Department of Poultry
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"Some Studies on Newcastle Disease Virus (NDV) in Ducks in Assiut Governorate"

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List of Abbreviations

AAF	Allantoic/amniotic fluid
Ag	Antigen
AGID	Agar gel immunodiffusion
AIV	Avian influenza virus
APMV	Avian paramyxoviruses
BHI	Brain-heart infusion
Ct	Cycle threshold
C-Ve	Control Negative
C+Ve	Control Positive
DPC	Day post challenge
ECE	Embryonated chicken eggs
ELISA	Enzyme-linked immunosorbent assay
ER	Endoplasmic Reticulum
F	Fusion Protein
GP	Groups
HA	Hemagglutination
HI	Hemagglutination-inhibition
HN	Hemagglutinin-neuraminidase protein
IBDV	Infectious bursal disease virus
ICPI	Intracerebral pathogenicity index
IM	Intramuscular
IN	Intranasal
IVPI	Intravenous pathogenicity index
Kb	Kilobases
LBMs	Live bird markets
M	Matrix Protein
MAbs	Monoclonal Antibodies
MDA	Maternally-derived antibodies
MDT	Mean death time
Mg/ml	Milligram/milliliter
µl	Microliters
National Veterinary Service Laboratories	NVSL
ND	Newcastle disease
NDV	Newcastle disease virus

NO	Numbers
OIE	Office International des Epizooties
pAbs	Polyclonal antibodies
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
PM	Post-mortem lesions
RBC	Red blood cells
rRT-PCR	Real-time reverse transcription polymerase chain reaction
RT-PCR	Reverse transcriptase-Polymerase chain reaction
SA	Sialic Acid
SPF	Specific pathogen free
TBTB	Tris-buffered tryptose broth
VI	Virus isolation
vNDV	Virulent Newcastle disease virus
vnNDV	Velogenic neurotropic Newcastle disease virus
vVND	Very virulent Newcastle disease
vvNDV	Velogenic viscerotropic Newcastle disease virus

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6.Summary

A total no. of 151 cloacal swab samples was collected from different LBMs in Assiut Governorate during the years 2019 and 2020. Virus isolation was done through inoculation of ECG (9-11) day via allantoic sac route; the harvested fluids underwent rapid and slow HA tests, and the positive ones exposed to HI test using specific HIS previously prepared against NDV. Only 5/151 were positive for the properties of hemagglutination and 4/5 were positive for HI test. The 4 positive samples further subjected to RT-PCR for more confirmation using a specific primer. None of the samples gave the target band.

Experimental infection was done using Muscovy species which was vaccinated with different inactivated vaccines using matched and mismatched NDV genotypes and also one group treated with HIS (previously prepared against NDV). These ducks were challenged 3 weeks after vaccination and administration of HIS with chicken highly virulent strain previously characterized. The signs, p/m lesions, serological effects, and viral shedding were investigated.

The experiment revealed that Muscovy ducks of all groups except negative control (G5), showed depression started from 3DPC. G1 and G4 also showed slight watery greenish diarrhea. Decreased feed intake and activity appeared at 4DPC for all groups. By 5DPC all groups had watery greenish diarrhea. Labored respiration appeared in all groups at 6DPC which rapidly disappeared. At 13 DPC all groups started to recover and signs started to hide.

Muscovy ducks from all groups vaccinated or not, did not show any mortality during the experiment. PM examination was performed at

17DPC. Severe congestion and inflamed lung were observed in all treated groups except the G5. Slight inflamed, enlarged gall bladder in all groups except G3 (HIS treated).

Slight inflamed with some petechial hemorrhage on the intestinal serosa and caecal tonsils in all groups except G2 (Dalguban treated) and G3 (HIS treated). Slight inflamed lower part of the liver and enlarged spleen. The proventriculus, trachea, and heart appeared normal. Only G5 (negative control) appeared normal through the experiment.

The serum samples were obtained from ducks before and after the challenge were used to assess the immune status of birds through HI test, and evaluate the level of protection from used vaccines and HIS against virulent challenge. The used vaccines and HIS induced a higher immune response.

It was observed that ducks of different groups increased in antibody titers after challenge, indicating replication of the challenge virus, which was observed after that as viral shedding in swab samples. Antibody titers using HI test of all groups at 7^{th}, 15^{th},17^{th}, 18^{th} day after challenge using different antigens LaSota and Genotype VII antigens. A comparison between values of HI titers using different antigens clearly showed that titers measured with NDV-VII antigen (matched) were slightly higher than with LaSota antigen (mismatched).

All groups were tested for NDV by rRT-PCR pre-challenge and all were negative for the virus and also tested after challenge at 2,4,6,8,10,15 DPC. Infected ducks (G1) (LaSota treated) started to excrete NDV from 4DPC until 10 DPC, while G4 (non-vaccinated, Challenged) started excretion by 2DPC and continued to 15DPC, whereas G2 (Dalguban

treated), G3 (HIS treated), and G5 (non-vaccinated, non-challenged) did not excrete NDV through the experimental period.

Keywords: Newcastle disease virus; Muscovy species; different vaccines, HIS, rRT-PCR, viral shedding.