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Humoral and Interferon-γ Immune Response to DNA Vaccine Encoding The surface Glycoprotein B of Infectious Laryngotracheitis Virus

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DNA vaccines continue to be a suitable safe and potent alternative particularly for controlling the infection with pathogens that rely on the cell-mediated type of immune response and for the ability to eliminate viral shedding Infectious laryngotracheitis virus causes a contagious respiratory disease with a considerable economic impact. The aim of current study was developing of a DNA vaccine coding for the surface glycoprotein B gene (gpB) from locally isolated strain. The developed vaccine (pcDEST40-gpB) could elicit potent antibody titers positively correlated with the commercially available live TC-propagated ILT vaccine. IFN-γ gene transcript revealed that both DNA vaccine and live vaccine initiate a powerful fold change in IFN-γ till the 15 days post-vaccination, however, by day 7 post-challenge, the use of a booster dose of DNA vaccine resulted in an abrupt increase in the level of IFN-γ gene transcript, which was significantly higher than that of the live vaccine. Surprisingly, the data present here revealed that the DNA vaccine, but not the live vaccine, could prevent virus shedding after a challenge.

Keywords: DNA Vaccine, Glycoprotein B, Infectious laryngotracheitis.

Introduction

Infectious laryngotracheitis virus (ILTV) is a member of the subfamily Alphaherpesvirinae of the family Herpesviridae, genus Iltovirus ,Gallid herpesvirus 1 species [1]. The virus causes highly contagious respiratory disease that primarily affects chicken, with mortalities ranging from 5-70% [2]. In severe cases of infection, the respiratory distress is distinguished by tracheal hemorrhage and the formation of a mucoid plug, which causes the bird to asphyxiate. The infection is deemed one of the causes of economic losses in the poultry industry [3].Latency after ILT infection is of great concern as it maintains the virus in a dormant state in the trigeminal ganglia and causes later reactivation of the virus and outbreaks [4].

The most effective approach for controlling ILT infection is the massive use of vaccination. Currently live attenuated vaccines propagated either on tissue culture (Live TC propagated ILT vaccines) or egg-adapted vaccines might contain residual virulent viral particles or have a greater propensity to revert to virulence following bird-to-bird passages, culminating in later outbreaks [3, 5].

Although vector-based vaccines and recombinant/mutant vaccines are considered safer alternatives, they have several limitations, including the inability to stop complete viral shedding [2] and the pre-existence of antibodies against vectors, which can neutralize the vaccines, particularly in endemic areas [6, 7].

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DNA vaccine against ILT virus involves the use of plasmid DNA encoding viral surface glycoproteins to stimulate an immune response in chickens, resulting in protection against the virus. This type of vaccines has shown promising results in preclinical investigations and could potentially be applied as a preventative measure in poultry farming industry [8, 9]. DNA vaccine is a mammalian expression plasmid-based vector in which the gene (s) of infectious viruses or bacteria can be cloned, for such reasons DNA vaccine is safe and no concern of infection is present, DNA plasmids themselves are not immunogenic[10], as a result, it is possible to administrate booster doses after DNA vaccination without eliciting a heterologous immune response to the vector.

Surface glycoproteins antigens of ILT virus (gB, gC, gD, gE, gG, gH, gI, gJ, gK, gL and gM) play a very crucial role in viral attachment to the host target cells and entry and replication [2, 11]. These protein antigens can elicit powerful immune responses either humoral or cell-mediated immune response. Surface glycoprotein B(gpB) antigen showed a high conservancy between different isolates in Egypt [12, 13]. Different B- and T-cell epitopes have been extensively studied using synthetic peptide and bioinformatics approach study [13].

The current study was carried out to develop a DNA vaccine coding for the ILT virus's gpB antigen and to scrutinize the immunogenicity and protective efficacy of the prepared vaccine in controlling ILT infection and preventing viral shedding after challenge.

Material and Methods

Experimental study was conducted in Central Laboratory for Evaluation of Veterinary Biologics (CLEVB), Agricultural Research Center {ARC}, Cairo, during the period from 2016 till 2020.

Virus Samples

Infectious laryngotracheitis virus, Egyptian isolate (ILT-YA/18) was used in the current study for both preparation of the DNA vaccine as well as the challenge of the experimental SPF chicken, this isolate was completely identified and characterized [12] and the glycoprotein B sequence was published on the Gene Bank under accession number (MT265067)

Amplification of the full-length gpB if ILT virus
Purification of ILT virus by sucrose cushion:

ILT-YA/18 isolate was inculcated on chorio-allantoic membrane of 10 days old SFF-chicken and incubated at 37°C/5 days, developed lesions on the chorio-allantoic membranes were removed ,homogenized mechanically (PRO 200homogenizer - Pro Scientific USA) and clarified first by low speed centrifugation (500rpm/5min/4°C) , then the virus suspension was laid onto sucrose cushion 3 mL 20% and 3 mL 50% sucrose prepared in TNE buffer [9], centrifuged for 5h at 30000rpm using Sorvall WX 100 ultracentrifuge (Thermo Fisher scientific, USA). The layer containing the virus was aspirated and the virus particles were sedimented at 50000 rpm/3h/4 °C. The sedimented virus was re-suspended in 1 mL of TNE at 4 °C/24h with shaking and stored at -80 °C [12] . One hundred microliters of the purified virus was subjected to qPCR to ensure the presence of the virus and the viral titer was measured using the standard EID_{50} [14, 15]

Viral DNA extraction

Viral DNA was extracted from the purified ILT virus using Gene JET Genomic DNA Purification Kit (Cat.No.K0721, Thermo Scientific, USA) according to the manufacturer's instructions. Briefly, the purified virus suspension was subjected to enzymatic digestion using 20 µl of proteinase K in 180 µl of enzymatic digestion buffer and incubation takes place at 56°C for 30 min, 20µl of RNase solution was then added and the mixture was incubated at 37 °C for 10 min. Two hundred µl of lysis buffer was then added and mixed for 15 sec till a complete homogeneous solution was formed. The whole content was mixed with 400 µl of 50% ethanol and transferred to the spin column. After centrifugation at 10,000 xg for 1 min, the spin column was washed with 500 µl of wash buffer 1 and then wash buffer 2. After centrifugation after each was, the DNAwas then eluted in 50 µl of elution buffer and centrifuged at 13,000 x g /60sec. The eluted DNA was quantified using Qubit 2 Fluorometer and the dsDNA BR assay kit (Cat No. Q32853, Thermo Scientific, USA) according to the manufacturer's instructions.

Full-length amplification of the surface glycoprotein B gene:

The full-length orf of gp B gene was amplified using Phusion High-Fidelity DNA Polymerase (Cat No. F530S, Thermo Scientific, USA). Three µl of ILT viral DNA was mixed with 25 µl of 2X master mix and 100nM of each primer (Table 1) in total volume of 50µl.The reaction

was adjusted at 98 °C/1 min initial denature and enzyme activation, and 35 cycles of denature at 98°C/30 sec, annealing at 60°C/20 sec, and extension at 72°C/ 3min, a final extension at 72°C/10 min using T professional 3000 thermal cyclers (Biometra , Germany).The amplicon was subjected to electrophoresis on low melting agarose, gene Ruler 1Kb DNA ladder (Cat No. SM0311, Thermo Scientific, USA) was used as DNA marker. The 2.3 Kbp amplicon was spliced off and purified using PureLink™ Quick Gel Extraction Kit (Cat No. K210025, Thermo Scientific, USA) according to the manufacture instruction.

Preparation of DNA vaccine:
Generation of the entry clone pENTER SD/D-gpB vector.

The full-length purified gpB amplicon was cloned in pENTER SD/D topo donor vector(Cat No. K2420-20, Invitrogen, USA)to generate pENTER SD\D-gpB, and transformed into the chemically competent TPO10 E. coli then plated onto ,LB broth containing 50µL /ml Kanamycin and incubated overnight at 37°C. Few grown colonies were picked up and incubated overnight in 10ml LB broth with antibiotic then mini prep was purified (Cat No. K210003, Thermo Scientific, USA) and subjected to qPCR to detect the presence of the insert in the entry vector.

-qPCR

The purified plasmids from different colonies were analyzed for the presence of the gpBgeneinsert in the entry clones using real-time PCR. Briefly, 5µl of plasmid preparation was mixed with 2X Brilliant II SYBR® Green qPCR Master Mix (Cat No. 600828, Agilent, USA) and 100nM of each primer listed in Table 1. The reaction was set at 95°C initial denature, and a 40 cycle of 95°C/20sec, 60°C/20sec for primer annealing and 72°C/30 sec for extension. Dissociation curve was included at the end of the cycles.

Cloning of surface glycoprotein B gene in the destination vector.

Colonies that gave +ve Cq value with the qPCR were subjected to homologues recombination with mammalian expression destination vector (pcDNADEST40)to generate pcDEST40-gpB vector. Briefly 1µl of the pENTER SD/D-gpB was mixed with 1µl of the destination vector (Cat No. 12274-015, Invitrogen, USA) in 8µl final reaction volume to which 2µl of LR Clonase™ II enzyme mix (Cat No. 12538120, Thermo Scientific, USA) was added and incubated at 37°C /30 min, then Two µl was added into a vial of One Shot Chemically Competent *E. coli* and mixed gently and incubated on ice for 30 minutes. The cells were subjected to heat shock for 30 seconds at 42°C without shaking and immediately transferred to ice. Then 250 µl of room temperature S.O.C. Medium was added and incubated at 37°C for 1 hour with shaking (200 rpm). The transformation cells (50–200 µl) was Spread on a pre-warmed selective plate (LB agar plate containing 100IU/mL ampicillin) and incubated overnight at 37°C. On the second day, a few colonies were picked up and cultured individually in a 50ml centrifuge tube containing 1mL LB broth with 100 IU/mL ampicillin and incubated overnight at 37°C with shaking at 200rpm for miniprep and qPCR analysis as before.

Maxiprep:

A single colony of *E.coli* harboring pcDEST40-gpB was used to inoculate 10mL of LB-ampicillin broth and incubated overnight at 37°C while shaking (200rpm) and scaled up to 1litter. The culture was then cooled down on the ice and subjected to Maxiprep (BioBasic cat # BS466) according to the manufacture's instruction. Briefly, the cells were collected by centrifugation at 4000rpm/10 min/4°C, washed with cooled PBS pH 7.2. The cell pellet was then re-suspended in 10mL of re-suspension buffer containing RNaseA and then lysed for 3 min in 10mL of lysis buffer. The clear cell lysate was then neutralized by

TABLE 1. The sequence of the primers and probe were used for amplification and detection of gpB:

Gene	Name of primer/ probe	5'>3' sequence	Product size
Complete *orf*gpB	ILTB cl-F	5'-CACCATGGCTAGCTTGAAAATGC-3'	2300 bp
	ILTB cl-R	5'-TTCGTCTTCGCTTTCTTCTGCC -3'	200bp
Real-time detection of gpB gene	QILTB-F	5'-CGACTAACATGACTGAAGGA-3'	200bp
	QILTB-R	5'-ATGATACGTTGCTTTGGATGAG-3'	200bp

10mL of neutralization solution and centrifuged at 10000rpm/ 30min/ 4°C. The clear supernatant containing the plasmid was then transferred to the Maxi spin column pre-equilibrated with 10 ml of equilibration buffer and the column was left till all the solution pass through by gravity. The column was then washed 2 times with 10mL of washing solution and the plasmid was eluted with 4mL of elution buffer. The plasmids were quantified by Qubit 2 Fluorometer.

Immunization of the SPF chicken with the DNA vaccine encoding gpB

Seventy-five, SPF chicken 21 days old (Komoshim-Fayoum) were kept in bio-isolators, fed on sterile maintenance dry ration and sterile water. The chicken was divided into 3 groups (Table 2), the first group received the DNA vaccine (2 doses of 20µg/0.1ml, 21 days apart) coding for surface glycoprotein B(gpB), group two received the commercially available tissue culture ILT vaccine and group three remained unvaccinated (Negative control group). Chicken groups were challenged (intra-tracheal) at day 28 (one week after the booster dose of DNA vaccine) with 103/ 0.1ml EID_{50} of the virulent ILT virus

Serum samples (for ELISA) were taken at 0, 15, 21, 28 days post-vaccination and 7 and 15 days after challenge. Heparinized blood samples (for IFN-γ gene transcripts quantification) were taken 0, 3, 5,7,15, and 21 days post-vaccination and 3, 5, and 7 days after challenge.

Evaluation of immune response to DNA vaccine encoding gpB :

The protective level of the DNA vaccine in comparison to the commercially TC propagated ILT vaccine was evaluated at the level of humoral immune response by ELISA and cellular immune response by measuring the level of IFN-γ transcripts by real-time quantitative RT-PCR, and finally by measurement of the shedding.

ELISA

The antibody titers were measured by Indirect ELISA using ID screen ILT indirect kit (ID. Vet). Data were expressed in the S/P ratio according to the manufacturer's recommendation, samples were considered positive when S\P exceeds 0.3

Quantification of IFN-γ gene transcript using real-time quantitative RT-PCR:

Extraction of ILT viral protein antigen:

ILT virus protein antigens were extracted using Trizol® (Cat No. 15596026, Invitrogen, USA) , the purified protein was re-suspended in 1% SDS containing CAHPS and quantified using Qubit II assay (Cat No. Q33211, Invitrogen, USA) and the concentration was adjusted at 10µg/ml, kept in Low bind polypropelling tubes at -80 °C till used.

Purification of peripheral blood mononuclear cells (PBMCs) from chicken groups:

Peripheral blood mononuclear cells from all vaccinated and unvaccinated chicken were purified by density-gradient centrifugation using Ficoll-Histopaque (density, 1.077 g/ml; Biowest cat# L0560-500). Cells concentration was determined using trypan blue dye exclusion dye and adjusted at 0.5-1x106/ml. cells were then pulsed with either 5 µl of ILT protein (specific stimulant) or with PBS (-ve control), and incubated at 37 °C/ 20 h in a 5% CO_2 incubator. After 20h, 300µl of Trizoal® was added and total RNA was extracted and treated with RNase-free DNase I[16].

Quantification of the IFN- γ gene transcript:

The level of IFN-γ mRNA from cultured PBMCs pulsed with ILT proteins or the un-pulsed culture was measured using Q-PCR assay [16-18]

The first strand cDNA was synthetized using Omniscript cDNA Synthesis kit (Qiagen Cat # 205111. USA) as described by the manufacture manual. The real-time quantitative PCR was performed using brilliant II QPCR master mix (Agilent cat # 600804). The reaction was carried

TABLE 2. The vaccination regimen of the SPF chicken groups.

Groups	Designed code	Vaccine	Dose
Group 1	G1	DNA vaccine coding for gpB	20µg/0.1ml Booster dose at day 21
Group 2	G2	Commercially available T.C propagated ILT vaccine	0.1 ml
Group 3	G3	Placebo (sterile pyrogen-free water)	0.1 ml

out on Agilent Ariamx instrument. the cycle parameters was adjusted for initial denaturation at 95°C/10 min; and then 40 cycles of denaturation at 95°C/30 sec , annealing at 50°C/30 sec and extension at 60°C/40 sec.

For the normalization of target gene expression, β actin gene was used as reference gene. Results were analyzed using the comparative Ct method. Relative abundance of the IFN-γ gene transcript was calculated as ΔCq = Cq reference – Cq target. Relative fold changes in IFN-γ gene transcript were expressed as 2^-ΔΔCq values between vaccinated and negative control group [19, 20].

Clinical signs and Shedding

Clinical signs:

Chicken remained under observation for 15 days post challenge. Clinical signs were recorded including lethargy, gasping, extended neck, and swollen of the sinuses. Mortality percentages were also recorded by the end of 15th day post challenge

Measuring ILT viral shedding:

Swabs of the trachea from all living and perished birds 15 days after the challenge were collected individually and virus titration was done following the standard EID_{50} assay [9, 14, 16].

Statistical analysis

Analysis of variant (ANOVA) with post hoc was analyzed using SPSS V 21 package.

Results

For purification of the ILT virus isolate used in the current study (ILT-YA/18), a 2-step ultracentrifugation procedure was adopted. First sucrose gradient centrifugation was done to concentrate the virus in a single layer away from all other particulate and membrane proteins (chorio-allantoic membrane proteins on which the virus was propagated), then the virus participated at 50000rpm/3h/4°C. The re-suspended virus was tested using qPCR to ensure the presence of the virus (Fig. 1). Virus titration using EID_{50} revealed that the purified virus sample had a virus titer of $log^{10} = 6.5$.

DNA vaccine preparation

The full-length gpB gene was amplified from the DNA extracted from the purified virus preparation, the 2.3Kbp amplicon (Fig. 2A) was spliced off and purified then cloned into pENTER SD/D topo vector to generate pENTER SD/D-gpB vector and transformed in E.coli-topo 10 chemically competent host strain. After 24h of incubation, the developed colonies

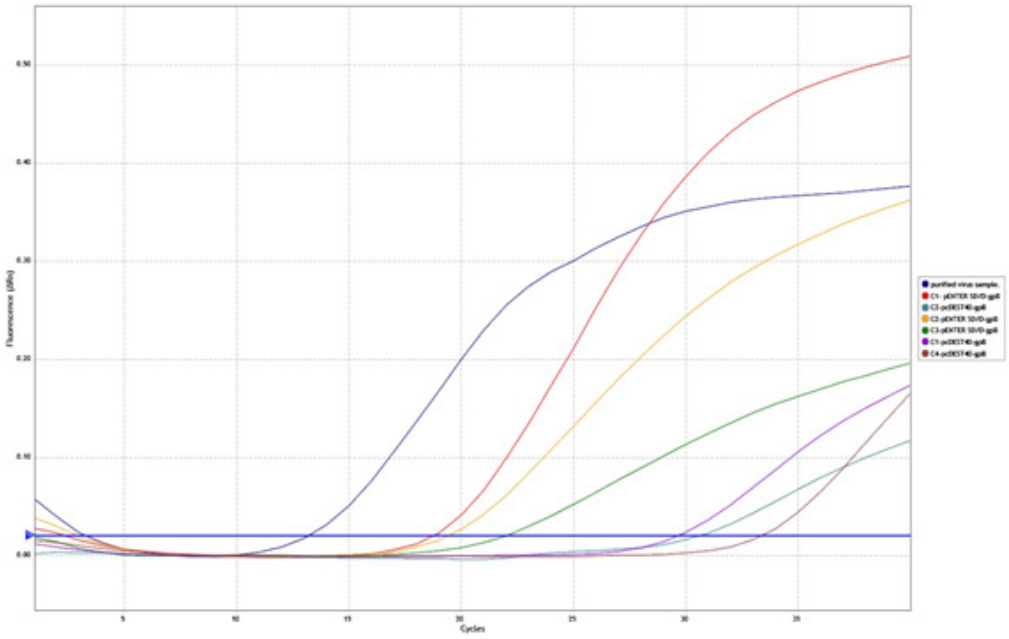

Fig. 1. The amplification of gpB gene from ultracentrifuge-purified virus sample, pENTER SD/D-gpB cloning vector, and PCDEST40-gpB mammalian expression destination vector. Note that the purified virus sample gave a very early Cq value denoting the high virus titer

(Fig. 2B) were sub-cultured and the miniprep was subjected to qPCR to ensure the presence of gpB gene insert within the cloning vector. As seen in Fig. 1, different gave Cq vary from 18.89-22.18indicating the presence of the insert within the entry cloning vector. Positive colonies were selected and homologous recombination between the entry vector and destination vector was done to generate pcDEST40-gpB vector. After transformation of E.coli-topo10 (Fig. 2C), the miniprep from different colonies were subjected to qPCR to investigate the presence of the gpB insert within the destination vector. The Cq value of amplification of gpB gene from the destination vector was found to vary from 29.89-33.54(Fig. 1). Colony with low Cq (high expressing colony) was selected and scaled up for the preparation of the DNA vaccine.

Evaluation of the protective efficacy of DNA vaccine coding for gpB :

DNA vaccine evaluation was done on the level of both humoral and cell-mediated immune response in comparison to the commercially available live TC-propagated ILT vaccine.

ELISA:

DNA vaccine showed to induce powerful antibody titer by day 15 that was comparable to the live TC propagated vaccine. The titer reaches its maximum after 1 week of boostering (28 days post-vaccination). After the challenge, the titer declined (at 7 days post-challenge) manly due to the consumption of the circulation antibodies with the challenge virus and then memory cells began to be activated and resulting in a second wave of increased anti-ILT titer (Fig. 3) .

Quantitation of IFN-γ transcripts:

After vaccination, G1and G2 showed elevation of the transcript level by the 5th day and seen until the 7th day post-vaccination (Table 3). G2 that receive the live TC ILT vaccine showed much elevation till day 21 where there was significant higher ($P < 0.05$) than G1 that receive the DNA-gpB vaccine. At the time of the booster dose of G1 (at day 21), there were an abrupt increase in the level of IFN-γ transcript, which was nearly as high as the G2 group. Surprisingly however, the G1 group that receive the DNA vaccine showed sustained elevation in the level of IFN-γ transcript even after 7days post-challenge and become sig higher than G2.

Control negative group (G3) showed an elevation in the level of IFN-γ gene transcript by the 3rd day after challenge due to the effect of the challenge virus itself, and showed a moderate increase in the fold change of IFN-γ gene transcript from 3rd to the 7th day post-challenge (Fig. 4)

Clinical signs and shedding:

Chicken in the unvaccinated group (G3) showed severe clinical signs including swelling of the sinuses, closed eyes, extended neck and gasping, mucoid and hemorrhagic mucous on the wall of the isolators, and in the letter. Deaths (32%) have been noticed by day 4 to 15 post-challenge.

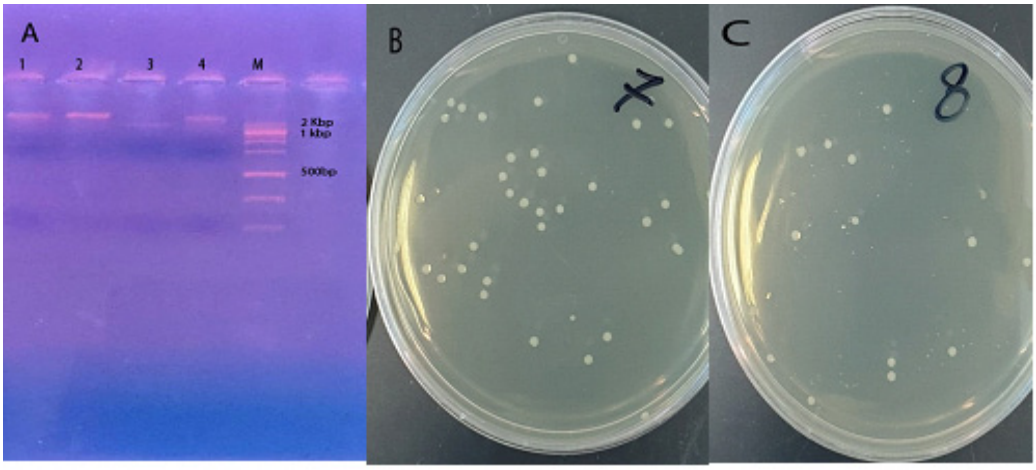

Fig. 2. Amplification of the full–length gp B from the purified virus preparation using different DNA concentrations (A), Transformed E.coli-Topo10 competent cells with the pENTER SD/D-gpB entry vector (B) and pcDEAT4-gpB mammalian expression destination vector (C).

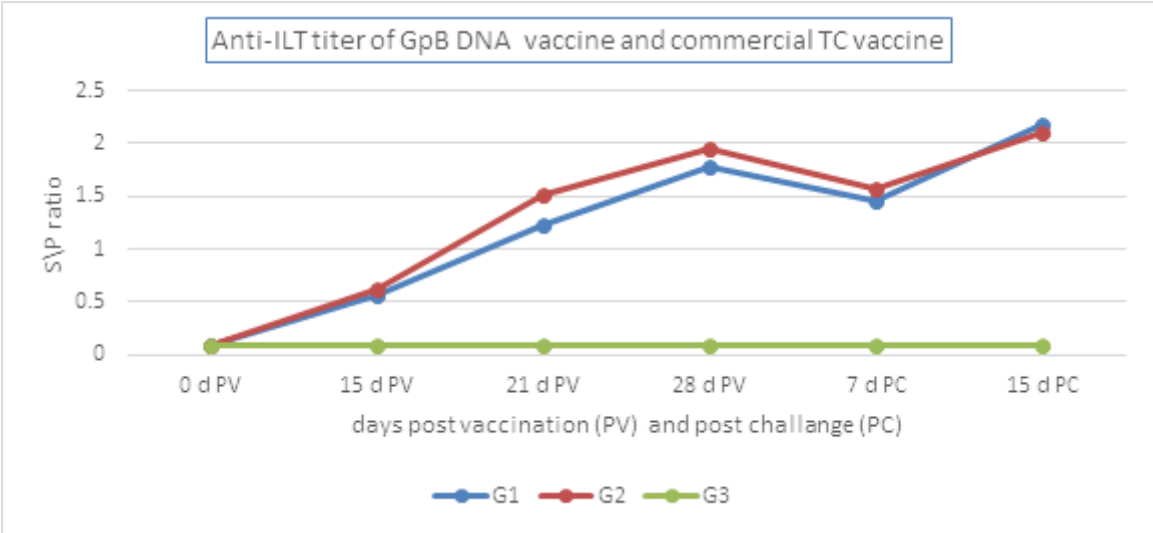

Fig. 3. Antibody titer of sera from SPF chickens vaccinated with either GpB DNA vaccine (G1) or commercially live TC propagated vaccine (G2) in comparison the unvaccinated groups (G3). The titer was expressed in S\P ration (S\P >0.3 considered positive as manufacture instruction). PV= post-vaccination, PC = post-challenge.

TABLE 3. Fold change of IFN-γ transcript after vaccination and post-challenge as analyzed using ΔΔCt method.

Groups	Fold change of the target IFN-γ transcripts							
	Post-vaccination (PV)					Post-challenge (PC)		
	3d-PV	5d-PV	7dPV	15d-PV	21d-PV	3d-PC	5d-PC	7d-PC
G1	0	2,010.03	4,396.07	2,010.03	269.29	29,995.06	37,510.32	54,308.59
G2	0	1,986.48	4,087.49	10,029.76	12,161.22	30,552.44	16,742.18	20,562.22
G3	0	0	0	0	0	12,498.77	13,293.05	12,133.66

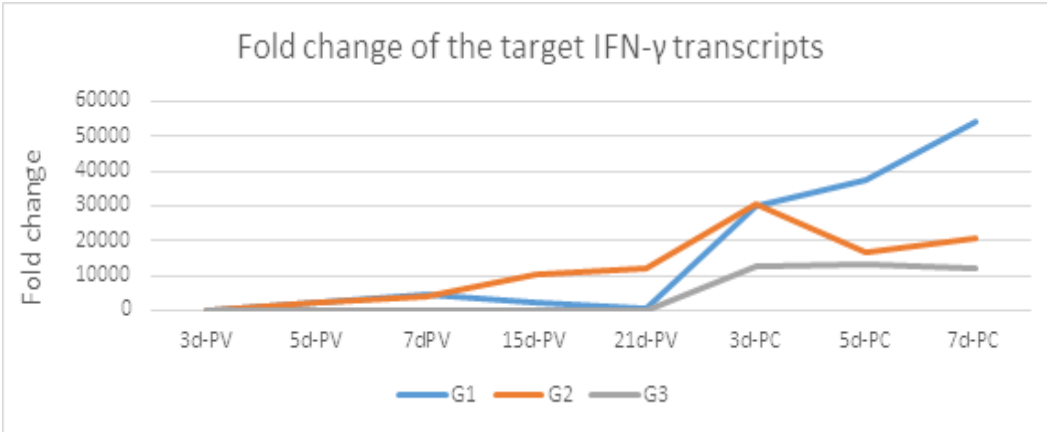

Fig. 4. Fold change in the IFN-γ gene transcript of the peripheral mononuclear cells in response to ILT viral proteins for the groups vaccinated with either the DNA vaccine coding for gpB (G1) or live TC ILT vaccine (G2) and the negative control group (G3).

The vaccine groups, however, showed no deaths at all yet the clinical signs in the groups receiving sing DNA vaccine (gpB) or vaccinated with the commercially TC propagated vaccine showed only decreased activities for about 3 days post challenge and mild cough in the live TC ILT vaccine.

ILT virus titration in the trachea collected from different groups revealed that (Table 4) , the group received the DNA vaccine had nearly no detectable level of the virus in the tracheal swabs, where chicken received the live TC-ILT vaccine however, the viral titer was 2e-02 Log^2. The unvaccinated group showed a very high titer reached Log^2= 3e-06.

Discussion

The developed DNA vaccine coding for the gpB gene of ILT (pDEST40-gpB) was used to vaccinate SPF chicken at 21 days in comparison to the commercially available TC propagated ILT vaccine. The antibody response against the DNA vaccine, as measured by ELISA, showed high antibody titer that increase by time. There was no discernible difference in antibody titer when compared to the live TC ILT vaccine. This high level of antibody titer may be attributed to the high dose of DNA vaccine used for immunization (20g/dose in two doses) that span the replication advantage of the live TC ILT vaccine used. the ability of gpB DNA vaccine to induce such powerful humoral immune response was reported earlier [32,33] . Bioinformatics' analysis of gpB of ILT virus revealed the presence of more than 30 unique linear epitopes based on B-lymphocytes binding affinity [13,34],So DNA vaccines exhibit many advantages[10], such as long persistence of immunogenicity and induction of a wide range of both humoral and cell-mediated immunity [30], and lack of the risk of infection (in contrast to the live tissue culture or egg propagated ILT vaccines which have an issue in the presence of residual virulence, which might be exacerbated with the passage in the chicken farms after vaccinal application [24] and the revertants to the virulent state that attributes to later outbreaks [25,35]). DNA vaccines also have the ability for polarization of T- helper cell type towards Th2 immune response[31]

On the level of cell-mediated immune respace the fold change of IFN-γ gene transcript were highly correlated in both G1 and G2 starting at 3rdday till 7th days post vaccination, however at 21st days post vaccination the IFN-γ transcript level was significantly elevated ($P < 0.05$) in G2 that received the live vaccine. Booster dose of DNA vaccine showed a powerful positive effect on the IFN-γ transcript and the fold change was significantly higher in G1 that received the DNA vaccine (5days post challenge and onward). Such intense induction of IFN-γ may contributes to the presence of several dominant T-cell epitopes (at least 6) on the gpB protein antigen that bind to MHC II and MHC I alleles with the top ranked three 301$FLTDEQFTI$ 309 , 277$FLEIANYQV$ 285 and 743 $IASFLSNPF$ 751[13]. This high level of IFN-γ which elicit powerful CTL found to be very crucial for limiting viral replication and elimination through expansions of both CD4+ and CD8+ cell populations [38, 39] through recognizing and killing virus-infected targets. Several studies showed that Multiple intra-muscular immunizations (boostering) might drive the immune responses toward a Th1 phenotype with MHC-I restricted antigen specific CTL that might last for long time post final boostering dose [40].

In response to such intense humoral and cell-mediated immune response , very mild clinical signs observed after challenge in comparison to the group received placebo, where typical and sever symptoms of ILT virus infection have been developed , however the most pronounced advantage of using of the DNA vaccine (pDEST40-gpB) however, was its ability to prevent viral shedding completely after challenge with the virulent strain which is an intrinsic property of most DNA vaccines [9, 39-42].

Conclusion

The produced DNA vaccine encoding the surface glycoprotein B(gpB) gene of the Egyptian strain of ILT virus (ILT/YA-18) in the current

TABLE 4. The EID_{50} of the virus titer from the tracheal swabs of vaccinated and control groups expressed in Log^2 ±SD.

Group	EID_{50}/100µl
G1	0
G2	2.00e-02±0.8
G3	3.08e-06 ± 0.5

study, could induce a powerful humoral immune response comparable to the live TC propagated ILT vaccine. The vaccine also enabled induction of cell-mediated immune response (as measured by quantification of IFN-γ gene transcript) that was significantly higher than that induced by the live vaccine, particularly 5 days post-challenge and onwards. The DNA vaccine could prevent viral shedding in the tracheas of the challenged chicken, which the live vaccine could not, and this is considered the main advantage of the current study's DNA vaccine (pDEST40-gpB) over the commercially available live TC ILT vaccine.

Conflicts of Interest

The authors declared that they have no conflicts of interest regarding the publication of this research paper.

Funding statement

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Animal Ethical approval

Vaccination and challenge of the SPF chickens approved by the Institutional Animal Care and Use Committee (Vet. CU. IACUC permit number Vet. CU 8/03/2022/411),

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الاستجابة المناعية الخلطية والانتيرفيرون جاما للقاح الحمض النووي المشفر للجيليكوبروتين بي لفيروس التهاب الحنجرة والقصبة الهوائية المعدي

مها ابوالنجا جمال و يوسف عادل سليمان
المعمل المركزي للرقابة علي المستحضرات الحيوية البيطرية - مركز البحوث الزراعية - القاهرة - مصر.

تستمرا للقاحات المهندسة وراثيا في كونها بديلاً مناسبًا وآمنًا وقويًا ، خاصةً للسيطرة على العدوى بمسببات الأمراض التي تعتمد على نوع الاستجابة المناعية التي تتوسطها الخلايا والقدرة على القضاء على تساقط الفيروس. يسبب فيروس التهاب الحنجرة والقصبة المعدية مرضًا تنفسيًا معديًا له تأثير اقتصادي كبير. تم تطوير لقاح DNA لترميز لجين السطحي للبروتين السكري (gpB) من سلالة معزولة محليًا. اللقاح المطور (pcDEST40-gpB) يمكن أن يثير عيارات الأجسام المضادة القوية المرتبطة بشكل إيجابي بلقاح ILT الحي المتوفر تجاريًا والذي ينتشر عن طريق TC. كشفت الاستجابة المناعية الخلوية (كما تم قياسها عن طريق تقدير نسخة الجين IFN-) أن لقاح الحمض النووي واللقاح الحي يبدآن تغييرًا قويًا في IFN-حتى ١٥ يومًا بعد التطعيم، ومع ذلك ، فإن استخدام المعزز أدت جرعة لقاح الحمض النووي إلى زيادة مفاجئة في مستوى نص IFN-الذي كان أعلى بكثير من مستوى اللقاح الحي بحلول اليوم السابع بعد التحدي وما بعده. لقاح الحمض النووي وليس اللقاح الحي يمكن أن يقضي على إفراز الفيروس بعد التحدي.