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ORIGINAL ARTICLE

Assessments of Required Levels of Immunity to Protect Against Genotype VII Newcastle Disease Virus in Broiler Chickens

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Abstract

OBJECTIVE: To assess the levels of immunity required to protect against genotype VII Newcastle disease virus.
DESIGN: Randomized experimental design.
ANIMALS: Hundred fifty chicks.
PROCEDURES: One hundred fifty chicks at one-day-old were randomly divided into six groups and subgroups (25 chicks each). G1 was considered the control group that was unchallenged with the virus. G2-G4, birds challenged with the virus at 1, 7, and 14 days old via the oculo-nasal route at a dose of $10^{7.5}$ EID_{50}. However, G5 was subdivided into two sub-groups inoculated at 21-day-old via the oculo-nasal (G5-I/N) and intramuscular routes (G5-I/M). All experimental birds were monitored and recorded for clinical signs, mortality rates, and gross pathological lesions within 5 weeks period. Blood samples and body weights were measured weekly. Cloacal swabs for viral shedding were detected 3 and 7 days postchallenge (PC).
RESULTS: All birds in G5-I/N and G5-I/M groups died within 7 days of PC. Infectivity and mortality rates were reversibly correlated with maternally drived antibodies MDA titers of 20, 28, 48, 0.0, 8, and 28 % for−G2-G4, respectively. Clinical signs starting within 2−3 days PC for all challenged birds were more severe at 7 and 14 days of age. The signs observed were compatible with those of Newcastle disease (ND), namely marked depression, severe respiratory signs, torticollis, and a marked decrease in feed intake. Gross pathological changes revealed an ideal lesion for ND, most commonly hemorrhage at the tips of the proventricular gland, congested tracheitis, and congested liver and spleen. Birds challenged at 14 days of age G4 showed prominent kidney lesions.

The HI titer assays revealed that the virus broke down the maternally derived antibodies level by 3.8 log2 at 28 days of age, as it recorded a significant elevation of HI titers. However, in birds challenged at 7 and 14 days old, significant elevation in HI titer was shown at 2 weeks, i.e. 6.10 log2 and 6.0 log2, respectively. Cloacal shedding was detected in cloacal swabs at 3 and 7 d PC at similar rates in birds in G2 and G3 (33.5 and 66.7 %, respectively). However, virus shedding in birds challenged at 14 days of age at 3 and 7 days PC was 66.7 and 110 %, respectively.
CONCLUSION AND CLINICAL RELEVANCE: The causative agent of recent ND outbreaks in most vaccinated flocks in El-Dakahlia governorate, Egypt, was characterized as velogenic genotype VII Newcastle disease virus (NDV), which is highly pathogenic to commercial broilers.

Keywords: Egypt, Maternal immunity, Newcastle disease virus genotype VII, Poultry, Virus shedding

1. Introduction

Newcastle disease (ND) has been concerned as one of the most important devastating disease of poultry because of its wide range host of birds and worldwide distribution with severe economic nature of the disease. ND is included as a list A disease [1].

The ND virus is synonymous with avian paramyxovirus serotype 1, a member of the

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Paramyxoviridae family and genus Avulavirus [2]. Based on their virulence, Newcastle disease virus isolates are divided, from least to most virulent, into asymptomatic enteric, lentogenic, mesogenic, and velogenic isolates [3].

Genetically, NDV strains are divided into two distinct classes: class I and II [4]. Strains of genotype VII within class II have been most commonly associated with ND outbreaks in the Middle East (including Egypt) and Asia [5].

Current NDV vaccines for live and inactivated genotype I and/or II NDV. Repeated outbreaks of virulent NDV among vaccinated chickens indicate the need to revise NDV vaccination strategies. Through recombinant technology, newer NDV vaccines have been developed in some countries. These novel vaccines are based on recombinant herpesvirus vectored [6] and reverse genetic LaSota NDV vaccines expressing velogenic F and/or HN genes [7], which can protect chickens from virulent strain challenges [8].

2. Materials and methods

2.1. Ethical approval

All applicable international, national, and institutional guidelines for the care and use of animals were followed.

2.1.1. Fertile chicken eggs

Seventy eggs were used; embryonated chicken eggs (aged 9 days) were obtained from commercial and unvaccinated flocks of local private hatcheries for isolation and pathogenicity of the NDV strains isolated in this study.

2.2. Viral titration

The live ND vaccine (clone 30) and challenge virus (NDV VII) were 10 fold serially diluted in sterile saline, and gentamycin sulfate was added to saline at 1000 μg/ml prior to its usage.

Five eggs were injected from each dilutions (10^{-1}–10^{-11}) of the vaccine of the challenge virus (0.1 ml/egg). Eggs were incubated at 37.5 °C for 5 days with daily observation. The eggs were observed daily, and the HA activity was checked using the slide HA test. Eggs with live embryos were kept in the refrigerator by the end of the 5 days overnight then opened, and the HA activities were performed by slide HA test and the EID_{50} and the mean death time was calculated according to [9].

2.3. Pathogenicity in chickens

To examine the pathogenicity of the NDV/CH/9/2018 virus in chickens, 150 1-day-old commercial Ross-308 broiler chicks were used.

2.4. Experimental chickens

A total of 150 1-day-old broiler chicks (Ross 308) were used to evaluate the pathogenicity of the isolate.

2.5. Newcastle disease virus

The velogenic Newcastle disease virus (VNDV) used in the pathogenicity experiment as well as in the challenge of the vaccinated groups was isolated from field cases in Dakahlia governorate during the period from September 2018 to March 2019. Twelve chicken-vaccinated flocks (seven broilers, three commercial layers, and two broiler breeders) of different ages demonstrated respiratory and nervous symptoms in accordance with postmortem lesions. The isolated virus was confirmed by real-time reverse transcriptase-polymerase chain reaction (RT-PCR) and was characterized by sequencing as NDV genotype VII (NDV/CH/9/2018). It was titrated in embryonated chicken eggs, as described by [10], and the EID_{50} was calculated according to [9]. The inoculation dose was estimated as $10^{7.5}$ EID_{50}/chick.

2.6. Virus shedding

To examine the shedding of NDV genotype VII (NDV/CH/9/2018) in challenged and vaccinated chickens, cloacal swabs were collected at 3- and 7-days postchallenge (PC). Samples were placed in 1 ml of PBS containing penicillin G (10 000 IU), streptomycin (10 000 μg/ml), and mycostatin (156 U) and incubated overnight at 4 °C. The processed swab samples were centrifugated at 1000×*g* for 5 min. Genomic viral RNA was then directly extracted from cloacal swabs using QIAamp viral RNA extraction kits, according to the manufacturer's protocol. Standard RT-PCR was performed using a one-step RT-PCR kit (QTAGEN; Valencia, CA, USA) according to the manufacturer's instructions. The primers used were NDV-F 5′ GCA GCT GCA GGG ATT GTG GT3 and probe-p 5′ TCT TTG AGC AGG ATG TTG3.

Chicks with maternal NDV antibodies were provided by a local hatchery. They were housed in separate pens with *ad libitum* access to food and water provided *ad libitum* at a bio-secured experimental facility. The chicks were randomly divided

into five groups (G1–G5) of 25 chicks each. G1 received no viral challenge and was maintained as a control. G2 birds were exposed to the challenge virus at 1-day-old. G3 was challenged with the virus at 7 days of age. G4 received a viral infection at 14-day-old. All birds in G2, G3, and G4 were inoculated with the challenge virus via the oculonasal route. However, birds in the G5-ON group received the challenge virus at 21-day-old via the oculo-nasal route. Birds in G5-IM at the same age (i.e., at 21 days old) received the infectious virus via the intramuscular route.

3. Results

3.1. Infectivity rate of NDV genotype VII (NDV/CH/9/2018)

The infection rate in the control group G1 was zero, whereas it was 100 % in group 5, regardless of the route of infection, that is, intranasal (IN) and intramuscular (IM). The infection rates of NDV genotype VII (used in this study) challenged the chicks at one and 7 days of age at 20 and 28 %, respectively. However, the infection rate of this isolate was 48 % when birds at 14-days old (Table 1).

3.2. Mortality rate of NDV genotype VII (NDV/CH/9/2018) at different times

The mortality rate in the control group G1 and G2 that was challenged at 1-day old was nil (zero), while it was 80 % (G5-IN) and 92 % (G5-IM) in the challenged groups at 21 days of age. The mortality rate was 8 % in the bird group G3 challenged at 7 days of age, and 28 % in birds challenged at 14 days of age. In G3, G4, and G5, mortality appeared on the 5th day PC and continued for 2 weeks (Table 2).

Table 1. Infection rate of Newcastle disease virus genotype VII (Newcastle disease virus/CH/9/2018) at different ages of broilers.

Groups	No. of chickens	Infected No. (%)	Noninfected No. (%)
G1 Control	25	0	25 (100.0)
G2 Challenge at 1 day old	25	5 (20.0)	20 (80.0)
G3 Challenge at 7 days old	25	7 (28.0)	18 (72.0)
G4 Challenge at 14 days old	25	12 (48.0)	13 (52.0)
G5 IN Challenge at 21 days old	25	25 (100.0)	0
G5 IM Challenge at 21 days old	25	25 (100.0)	0
$\chi^2 = 99.386$, $P < 0.001$			

Table 2. Mortality rate in broilers challenged at different ages with Newcastle disease virus genotype VII (Newcastle disease virus/CH/9/2018).

Groups	No. of chickens	Live No. (%)	Dead No. (%)
G1 Control	25	25 (100.0)	0
G2 Challenge at 1 day old	25	25 (100.0)	0
G3 Challenge at 7 days old	25	23 (92.0)	2 (8.0)
G4 Challenge at 14 days old	25	18 (72.0)	7 (28.0)
G5 IN Challenge at 21 days old	25	5 (20.0)	20 (80.0)
G5 IM Challenge at 21 days old	25	2 (8.0)	23 (92.0)
$\chi^2 = 94.750$, $P < 0.001$			

3.3. Clinical signs

No clinical signs of disease or mortality were observed in the broilers in the control group G1. In contrast, 100 % of the birds in G5 (either IN or IM) were depressed, listlessness (Fig. 1), increased respiration, greenish diarrhea, soiled feathers of vents, conjunctivitis (Fig. 2), prostration, and mortality within 10 days of PC. In G2, only five (20 %) birds showed signs of torticollis (Fig. 3) at the 4th week PC, and other birds were observed in a normal status (Table 3).

3.4. Gross lesions

Gross lesions observed in the dead infected chickens were typically velogenic viscerotropic ND. The lesions were characterized by emaciation and dehydration, with deep congestion of the breast musculature (Fig. 4). Multifocal and diffuse hemorrhages around the proventricular gland (Fig. 5) and

Fig. 1. A bird challenged by Newcastle disease virus from group 3 showed dullness weakness and depression after 7 days post–challenge.

Fig. 2. A bird challenged by Newcastle disease virus at 7 day old from group 3 showed nasal discharge, periorbital swelling, and conjunctivitis after 7 days postchallenge.

Fig. 3. A bird challenged by virus at one day old from group 2 showed torticollis after 14 days postchallenge.

Fig. 4. A chicken challenged by Newcastle virus at 14 days old showed severe congestion in breast muscle after 5 days postchallenge.

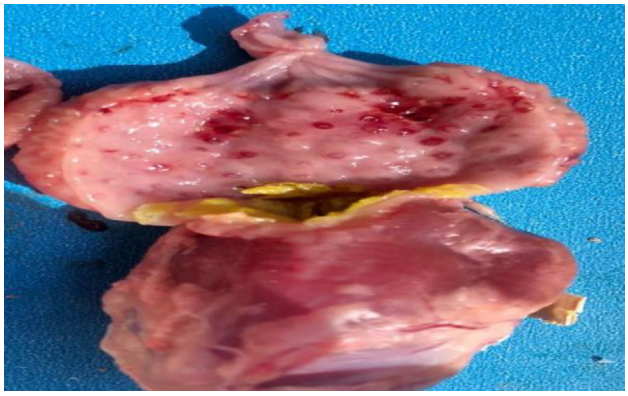

Fig. 5. A chicken from group 4 challenged by newcastle virus at 14 day old showed Petechial hemorrhage and echymotic hemorrhage on tips of proventricular gland after 7 days postchallenge.

necrotic hemorrhagic ulcers throughout the intestine and cecal tonsils are shown (Fig. 6). A congested enlarged liver with subcapsular hemorrhages is shown (Fig. 7). Multiple disseminated foci of necrosis with pinpointed hemorrhages were observed in the splenic parenchyma (Fig. 8). Kidney lesions were seen in birds of G5 that were challenged at 21 days and varied between paleness and enlargement (Fig. 9) and hemorrhagic (Fig. 10; Table 4).

3.5. Evaluation of immunogenicity

Experimental birds in this study showed the persistence of maternal antibodies throughout the entire study period. The level of age, the HI titers ranged from 7.7 ± 0.2 at 7 days old to 2.9 ± 0.27 at 35 days old. Results revealed that the challenge at 1-day old G2 breakthrough the maternal immunity at the level of 3.8 ± 0.24 (28 days old) as it showed

Table 3. Clinical signs in broilers challenged by Newcastle disease virus genotype VII (Newcastle disease virus/CH/9/2018) at different ages.

Groups	Total No.	Affected No.	%	Respiratory[a]	Nervous[b]	Others[c]
G1 Control	25	–	–	–	–	–
G2 Challenge at 1 day old	25	5	20	–	5	–
G3 Challenge at 7 days old	25	7	28	5	2	–
G4 Challenge at 14 days old	25	12	48	8	–	4
G5 IN Challenge at 21 days old	25	25	100	18	5	2
G5 IM Challenge at 21 days old	25	25	100	14	11	–

[a] Respiratory signs = coughing, sneezing and swollen eyelids.
[b] Nervous signs = incoordination, paralysis, torticollis.
[c] Other signs = ruffled feather, depression, diarrhea.

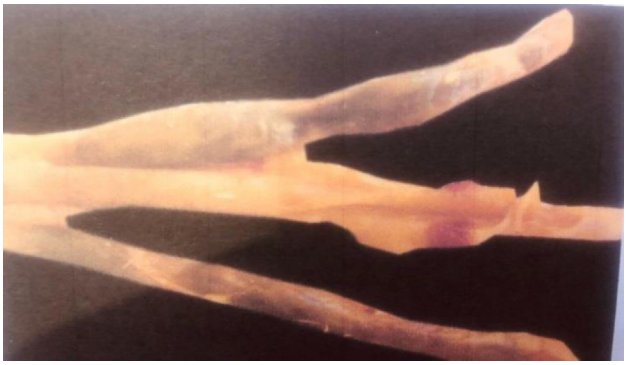

Fig. 6. A chicken from group 4 challenged by Newcastle disease virus at 14 days old showed hemorrhagic ulcer in the lower third of the ileum after 7 days postchallenge.

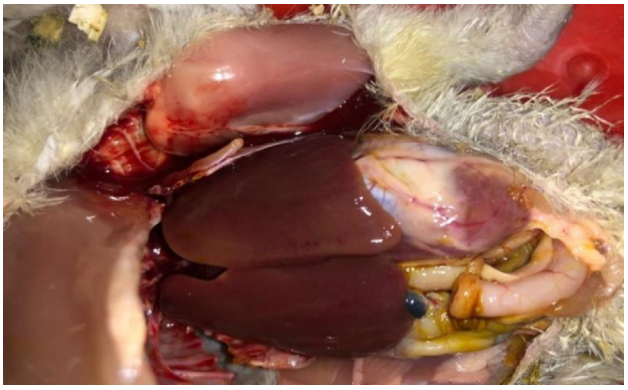

Fig. 7. A chicken from group 3 challenged by Newcastle disease virus at 7 days old showed congested liver with subcapsular hemorrhage after 7 days postchallenge.

significant elevation 5.30 ± 0.42. However, the challenge at 7 days of age revealed a significant elevation in the HI titers at 21 days old (6.10 ± 0.37) compared with the control group (4.40 ± 0.16). In regards to the challenge at 14 days old, birds revealed immunity response significantly elevated than that of the control group in 28 days old, i.e. 6.0 ± 0.47 versus 3.8 ± 0.24 (Table 5).

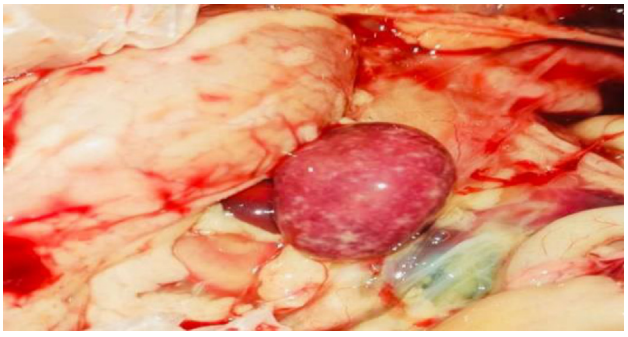

Fig. 8. A chicken from group 5 challenged by Newcastle disease virus at 21 days old showed Spleen suffering from hemorrhage and enlargement and multiple foci after 3 days postchallenge.

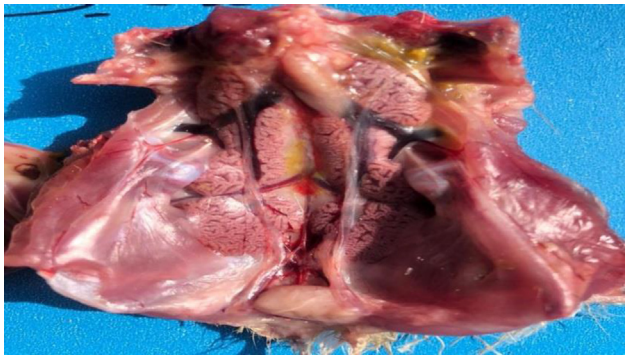

Fig. 9. A chicken from group 5 challenged by Newcastle disease virus at 21 days old showed paleness and enlagrement of Kidney after 5-days postchallenge.

3.6. Effects of body weight

All one-day-old challenges induced a significant reduction in the body weight in comparison to the control group i.e. 152.41 ± 6.53–1706.33 ± 20.83 versus 171.08 ± 4.59 – to 1860.07 ± 27.52.

In the challenge at 7-day old, the virus induce reduction in body weight since a week postinfection, but the reduction showed highly significant at the period of 3- and 4-weeks postinfection in comparison to control group 1103.53 ± 14.15, 15533.26 ± 21.04 versus 1305.26 ± 24.07, 1860.07 ± 27.52, respectively.

In regards to G4 i.e. challenge at 14 days-old, results revealed severe body weight reduction since 1-week PC, i.e. 21 days-old in comparison to the control ones 642.39 ± 26.63, 918.52 ± 13.68, 1120.19 ± 24.82 versus 791.16 ± 17.55, 1305.26 ± 24.07, 1860.07 ± 27.52, respectively (Table 6).

In G3 challenged at 7-day-old, only 7 (28 %) birds showed respiratory signs after 5 days of PC, which persisted for the entire period of observation. Signs of torticollis were observed at the ages of 3, 4, and 5 weeks-old.

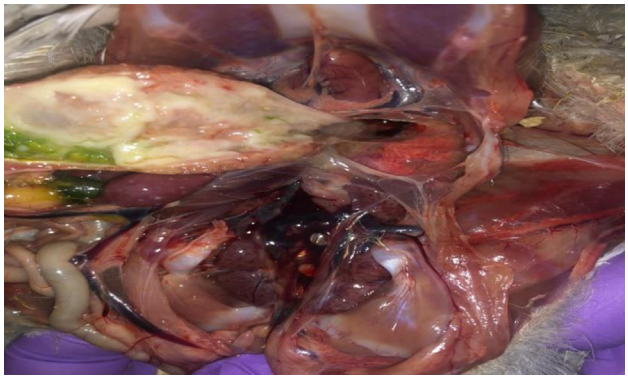

Fig. 10. A chicken from group 5 challenged by Newcastle disease virus at 21 days old showed congested kidneys after 5 days postchallenge.

Table 4. Gross pathological lesions in birds challenged by Newcastle disease virus genotype VII (Newcastle disease virus/CH/9/2018) at different ages.

Group	Weeks post infection									
	1	No	2	No	3	No	4	No	5	No
G1 Control	None	–	None	–	None	–	None	–	None	–
G2 Challenge at 1 day old	None	–	None	–	None	–	None	–	None	–
G3 Challenge at 7 day old	Hemorrhagic liver Hemorrhagic trachea	2	Same in 1-week post infection	2	Mortality	2	–	–	–	–
G4 Challenge at 14 day old	Hemorrhagic liver Hemorrhagic trachea Congested spleen	6	Same in 1-week post infection	7	Mortality	7	–	–	–	–

Table 5. Humoral immune response experienced as HI unit induced by Newcastle disease virus genotype VII (Newcastle disease virus/CH/9/2018) in broiler challenges at different ages.

Groups	Mean ± SE of Newcastle titer				
	7-Days	14-Days	21-Days	28-Days	35-Days
G1 Control	7.70 ± 021 a	6.10 ± 0.23 a	4.40 ± 0.16 b	3.80 ± 0.24 b	2.90 ± 0.27 b
G2 Challenge at 1 day old	7.50 ± 0.31 a	6.50 ± 0.26 a	5.10 ± 0.50 ab	5.30 ± 0.42 a	5.90 ± 0.64 a
G3 Challenge at 7 day old	–	6.80 ± 0.32 a	6.10 ± 0.37 a	5.00 ± 0.49 ab	5.90 ± 0.43 a
G4 Challenge at 14 day old	–	–	5.10 ± 0.65 ab	6.00 ± 0.47 a	6.60 ± 0.49 a
Level of Sig.	NS	NS	a	a	a

Means having with the different letters in same column differed significantly.
[a] ($P \leq 0.05$), NS: Nonsignificant.

Table 6. Effects of the Newcastle disease virus genotype VII (Newcastle disease virus/CH/9/2018) on the body weight of the experimental group.

Groups	Mean ± SE of body weight (gm)				
	7-Days	14-Days	21-Days	28-Days	35-Days
G1 Control	171.08 ± 4.59 a	392.36 ± 8.73 a	791.16 ± 17.55 a	1305.26 ± 24.07 a	1860.07 ± 27.52 a
G2 Challenge at 1 day old	152.41 ± 6.53 b	356.00 ± 11.06 b	742.28 ± 13.94 b	1200.49 ± 13.62 b	1706.33 ± 20.83 b
G3 Challenge at 7 day old	–	348.29 ± 8.91 b	710.12 ± 9.67 b	1103.53 ± 14.15 c	1533.26 ± 21.04 c
G4 Challenge at 14 day old	–	–	642.39 ± 26.63 c	918.52 ± 13.68 d	1120.19 ± 24.82 d
Level of Significance	a	a	b	b	b

Means having with the different letters in same column differed significantly.
[a] ($P \leq 0.05$).
[b] ($P \leq 0.01$).

In G4, 12 (48 %) birds showed clinical signs from the 5th day PC, characterized mainly by respiratory signs concomitant with depression and diarrhea.

3.7. Virus shedding

Virus shedding was examined in broilers on days 3 and 7 postchallenge by detecting viral RNA in the cloacal swabs. As shown in Table 7, swabs collected from the nonchallenged birds G1 revealed no virus detected in the two periods, whereas 100 % positive results were observed in the swabs collected from the birds in G5, that is challenged at 21 days at the two periods of collection and only at 7-day PC from birds in G4 that challenged at 14 days of age. Similar findings were obtained in G2 (challenged at 1-day-

Table 7. Real-time reverse transcriptase-polymerase chain reaction results of cloacal viral shedding (n = 3).

Groups	Three days postchallenge			Seven days postchallenge		
	No. birds + ve	No. birds −ve	% +ve	No. birds + ve	No. birds −ve	% +ve
G1 Control	0	3	–	0	3	–
G2 Challenge at 1 day old	1	2	33.3	2	1	66.7
G3 Challenge at 7 days old	1	2	33.3	2	1	66.7
G4 Challenge at 14 days old	2	1	66.7	3	0	100
G5 IN Challenge at 21 days old	3	–	100	3	–	100
G5 IM Challenge at 21 days old	3	–	100	3	–	100

old) and G3 (challenged at 7-day-old) for the two periods of collection: 33.3 % at 3 days PC and 66.7 % at 7-day PC (Table 7).

4. Discussion

Newcastle disease is a highly contagious poultry disease and one of the major causes of economic loss in the poultry industry. Although an intense vaccination strategy has been implemented in Egypt, virulent NDV strains are still constantly isolated in vaccinated and nonvaccinated poultry flocks in different regions since 2011 causing severe outbreaks and economic losses in chickens [11,12]. In the Middle East, Africa, and Asia, NDV genotype VII was associated with a vast majority of recently reported outbreaks [13,14]. The emergence of virulent NDV genotypes and repeated outbreaks of NDV in vaccinated chickens have raised the need for fundamental studies on virus–host interactions.

The present study was carried out to study the pathogenicity of NDV genotype VII (NDV/CH/9/2018), recently isolated from samples collected from the Dakahlia governorate from vaccinated chicken flocks, including broilers (seven flocks), layers (three flocks), and broiler breeders (two flocks), in commercial broilers. The virus was isolated from farms with different vaccination programs, and the mortality rate ranging 10–25 % (only one reported 45 %) in broiler, 5–10 % in layers, and 2 % in broiler breeders. The virus isolates were investigated and characterized by the pathogenicity index (ICPI) and molecular pathogenicity as virulent NDV genotype VII (personal communication with Dr. Kamel Ibrahim Mahmoud Abu-Alazim).

The genetic diversity of class II NDV probably originates from intrinsic errors of the viral polymerase during genome replication. These alterations are believed to create a large number of genetic variants known as quasispecies, on which natural forces act to select the determined characteristics of the NDV genome. Except for a few sites mapped on selected isolates [15], the roles of.

Minimal RT-PCR-positive cloacal swabs among the early samples may be due to the presence of PCR inhibitors in these samples [16], but it is more likely that the virus had not yet circulated systemically at this stage (3 days PC), and extensive shedding in the cloaca was therefore minimal [17]. Previous investigations have suggested that NDV isolation frequencies decrease following increased NDV antibody titers [18,19]. Therefore, our results suggest that the NDV/CH/9/2018 NDV genotype VII strain can be efficiently transmitted between chickens via direct contact.

In this study, we evaluated the immune responses (as HI titers) induced strain in broilers by NDV genotype VII (NDV/CH/9/2018). This strain induces stronger immune response 6.0 log2 HI unit (Table 5), concomitant with a higher viral RNA level in cloacal swabs at early stage of infections (Table 7). Consistent with these observations, it was previously reported that some strains of NDV (mostly genotype VII) replicated more efficiently and induced a stronger immune response in splenocytes of chickens at 6, 12, and 24 h postinfection *in vitro* [20] or in thymus and bursa tissues of chickens at 24, 48, and 72 h postinfection *in vivo* [21]. These observations revealed that the strong immune response caused by the NDV/CH/9/2018 genotype VII strain might be associated with high levels of viral load in infected tissues or cells at the early stage of infection; however, this suggestion requires more observations.

In Egypt, sub-genotype VIId is predominant and causes several ND outbreaks [22,23]. GVII strains have been reported to cause more severe lesions in lymphoid organs than genotype I strains [24]. Moreover, previous studies have indicated that GVII strains produce more severe clinical and pathological signs in the spleen than other virulent strains do.

The innumerable mutations that exist in circulating viruses in the pathogenesis and host range remain unknown [25]. The first part of the current study deals with the pathogenicity of the NDV/CH/9/2018 isolate of genotype VII NDV in commercial broiler chickens with considerable maternally derived antibodies (MDA).

Our results indicated that the infection and mortality rates due to the NDV genotype VII isolate were correlated with persistently high levels of maternal antibodies. experimental chicks (Tables 1, 2 and 5). Therefore, the infectivity and mortality rates in one-day-old G2 broilers challenged with MDA (8.8) were 20 % with no mortality; however, in G3, which was challenged at 7-day-old 28 and 8 %, respectively. In G4 birds challenged at 14-day-old with HI titer more than 6.0 $\log_2$, infectivity rate reached 48.0 % with mortality rate 28 %. In birds, immunoglobulins are deposited in the egg yolk, and chicks absorb these antibodies into their developing systems. The yolk contains mainly IgG (Igγ), which becomes the circulating antibody in the chick, while albumin contains predominantly IgA, which is swallowed by the developing chick, coating its mucous membranes with IgA [26]. High levels of maternal antibodies in young chickens may interfere with the multiplication of the field virus or live vaccine strains, reducing the level of immunity

provided and leaving birds susceptible to field challenges.

Previous reports have demonstrated that the combination of both a live attenuated vaccine and an inactivated adjuvant vaccine, as the regimen of vaccination in broiler breeder flocks in Egypt and other parts of the world, was able to activate a high level of antibody production in dams [27]. The levels of passively acquired maternal antibodies in the serum of a day-old-chick are approximately the same as those found in the serum of a hen [28]. The MDA titer (mean < 6 logs) detected on the day of challenge protected susceptible chickens from death in G2 group. Our results agree with previous reports of low titers [29,30]. This finding could be in accordance with other experiments demonstrating that some NDV strains are more antigenic than others, from varying HI antibody titer levels to different antigens after equal amounts of vaccines are administered [31]. Over time, the MDA titer wanes and eventually cannot protect against infection with wild viruses. Similar findings have been reported previously [32,33]. Our results confirmed that circulating vNDV strains are capable of causing high mortality in unvaccinated susceptible flocks, which is consistent with the suggestions of [34]

Strains [35,36]. These findings suggest that neither the fusion protein (F-protein) cleavage site sequence nor the intracerebral pathogenicity index are sufficient tools to fully predict clinicopathological outcomes associated with virulent viruses [14].

The clinical signs (Table 3), mortality patterns (Table 2), and postmortem lesions (Table 4) observed in the present study were suggestive of very virulent Newcastle disease. The NDV genotype VII strain used in this study broke maternal immunity at a level of 4.4 log2 HI titer in birds at age of 21 days, causing 100 % mortality (Table 2). Lesions, such as multifocal to diffuse hemorrhages around the proventricular glands, necrotic hemorrhagic ulcers throughout the intestine, and cecal tonsils, were similar to the findings observed by [37]. Congested and enlarged livers with subcapsular hemorrhage and disseminated multiple foci of necrosis with pinpointed hemorrhage that were observed in the spleen were also in agreement with the findings observed by others [12,13,20,38–40].

The severe lesions and probably extensive viral replication observed in the intestine may account for the high levels of virus shedding in cloacal swabs. In the kidneys, multifocal necrosis may support the notion that viral shedding may occur through urine [41].

The present study elucidated a significant reduction in the body weight of broilers infected with NDV genotype VII strains at different ages (Table 6). The severity of the reduction concomitant with the reduction in food consumption and severity of the disease induced, that is, 1120.19 ± 24.82 (G4), 1533.26 ± 21.04 (G3), and 1706.33 ± 20.83 (G2) as compared with the control in the noninfected group G1 1860.07 ± 27.52. These results are in accordance with those reported in [42].

Reactive species (RNS), generally known as reactive oxygen species and reactive nitrogen species, are produced during normal metabolism in the host and are required for many cellular processes such as cytokine transcription, immunomodulation, ion transport, and apoptosis [43]. Intriguingly, both RNS and reactive oxygen species are commonly triggered by pathogenic viruses and are known for their dual roles in the clearance of viruses and their pathological implications. Uncontrolled production of reactive species results in oxidative stress and damages proteins, lipids, DNA, and cellular structures. Mesogenic and velogenic NDV causes oxidative stress, increases the levels of malondialdehyde (MDA), and decreases glutathione in infected and [44].

On the other hand, the mortalities recorded in our study seem to be compatible with the ratio recorded in the flocks affected by this isolate in the Dakahlia governorate [38]. reported a morbidity rate of 80 % in 28-day-old broilers infected with NDV genotype VII. These unusual mortality patterns caused by different isolates of the same genotype (VII) may contribute to the genomic differences in each virus.

Virus shedding can be an important source of environmental contamination, which in turn acts as a mechanical transmission vector for viruses spreading between poultry houses on farms and/or between farms [45]. NDV cloacal shedding was detected at 3- and 7-day PC with NDV genotype VII by examining cloacal swabs (Table 7) from infected broilers for detection of viral shedding using RT-PCR; the positive results were 33.3 % at 3-days PC and 66.7 % at 7-day PC for birds in G2 and G3. In G4 birds, when the challenge with NDV was at 14-day-old, virus shedding was 66.7 % and 100 % for 3- and 7-days PC, respectively. These results are in agreement with those of previous studies [38,46,47] chickens [48,49].

Similarly, increased concentrations of the Nitric oxide and MDA were observed in NDV-infected chickens [24].

The findings of the present isolate obtained from chicken flocks at Dakahlia governorate (NDV/CH/9/2018) genotype VII by the F gene sequence and whole genome sequence (data not shown) confirmed that mortality and production losses in

the present study were due to the highly virulent nature of the genotype VII pathotype of NDV, which supports the findings of earlier reports by [50] who reported that the current vaccination program against ND in poultry, including genotype II virus strain like-B1, LaSota, etc., does not prevent the clinical disease against velogenic pathotypes VII, and the resultant outbreaks.

5. Conclusion

The causative agent of recent ND outbreaks in most vaccinated flocks in Dakahlia governorate, Egypt, was characterized as velogenic genotype VII NDV, which is highly pathogenic for commercial broilers with high maternal antibody to NDV at 7, 14, and 21 days of age. Our study demonstrated that recombinant viral-vectored NDV vaccines have promising protective efficacy, even in the presence of significant maternal antibody titers. To date, the most promising vaccines against virulent NDV genotype VII infection in poultry are recombinant genotype-matched live attenuated vaccine candidates generated by reverse genetics. They specifically target the prevailing genotype in a particular region and are, therefore, rationally designed to fulfill the criteria of an excellent NDV vaccine.

Animal ethics committee permission

The current research work was permitted to be executed according to the standards of the Animal Research Committee of the Faculty of Veterinary Medicine, Mansoura University.

Author contributions

Fahad and El-Azm performed experiments. Ahmed and Hassan revised the manuscript.

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Data access statement

The data are available within this study.

Conflicts of interest

The authors declare that there is no any conflict of interest.

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