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SOME STUDIES ON AVIAN INFLUENZA H9N2 IN BROILER CHICKENS AT ASSIUT GOVERNORATE

Thesis

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List of abbreviation

AI	**Avian influenza**
BHI	**Brain heart infusion**
CCRD	**Complicated chronic respiratory diseases**
dpi	**Day post infection**
ECE	**Embryonated chicken egg**
EID	**Embryo infective dose**
HA	**Haemaglutination test**
HI	**Haemaglutination inhibition test**
HPAI	**Highly pathogenic Avian influenza**
HPNAI	**Highly pathogenic notifiable avian influenza**
IB	**Infectious bronchitis**
IBD	**Infectious bursal disease**
IVPI	**Intravenous pathogenicity index**
LBM	**Live bird market**
LP	**Low pathogenic**
LPAI	**Low pathogenic avian influenza**
LPNAI	**Low pathogenic notifiable avian influenza**
MT	**Mean HI titre**
NAI	**Notifiable avian influenza**

List of abbreviations

ND	**Newcastle disease**
OIE	**World Organization for Animal Health**
ORT	**Ornithobacterium rhinotracheale**
PM	**Post mortem**
RBCs	**Red blood cells**
SPF	**Specific pathogen free**
WHO	**World Health Organization**

Summary and **conclusion**

This thesis aimed to seroprevalence of H9N2 infection in Assiut Governorate, study pathogenicity of recent H9N2 Egyptian isolate 2020 in broiler chicken, study the role of maternal antibody in protection of chicks from infection and or mortality, study the role of H9N2 as immunosuppressed agent, study effect of live vaccines used in broiler chicken on pathogenicity of H9N2 and on severity of infection and study role of hyper immune sera in protection from H9N2 infection. For serological diagnosis of H9N2 virus serum samples were collected from 70 different broilers farms which all show respiratory sign, different level of mortality, from Assiut Governorate in a period from January 2019 to march 2020. Oropharyngeal or tracheal swabs were collected, tested by real time PCR the positive sample with the lowest ct was isolated in ECE and retested by real time PCR for H9, H5, ND, and IB, sequenced and then titrated in ECE to be used in experimental trial where 120 day old chicks were divided into 6groups G1:was challenged at 5 day old G2: challenged at 12 days old G3: challenged at 12 days old and vaccinated with live vaccines against ND, IB and IBD as follow (Hitchner IB at 7day old by eye drop, IBD (intermediate) at 12 and 22 days old and Lasota at 19 day old in drinking water) G4:injected with 2ml of H9N2 hyper immune sera at 11 days old and challenged at 12 days old then half of them (G4b) were injected with booster dose of hyper immune sera (3ml) after appearance of clinical sign (2dpi).G5: vaccinated only with live vaccines against ND, IB and IBD as

G3 G6: control group (not infected not vaccinated) at 28 days old 10 chickens were injected IV with diluted allantoic fluid of H9N2(1:10) to determine IVPI. Clinical signs, PM lesion, mortality were recorded daily. Pooled serum samples were collected at 1, 5, 9, 12,15 , 21 and 28 day from control group to measure maternal antibody. And collected from each group before infection and at 7, 14, 21 dpi. Also pooled serum samples were collected from vaccinated group and vaccinated challenged group to measure antibody titre against ND 21day post vaccination by HI test and using Lasota as antigen. Oropharyngeal swabs were collected before infection and at 3, 5, 7, 9, 14, 21dpi. Tissue samples (lung, trachea, pancreas, kidney, bursa, thymus, spleen, brain, cecal tonsil) were preserved in 10% neutral buffer formalin for histopathological examination they were collected from sick and morbid chicks. Serological diagnosis of H9N2 infection in broiler chicken in Assiut Governorate revealed that 27 farms were positive for H9N2 with percent 38.5%. and HI titer ranged from 2^5 to 2^9 about 12 sample from positive appear in winter, 8in autumn, 6in spring and one in summer. In experimental trial G1 with 2^7 maternal antibodies had showed mild respiratory signs without mortality. Antibody titre against H9N2 was 2^9, 2^5 and 2^8 at 7,14and 21 dpi respectively and shedding start at 3dpi and continuous to 14dpi. G2 with 2^4 maternal antibodies showed moderate to severe clinical sign with mortality 25%, antibody titre 2^4, 2^6, 2^7 at 7,14and 21 dpi respectively. Shedding start at 3dpi and continuous to 21dpi. G3 showed sever clinical sign with 35% mortality and antibody titre 2^5, $2^9$and 2^8 at 7,14and 21dpi and shedding start at 3dpi and continuous to 21dpi. Antibody titre against ND 21 day post vaccination was 2^3 compared to $2^{5.5}$ in G5. G4a (received one dose of hyper immune sera) showed mild

clinical sign with 5% mortality Antibody titre was less than 2^1 at 7,14and 21 dpi in some chicks (about 50%) where others (that showing sign) had antibody titre 2^4, 2^6, 2^6 at 7,14and 21dpi respectively. H9N2 shedding start at 3dpi and continuous to 21dpi and in G4b signs disappear after booster dose and no mortality appear antibody titre was less than 2^1 shedding appear only at 9dpi. In all group vascular disturbances, degeneration of epithelial lining affected organs and depletion of lymphocyte in immune organs is the main histopathological lesion appear in H9N2 infection.

The result of this study concluded that:

1. H9N2 AI infection occurs around the year and its appearance increase in winter and autumn and it occur in Assiut with a percent 38.5%.
2. The current H9N2 strain was intermediate in its pathogenic nature.
3. Maternal antibodies at a level 2^7 protects chicks from mortality and sever infection but can't prevent shedding of the virus.
4. Maternal antibodies at a level 2^4 can't protect chicks from H9N2 infection.
5. Live vaccines against (ND, IB, IBD) could increase severity of H9N2 infection and increase its pathogenicity.
6. H9N2 can play an important role as immunosuppressed agent.
7. Hyper immune sera can be used for protection of the young chicks during critical period (when levels of maternal antibody decrease and chicks immunity still in complete) but it should prepare in chickens or by using recombinant technology.

Recommendation

- ✓ Breeder chickens must vaccinated against H9N2 AI to provide adequate level of maternal antibody protecting chicks during early life.
- ✓ Breeder chickens must be free from vertical diseases (especially mycoplasma and ornitho bacteria) which can transmit to young chicks and increase severity of H9N2 infection
- ✓ Chicks must be reared in high biosecurity level during first 3 weeks of their life until it can depend on their immune system and hyper immune sera can be used during this period to help in protection.
- ✓ Don't vaccinate chickens showing respiratory sign especially with IB vaccines especially in winter and it is advisable to examine bird for H9N2 infection before vaccination.
- ✓ Chickens must be supported with vitamins regularly and when we vaccinate them we should use vaccines which have low virulence and high immunogenicity