(Co-infection effect of *Escherichia coli* and low pathogenic H9N2 avian influenza virus in broiler Chickens).

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6-Summary & Conclusion

This study was carried out to study the effect of *E.coli* O_{78} strain on the pathogenicity of H9N2 field strain in Luxor governorate in the south valley area.

In order to identify and clarify the effect of *E.coli* O_{78} to the H9N2 virus the agents of suggestive clinical cases in broiler farms in our area during 2015, and 2016, we carried out a combination of classic and modern molecular methods this study was conducted into parts, first part was the isolation and characterization of filed strain used in the co-infection, and the second part was the experiment which was conducted in specific isolators.

Firstly, screening of five hundred (n = 500) tracheal and cloacal swabs (pooled samples) collected from Fifty farms by using Real-time PCR using specific primers resulted into ten farms out of fifty farms was positive H9N2 avian influenza virus.

All ten positive samples were propagated in SPF ECE and confirmed by Real-Time PCR test.

We choose three samples for sequencing and characterization that these positive samples were higher in amplification plots (F18, F23, and F26) with cycle thresholds (16.19, 9.33, and 14.15) respectively, and the three isolates were published in GenBank.

Furthermore, molecular characterization was performed by direct sequencing resulting in a 963bp cDNA corresponding to the HA region.

With deduced amino acids analysis using A-Quail-HongKong-G1-97 the origin for comparison. The most important part of the pathogenicity detection was the HA gene. As well as there are three significant sites in the HA region which were important for the analysis and characterization of HA region called (Proteolytic cleavage site (PCS), receptor binding site (RBS), and presence or absence of glycosylation sites near to the (RBS)), these sites are important in pathogenicity determination that all sites revealed that our strains still low in pathogenicity.

The three local examined isolates used for nucleotides sequence, percent identity, and phylogenetic analysis revealed that our isolates were very close to the Egyptian strains from (2011 to2016) and also other Middle east, Asian, and north Africa strains and also related to Israeli viruses isolated in (2006, 2008, 2010 and 2012).

With the second part of our study, we choose A/chicken/Egypt/1618F/2016 as a sample for the experimental study with *E.coli* O_{78} strain.

The study clarified that the groups which inoculated *E.coli* O_{78} (after, before, or simultaneously) with the H9N2 showed increasing the severity of the clinical findings and mortality rates that observed in groups (2, 3, and 4).

The study indicated that the groups (3, and 4) that injected *E.coli* O_{78} after H9N2 inoculation or the both at the same time showed prolonged presence of H9N2 virus in the trachea and upper respiratory tract which the persistence extend to 9 days post the 1st infection, although other groups (1, and 2) the existence of the virus didn't exceeded the 6 days post the 1st infection and disappeared at 9, and12 days post infection.

These results give us an indication about the effect of *E.coli* O_{78} on H9N2 virus presence that if it comes with or after H9n2 infection increases it's duration in trachea and upper respiratory tract. However, H9N2 alone infection didn't persist more after 6 days and it disappeared at 9, 12 days.

In the case of virus shedding from cloaca the study implied that groups (1, 2, 3, and 4) showed positive results after 6 days and negative results in 9, 12 days post 1st infection

By using serological examination, the results showed that the higher antibody titer after 15 days post 1st infection especially in groups (3, and 4) with (7.1 and 7.3) geometric means.

Conclusion:

- This study reported that H9N2 avian influenza viruses are still widespread among poultry farms during 2015 and 2016 Luxor Governorate, south of the valley region in Egypt, remains low in pathogenicity and these viruses are closely related to some Middle Eastern, Asian, and Egyptian strains.
- Our fear of this disease is to become contagious to humans after time, should apply the adequate health measures in poultry flocks to dominate the birds from infection and keep humans from touch with the disease.
- It has been shown that the H9N2 virus remains low in pathogenesis if it comes separately without other respiratory pathogens, however, it seems more dangerous if co-infected with other bacterial infections, especially *E.coli* O_{78}
- In this study, the results showed that the relationship between H9N2 and *Escherichia coli* in chicken farms induces severe signs of respiratory and higher mortality rates in suspected broiler herds.
- Persistent surveillance and diagnosis of H9N2 are very important with good programs and strategies in experimental protocols to locate the situation of the virus and pursue its evolutions, particularly in the status of co-infections with Escherichia coli.

7. Abstract

This study was conducted to estimate the pathogenicity condition of H9N2 when co-infected with bacteria (*E.coli* O_{78}). Screening of five hundred pooled cloacal and tracheal swabs (250 from tracheal and 250 from cloacal) collected from fifty farms by using Real-Time PCR, resulted into ten farms out of fifty farms were positive for H9N2. All ten positive samples were propagated in SPF ECE 9-11 days old and then confirmed by real-time PCR detection. Sequencing of three samples from the ten positive samples for genetic characterization revealed that our field Egyptian isolates still low in pathogenicity and related to Egyptian strains and also, close to other Middle East, Asian, North Africa and some Israeli strains. The experimental study was conducted by using 60 SPF birds divided into 6 groups. Our H9N2 filed strain A/chicken/Egypt/1618F/2016 was used in the experiment with *E.coli* O_{78} strain. Using real-time PCR for the detection of the virus after co-infection and also the serological examination was done for all groups. The results of the experimental infection implied that the co-infection between H9N2 virus with *E.coli* O_{78} leading to increasing the clinical signs and mortality rates between poultry farms leading to high economic losses.