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"Protective Efficacy of Avian Influenza(H9N2) Commercial Vaccines in Chickens"

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SUMMARY

Avian influenza is a contagious disease caused by type A influenza viruses. In spite of the mild nature of H9N2 low pathogenic avian influenza virus, the virus was isolated from frequent disease outbreaks with high mortality in different parts of the world.

Nine chicken flocks of different breeds and ages during 2018 were examined in Sharkia Governorate. Samples were collected from farms that experienced respiratory distresses or drop in egg production.

The flocks were tested by real time PCR for determination of the H9N2 subtype in each sample, also they were tested for the presence of other viruses (H5, H7, NDV & IBV), which revealed four flocks were positive for H9AIV, negative for other avian pathogens; two were positive for IBV and three flocks were negative.

The examined broiler flocks showed mild respiratory signs: sneezing, swelling of periorbital tissues with conjunctivitis, nasal and ocular discharges. Whitish diarrhea also noticed. While, examined layer flocks showed depression, decrease in feed consumption with drop in egg production with thin-shelled, rough and misshapen eggs.

The postmortem findings of the examined broiler flocks revealed tracheitis, congestion of lungs and air-saculitis. Swollen Kidney was also detected.

The molecular characterization was carried out for HA and NA genes of the H9N2 isolates, at first the conventional PCR amplification for the HA and NA genes into 2 fragments was applied, the positive PCR products were sequenced for the nucleotides of the HA and NA genes, All of these sequences have been submitted to the GenBank database.

The HA identity % among our isolates shows high percent (96.9 – 99.8 %), also with other Egyptian isolates in gene bank (2011-2019) (94.3-98.4%). Egyptian viruses were closely related to the Israeli viruses with high identity% (94-96.2%).In the other hand, The NA identity % among our isolates shows (97.5 – 99.9 %)**,** also with other Egyptian isolates in gene bank shows very high percent (96-97.9%) and, were closely related to the Israeli viruses with high identity% (94.1-96.1%).

Phylogenetic analysis revealed that the HA and NA of our Egyptian H9N2 isolates are related to the Middle Eastern H9N2 isolates and maintained a direct out-group relationship to the prototype G1-like viruses, forming a distinct cluster.

The four samples were isolated and propagated in the SPF embryonated chicken eggs then the collected allantoic fluids were tested by HA assay for the detection of the virus titers which produce a titers ranged from 2^4 to 2^9. Then (S2 &S6) titrated in 10-day-old SPF embryonating eggs to determine the 50% embryo infectious dose titer (EID_{50}) calculated by the Reed and Muench. Virus (A/chicken/Egypt/S6ZAG/2018) was diluted with sterile phosphate buffered saline (PBS, pH 7.4) to adjust the

amount of inoculum to 1 x10^6 EID_{50} per bird. 100µl of diluted virus per bird was given.

Protection efficacy of commercial H9N2 vaccines were evaluated by two field available vaccines **(vaccine 1 A/chicken/Egypt/ME543V/2016 and vaccine 2 A/chicken/ Iran/Av1221/ 1998)** through groups each (15 birds) of SPF one day old chicks named (G1/G2/G3/G4/G5/G5/G6) respectively then challenged them with field isolate A/chicken/ Egypt/ S6ZAG/ 2018.

Protection was evaluated by clinical signs, histopathology, immune response weekly by ELISA and HI tests and tracheal swabs were used to monitor virus shedding at 3, 5 and 7 days post challenge by qRT-PCR test.

Our findings indicated that only some depression was obvious in positive control group. Also, there was not any mortality in all groups during the experiment, which confirmed the low pathogenic nature of H9N2 AIV.

It has been seemed that H9N2 vaccine 1 stopped shedding of the field virus at the 3rd day post challenge and the vaccine 2 succeeded to prevent any field virus shedding.

From this study, it concluded that H9N2 vaccines are efficient to decrease and also prevent AI shedding in specific pathogen free (SPF) chickens and give very good antibodies response against H9N2 after single vaccination with oil emulsion inactivated vaccines.