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Resistance to very virulent Newcastle disease virus among four different types of Egyptian chickens

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Abstract

Four phenotypes of local Egyptian hens were tested for their vulnerability to the highly pathogenic Newcastle Disease Virus (NDV). Serum samples were taken on days 0, 3, 7, 14, and 28 after the intraocular injection of vvNDV (NDV-EGYPT/18629F-2018) into eighty (80) village hens, twenty (20) of each type Dokki-4, Anshas, Mandarah, and El-Salam. For the Dokki, Anshas, Mandarah, and El-Salam kinds, there was a 100% morbidity rate and 100%, 100%, 70%, and 100% mortality rate, respectively. Prior to the infection, every phenotypic had a mean antibody titer of zero. On day 28 post-infection (PI), all phenotypes showed the highest Geometric mean titer (GMT) of NDV HI antibodies, with the exception of the Mandarah. Throughout the trial, the GMT in the control group stayed low. Therefore, it could be said that the Dokki-4 group was the most resistant phenotype to NDV infection, whereas the El-Salam and Anshas types were the least resistant and poor seroconverts. The most resilient phenotype was seen in the Mandarah group. It is therefore recommended that genetic selection techniques include the Mandarah group.

Keywords: *Newcastle disease virus, Phenotypes, Dokki, Anshas, Mandara, El-Salam, chickens.*

Introduction

Newcastle disease poses a major hazard to hens raised intensively and is the primary issue restricting rural chicken production in the majority of developing nations (Echeonwu et al., 2008).

In Egypt, poultry farming is a significant and affordable source of animal protein. In addition to native Egyptian breeds, certain locally created strains have been established for the production of both meat and eggs. Abd El-Gawad et al. (1983) made a pairing between Nichols x Maamourah to get El-Salam strain as well as Inshas strain. In 1966, co-breeding was made between Fayoumi x Barred Plymouth Rock to give Dokki-4 strain (El-Itriby and Sayed, 1966). Meanwhile, a crossing between Alexandriax Dokki-4 produced the Mandarah strain (Abd EI-Gawad, 1981). (a new Egyptian strain of chickens) which was developed by crossing strain of chickens) which was developed by merging between Sinai and White Plymouth Rock type **(Bakir *et al.,* 2002).**

Up to 80% of the total poultry population in developing nations in Asia and Africa are native birds (FAO, 2010). Endogenous hens have a significant role in maintaining gender equity, household incomes, and food security in Egypt (Kitalyi, 1998). According to Hossary and Galal (1994), native Egyptian breeds have been bred in rural regions in semi-intensive methods to produce more meat and eggs per bird.

Egyptian native chicken breeds are typically recognized for their high level of disease resistance. Fayoumi birds fare far better than White Leghorn, Rhode Island Red, or Mandarah breeds when it comes to coccidiosis infection, according to preliminary research (Hamet and Mérat, 1982). On the other hand, it has shown that the Fayoumi are more vulnerable to

IBDV **(Anjum *et al.,* 1993).**
Since the initial isolation in 1947, velogenic and a velogenic strain of NDV have been regularly found in Egypt and grouped as class II genotype II, VI, and VII (Daubney and Mansy, 1948; Hussein et al., 2005; Mohamed et al., 2009; Radwan et al., 2013). But class II genotype VII is thought to be the most common NDV isolates among hens, linked to outbreaks in Egyptian backyard and commercial poultry farms, and phylogenetically related to the NDV strains that were previously isolated in China (Mohamed et al., 2009; Hussein et al., 2014; Saad et al., 2017).

Egyptian breeds of native chickens have been reported to have natural resistance to infectious bursal disease and Newcastle's disease, and Hassan et al. (2004) has explored differences in immunocompetence for endemic diseases like ND within and between ecotypes. Similarly, several lines of White Leghorn hens have been shown to have inherent resistance to Salmonellae infections (Bumstead and Barrow, 1993). Because they receive less attention than other poultry species when it comes to disease prevention, village chickens are thought to be resistant to a wide range of common infections (Melewas, 1989; Chrysostome et al., 1995). This study's primary goal is to ascertain the four Egyptian crossbreeds (Mandarah, Inshas, El-Salam, and Dokki-4)'s resistance to experimental infection with highly virulent NDV by monitoring the birds' antibody titer following immunization as well as performance and mortality metrics.

Materials and Methods

Birds and Experimental design

Eighty one-day-old chicks from four locally enhanced Egyptian chicken breeds—Mandarah, Inshas, El-Salam, and Dokki-4—were used in this study. We bought chickens from the Animal Production Research Institute in Alexandria's Al-Sobaiha Farm. The 20 birds in each of the four groups—group 1: Mandarah, group 2: Inshas, group 3: El-Salam, and group 4: Dokki—were chosen at random and placed in a clean, well-ventilated room that had previously been formalin and potassium permanganate fumigated. Heaters were installed in the room to regulate the temperature based on the age of the chicks in order to facilitate experimental infection. The chicks were given water and ad libitum feed. HI and ELISA tests were used to screen all birds for NDV antibodies from day one to day five, weak, in order to demonstrate the chicks' freedom from NDV maternal antibodies and their attainment of MDA zero. Each group was split into two at the age of five: ten birds were infected with NDV-EGYPT/18629F-2018, while ten birds served as an uninfected control group. Each chicken was given 0.2 milliliters of the diluted virus to inject into its nostril and eyes.

VVNDV challenge virus

The highly pathogenic Newcastle disease virus (NDV-EGYPT/18629F-2018) was isolated in 2018 from broiler chickens in the governorate of Al Daqahlia. Each bird was given 106EID50 as a challenge virus. This strain was found at the Animal Health Research Institute in Cairo, Egypt, and was described as Very virulent NDV genotype VIId. The NDV-EGYPT/18629F-2018 virus, accession number MK604219, was released in the Genbank database.

The virus's Lethal Dose fifty (LD50) was determined by diluting it 1:10 with Phosphate Buffered Saline (PBS) with a pH of 7.2. Additionally, the chickens were challenged via intranasal injection with 0.2 cc of the dilution. For 14 days following infection, the hens were observed every day for clinical indicators, morbidity, and mortality. For chickens that passed away, postmortem examinations were performed. PM lesions were observed were recorded and organs were frozen at -20ºC for viral isolation.

Serum Samples

After infection, the experimental birds were bled on days 0, 3, 7, 14, 21, and 28. Each experimental bird had 3 ml of blood drawn into sterile vacutainer tubes via the wing vein using a sterile needle. In order for the blood samples to coagulate, they were stored at room temperature in the vacutainer tubes. Following ten minutes of centrifuging the vacutainer tubes at 1,500 rpm, serum samples were extracted and stored at -20 °C until analysis.

Chicken Red Blood Cells

Using a sterile syringe, four milliliters of chicken red blood cells were drawn onto one milliliter of Alsevers solution (an anticoagulant). Alsever's solution was used to first wash

the RBC solution three times. then afterwards with Phosphate Buffered Saline (PBS), pH 7.4, centrifuged for five minutes at 1,000 rpm. According to Allan and Gough's (1974) instructions, a 1% suspension of chicken red blood cells in PBS was made for use in the haemagglutination (HA) and haemagglutination inhibition (HI) assays.

Antigen

The "LaSota" vaccine against Newcastle disease served as an antigen for the HA and HI tests. The ND vaccine LaSota's HA titer was ascertained using the method outlined by Allan and Gough (1974), and the resulting 4HA unit was suitably diluted for use in the HI test. Each chicken serum's HI titre was calculated and expressed as the reciprocal of the final dilution at which the agglutination of the chicken red blood cell is totally inhibited.

Serological Test

According to (Allan and Gough, 1974), the haemagglutination inhibition (HI) test was utilized to identify and measure antibodies against NDV. Using beta-techniques (continuous virus and variable serum), the HI test was conducted against four HAUs calculated from the HA titration result. In summary, a microtitre plate was filled with 25µl of PBS in each well, and then 25µl of the test serum was added to the first well (A1). The test serum was then diluted across the plate by moving 25µl from the first well to the second well and finally to the last well (A12). To each well on the plate, 25 microliters (µl) of suitably diluted antigen (4HAU) were added. Following the addition of 25µl of 1% chicken RBC to each well, the plate was allowed to sit at room temperature for half an hour. Positive and negative NDV sera were also included on the plate. The titre was defined as the reciprocal dilutions at which the chicken RBC's ability to clump together was completely inhibited.

Results

According to the findings of the experimental vvNDV infection of four phenotypes of native chickens (NDV-EGYPT/18629F-2018), all of the infected birds developed the disease as early as day three (3) following infection, exhibiting mild to severe nervous symptoms and greenish diarrhea. Day 4 PI for El-Salam, Dokki-4, and Inshas, and Day 5 PI for Mandarah, marked the beginning of the mortality period. For every trait, a 100% morbidity rate was shown. For the Dokki-4, El-Salam, Inshas, and Mandarah kinds, the illness lasted 7, 7, 9, and 5 days, respectively (Table 1).Table 1 illustrates the high mortality rates (70%) and 100% morbidity rates (100%) resulting from the injection of 106 EID50 per bird of virulent NDV in Mandarah (70%), Inshas (100%), El-Salam (100%), and Dokki-4 (100%). The majority of the phenotypes began accumulating protective antibody titers as early as Day 7 post-infection, according to the geometric mean antibody titer for the experimental infection (Table 2). During the course of the experiment, none of the hens in the control group produced protective HI antibodies.

Table (1). Results of the morbidity and mortality rates among different phenotypes of village chickens challenged with (NDV-EGYPT/18629F-2018)

Parameters	Phenotypes			
	Dokki-4	El-Salam	Inshas	Mandarah
Onset of clinical signs (days)	3	3	3	4
Onset of mortality (days)	4	4	4	5
Morbidity (%)	100%	100%	100%	100%
Mortality (%)	100% (10/10)	100% (10/10)	100% (10/10)	70% (7/10)
Duration of the disease (days)	7	7	9	5

Table (2). Geometric mean titer (GMT) of NDV HI antibodies of four phenotypes of native chickens infected with (NDV-EGYPT/18629F-2018)

Phenotype	Tested birds	GMT of H1 Antibody					
		D_0	D_3	D_7	D_{14}	D_{21}	D_{28}
Dokki-4	10	0	2.6	3.2	0	0	1024
El-Salam	10	0	0	1.4	181	90.5	512
Inshas	10	0	2.3	3.6	45.2	128	128
Mandarah	10	0	2.6	3.2	10.3	40.3	128
Control group	10	0	0	0	0	0	0

Discussion

One of the most devastating diseases to plague chicken, particularly in large-scale production, is Newcastle disease (ND) (Alexander et al., 2003).

Newcastle disease virus (NDV) is endemic in Egypt, where long-lasting outbreaks of the virus cause severe economic losses in the poultry industry. This is because, despite intensive vaccination programs, high mortality rates from highly virulent strains of NDV can reach 100% (Mohamed et al., 2009; Radwan et al., 2013).

Since their initial isolation in 1947, velogenic and a velogenic strain of NDV have been regularly found in Egypt and grouped as class II genotype II, VI, and VII (Daubney and Mansy, 1948; Hussein et al., 2005; Mohamed et al., 2009; Radwan et al., 2013). Class II genotype VII, on the other hand, is thought to be the most common NDV isolates circulating among chickens. It has been linked to outbreaks in Egyptian backyard and commercial poultry farms, and it shares phylogenetic relationships with NDV strains that were previously isolated in China (Mohamed et al., 2009; Hussein et al., 2014; Saad et al., 2017).

For Egypt's domestic poultry production, NDV regulation is crucial. To reduce the financial losses, strategies other than immunization and medicine should be recommended. Accordingly, breed-specific differences in susceptibility to numerous chicken diseases have been reported (Bumstead, 1998). Therefore, it is vital to think about selecting for and enhancing genetic resistance to economically significant diseases. The specific objective of this study is to examine the vulnerability of four native Egyptian chicken phenotypes to the highly pathogenic Newcastle disease virus (NDV-EGYPT/18629F-2018) genotype VII. We decided to look into the sensitivity of the Egyptian breed to NDV further because a breed that is resistant to one virus may be more susceptible to another. The four chicken breeds used in this study—Mandarah, Inshas, El-Salam, and Dokki-4—were exposed to virulent NDV. The findings indicated that the Mandarah breed had a 70% death rate, while the Dokki-4, Anshas, and El-Salam breeds all showed 100% mortality. Breed or genetic stock seems to have relatively little bearing on a chicken's susceptibility to NDV, according to Cole and Hutt (1961). A hybrid between males from Alexandria and inbred females from Dokki-4 produced the dual-purpose breed known as the Mandarah (Abdel-Gawad, 1981). It is challenging to pinpoint the precise innate responses that might be in charge of the NDV resistance observed in the Mandarah hens based on the findings of this study. Resistance may be caused by elements like natural killer cells, macrophages, and secretory IgA (Gupta et al., 1989). To understand the mechanism of NDV resistance shown in the Mandarah hens, more research is required. Hence, it may be said that the Mandarah chickens group had the highest resistance phenotype, followed by the other three phenotypes. Selection programs should be adjusted to produce breeds of Mandarah chickens that are appropriate for raising in rural areas.

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