.1.1 showed no adverse effects. Additionally, the attenuated virus induced a strong humeral immune response after immunization.

Hemagglutination inhibition (HI) antibody titers are critical in evaluating the protection level induced by ND vaccines as it correlates well with protection level (Esaki *et al*., 2013). HI titers with 6 log2 or higher are typically protective. To prevent virus shedding and infection more than 6log_2 of antibody titers must be obtained (Kapczynski and King, 2005; Raghul *et al*., 2006; Han *et al*., 2017). Consequently, the attenuated virus was a potent inducer of an ascending titer of anti-hemagglutinin antibodies just 1 WPV (7 log2) in correlation with a high protection level in preventing mortalities and clinical manifestations and was consistent with earlier studies (Cheng *et al*., 2016; Bello *et al*., 2020).

Unlike the birds in the test group, clinical signs in unvaccinated challenged birds were observed on the 3rd day post-challenge and peaked severity on 5th day and 100% mortality on 7th day post-challenge (Ecco *et al*., 2011). The morbidity score of the positive control group was significantly higher than the test group group ($p < 0.05$), as stated earlier (Kapczynski and King, 2005), whereas full protection was achieved in the vaccinated challenged group.

The containment and control of AOAV-1 transmission among poultry are principally associated with the virus load. Hence, viral shedding estimation and quantification is a major indicator for assessing vaccine effectiveness. The birds from the test group disseminated lower virus amounts compared to the unvaccinated challenged group (Table 1). Likewise, vaccination of chicks with the attenuated genotype VII.1.1 starin that matches the challenge virus antigenotype diminished the quantity of virus shedding and consequently decreased the spread of ND infections among susceptible birds (Xiao *et al*., 2012; Liu *et al*., 2017; Wang *et al*., 2020).

Histopathological examination of bird organs from the test group on the 7th day post-challenge showed less degree of lesion severity compared to the positive control group, demonstrating the systemic protection induced by the immune response to the attenuated AOAV-1 (Figure 2). These findings assert that the attenuated AOAV-1 strain was able to inhibit the pathogenesis of the AOAV-1 challenge starin, which has been mentioned in other studies (Hu *et al*., 2015). Severe pathological damage that noticed in the lymphoid organs such as spleen necrosis and depletion of lymphocytes is due to high levels of replication of the challenge AOAV-1 strain and robust innate immune reaction impairing immune response and lymphoid organs (Wang *et al*., 2012). Besides, AOAV-1 pathology directly reflects apoptosis in chicken splenocytes via upregulating genes related to apoptosis (Harrison *et al*., 2011). Also, the antiviral activity of cytokines (cytokine storm) stimulates tumour necrosis factor that mediates death by motivating receptor-mediated cell death (Tisonick *et al*., 2012). The severe inflammatory response induced by genotype VII.1.1 reveals the significance of vaccines and immunity against virulent strains of AOAV-1 (Rasoli *et al*., 2014).

Collectively, the current study highlights the successful passage attenuation of the AOAV-1 genotype VII.1.1 (CH-EGY-GIZA-VVTNRC-2021) virus. This attenuated virus protected chickens from morbidity, mortality, and reduced virus shedding. The study insights collectively support further testing and identification of the attenuated AOAV-1 genotype VII.1.1 as a prominsing vaccine candidate.

CONCLUSIONS AND RECOMMENDATIONS

This research paper highlights the successful attenuation of AOAV-1 genotype VII.1.1 and its possible use as a living vaccine candidate against ND infections in poultry. The attenuated AOAV-1 confered full protection from clinical signs and mortalities against velogenic AOAV-1 genotype VII.1.1 challenge accompanied with reduction of virus shedding. This approach might be helpful in providing priming vaccine matching the antigenotype of circulating AOAV-1.

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NOVELTY STATEMENT

We provided the initial insights for the attenuation of the AOAV-1 GVII.1.1. there is no doubt that we need more tests and methods to complete this work to reach final product phase and in further studies we will address the sequence of attenuated NDV virus in comparison with the original isolate and identification of the diversity between the two viruses

AUTHOR'S CONTRIBUTION

WHE: Planned for this work and drafted the manuscript. ESI, EGS and MMK: Carried out the practical work. All authors revised and approved the final manuscript.

Ethical statement

The Ethical Committee for Medical Research at the National Research Center approved the study in accordance with local guidelines and regulations (IACUC No. 12020232/2022).

Conflict of interest

The authors have declared no conflict of interest.

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