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Study on clinical aspects of SPF chickens infected with H9N2 subtype of Avian Influenza virus

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ABSTRACT

Avian influenza virus (AIV) is prevalent in almost all countries with intensive poultry flocks. This disease mostly causes respiratory and gastrointestinal signs, but sometimes more complicated cases seen in commercial chickens. The aim of this study was to characterize the clinical aspects of A/chicken/Iran/m.1/2010 (H9N2) of AIV in infected SPF chickens. Forty two one-day-old SPF chicks were divided randomly into two groups (21 chicks each group). At the age of 12 days old the chicks in group1 were inoculated intra-ocularly with 10^6 EID₅₀ of the H9N2 AIV, and the group2 was as control group. Chickens in each groups was evaluated from 2 to 14 days post inoculation. The results of this study indicated that the H9N2 subtype of AIV cannot cause mortality, and only slight clinical signs such depression, pneumonia were seen in infected chickens. These results demonstrated that H9N2 subtype was a low pathogenic and probably concurrent infectious in field situation causes severe clinical signs and high mortalities.

Keywords: Avian Influenza Virus, H9N2 subtype, SPF chicks, Clinical signs

INTRODUCTION

Avian influenza (AI) is a contagious, able to spread in a susceptible population in a short period of time, and economically important viral disease of commercial chickens occurring at all ages, which is caused by avian influenza virus [24]. Avian influenza viruses (AIV) are members of *Orthomyxovirus* genus, and only type A influenza viruses causes disease in poultry, and at present 16 haemagglutinin and 9 neuraminidase subtype have been recognized [11].

H9N2 subtype of AIV was first reported in 1998 from commercial poultry flocks of Iran and causes serious economic losses in Iranian poultry industry, although it was characterized as the low pathogenic avian influenza (LPAI) [27]. Although earlier pathogenesis studies indicated that the LPAI viruses have only cause respiratory and gastrointestinal dysfunction [25], but there was some reports of systemic infections with the LPAI viruses [1, 16]. Although pathogenicity of AI viruses, associated with surface haemagglutinin (HA) antigens [12], some experimental studies indicates that the H9N2 LPAI virus in SPF chicks was low pathogenic and mortality was not

reported [10, 13], but in Asian and middle east countries high mortality rate reported in recent decades [18, 20, 28]. Co-infections of LPAI viruses with other bacteria or viruses also increase mortality rate and exacerbate clinical signs and gross lesions [2, 9, 10, 15, 21].

The aim of the study was to investigate the clinical aspects of H9N2 LPAI virus in SPF chickens as well as clinical signs and gross lesions. Serological response of chickens after infections were also evaluated challenged chicks.

MATERIALS AND METHODS

Virus: The H9N2 subtype of Avian Influenza virus (AIV) A/chicken/Iran/m.1/2010 (H9N2) was used in this study. The titer of virus was expressed as the 50% embryo-infective dose (EID₅₀) calculated by the method of Spearman-Kärber [4, 29]. Forty-two white Leghorn were obtained from the specific pathogen free (SPF) embryonated chicken eggs from Venky's company (Venky's, India), were divided randomly into two groups (21 chicks per group). They were kept in separate positive pressure isolators at Razi Vaccine and Serum Research Institute, Karaj-Iran. All chickens were provided feed and water *ad libitum*.

Experiments: At the age of 12 days-old, all birds of the experimental group were inoculated with chorioallantoic fluid containing 10⁶ EID₅₀/0.1 ml, AIV subtype-H9N2 by eye drop. The other group was kept as the control group. After challenge, all the chickens were monitored daily for clinical signs and mortality. From 2 to 14 days post inoculation (PI), three chickens from each group were randomly selected and were humanely euthanatized and necropsy was performed and gross lesions were recorded.

Serology: Serum samples were tested for the presence of antibodies against AI using the Hemagglutination Inhibition (HI) test.

Statistical analysis: The results obtained from HI test were analyzed by independent t-test, using PASW SPSS (version 18.0). The t-test was performed at 95% probability and p-value less than 0.05 was considered significant and less than 0.01 was considered as very significant.

RESULTS AND DISCUSSION

Clinical findings: Some chickens of the infected group showed mild depression at 4 days PI and immediately recovered form days 6 post inoculation. There was not any mortality in all groups during the experiment. There were not any clinical signs in control group.



Fig1- depression in H9N2 infected group

Gross Necropsy Findings: There were no detectable gross lesions in all organs of the control chickens therefore that were regarded as normal. The chickens exposed to Avian Influenza virus showed the following lesions. Trachea and kidneys were normal during study period, and there were not any gross pathological changes, but from days 6 PI, in lungs pneumonia was seen. Other organs also were normal and there were not any gross pathological changes.



Fig2- lungs, 6 days PI, pneumonia is obvious in lungs

Serological findings: Antibody titers against H9N2 subtype of AI in the serum samples that were collected on days 0, 2, 4, 6, 8, 10, 12, and 14 PI was measured by HI test. All serum samples on day 0 and 2 PI were negative to AI, serological results of AI are shown in Table-1, statistical comparison between two groups indicated that from days 4 to 14 there was significant difference between two groups and at days 6 to 14 PI there was very significant difference between groups. There was not any change in antibody titer against H9N2 AI virus in the control groups.

Table1: Antibody titers against H9N2 subtype of AI in experimental groups (Mean±SE)

Groups	Days Post Inoculation						
	2	4	6	8	10	12	14
H9N2 Infected	0.0±0.00	1.8±0.12	3.1±0.31	4.5±0.19	5.2±0.15	5.8±0.21	6.5±0.23
Control	0.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00
t-test p-value	1.00	0.02	0.01	0.01	0.01	0.01	0.01

The first outbreak of Avian Influenza in Iranian chicken flocks was reported in 1999 [27]. Since then Avian Influenza outbreaks in commercial poultry industry were reported by several researchers from Iranian poultry flocks [19, 20, 28] and Avian Influenza become an economically important disease in the Iranian poultry industry.

In field and experimental cases of AIV in broiler chickens anorexia, depression, coughing, sneezing, dyspnea and weight losses were reported [20] and mortality rate were 5% [16] to 20% in experimental studies and 65% in commercial chickens [20], and also in some cases mortality up to 80% was reported in commercial chickens due to concurrent bacterial infections [28]. In some other studies mortality were not reported [9, 14, 22, 26]. Our results indicated that H9N2 subtype alone could not cause any mortality in SPF chickens and it is in agreement with previous experimental studies that report no mortality in chickens after H9N2 subtype inoculation, but higher rate of mortality that was reported in some cases possibly because of secondary bacterial or viral infectious as same results was reported by Banani et al (2002) and Pan et al (2012) which in co-infected broilers mortality rate was increased [3, 21].

Previous findings noted that the replication of AIV in the chickens causes diarrhea, ruffled feathers, and slight depression in uncomplicated cases [6, 23, 28], but in some experimental studies mild tracheal rales, respiratory distress, swelling of the head, nasal and ocular discharge, coughing and sneezing in broiler chickens were reported [17, 20] and in field studies more complicated signs including swelling of sinuses, conjunctivitis, excessive lacrimation, dyspnea, and gasping were described [16, 19]. Also in necropsy severe congestion of trachea and upper respiratory tissues with formation of fibrinonecrotic casts in tracheal syrinx was reported in both field and experimental evaluations of broiler chickens infected with Avian influenza [7, 19], and decrease of bursa fabricius size and abnormal kidneys were described [8].

Some studies indicated that H9N2 AIV causes hemorrhage in small intestine, airsacculitis and kidney swelling and both clinical and gross signs were prominent at 6 days PI, they were reported subcutaneous hemorrhage, and hemorrhage in pancreas, intestine and bursa fabricius [1, 16]. Our findings indicated that only some depression and pneumonia in lungs were obvious and this results in agreement with previous reports that AI infectious in experimental situation, But severe clinical signs possibly due to difference in virus subtype, strain of birds,

concurrent bacterial or viral infections, and nutritional problems. In previous studies demonstrated that the co-infection of Avian Influenza with *Ornithobacterium rhinotracheale* or *Infectious Bronchitis* vaccine exacerbate clinical signs and gross lesions [3, 9, 21].

In natural cases of infectious with AI, weight gain significantly reduce up to 25 percent [19] and in experimentally infected broilers statistically significant difference between infected and healthy chickens were reported [5, 23]. In our study, weight gain was reduced significantly ($p < 0.05$) in comparison to control group during study period, and this results was probably because of impairment in pancreatic enzyme production.

The results of serological examination showed antibody increase in H9N2 infected groups and this results was in agreement with previous studies results [1, 7]

Our results indicate that the H9N2 subtype of AIV cannot cause mortality, sever clinical signs or gross lesions in infected chickens. The trypsin-like proteases found in restricted anatomical sites, such as respiratory and gastrointestinal tracts, are required to cleave the HA and thereby co-infections with bacterial or viral disease could facilitate AI virus replication and inducing clinical signs and gross lesions. Likewise further studies are required to confirm the role of birds strain and other pathogens like infectious bronchitis virus, *E. coli*, ORT and other bacteria on outcome of the disease.

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