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European Journal of Experimental Biology, 2013, 3(6):311-314

ISSN: 2248 –9215
CODEN (USA): EJEBAU

Serological and clinical survey of Newcastle disease in broiler chickens of east Azarbayjan by HI tests

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ABSTRACT

The objective of this study to investigate the clinical signs of Newcastle Disease (ND) in infected broiler farms of Iran Northwest and to determine serological status of this flocks and healthy flocks. Also the mortality was compared in healthy and infected flocks. From 22 broiler flocks blood samples were collected and examined with HI test. Mortality rate was documented in each flock. For Data analyzing Independent samples T test statistical method was used for compare infected and healthy flocks and statistical software was PASW SPSS 18th edition. Results of HI test showed that mean of antibody titers in healthy flocks was 5.36±0.20 and in infected flocks was 8.63±0.28. The data was demonstrated that there were significant differences between groups. The mortality rate in infected flocks was 33.20±4.11 and in healthy flocks was 12.99±1.12 percent ($p<0.05$). Because of economical losses causes by growth decrease, increase of feed consumption due to ND disease, it is necessary to applying exact vaccination programs in broiler flocks and observe of biosecurity to decrease mortality rate and losses due to decrease of growth.

Key words: Newcastle disease, broiler chicken, Hemagglutination Inhibition

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INTRODUCTION

Newcastle disease (ND), a highly contagious viral disease, affects domestic poultry and wild birds and characterized by respiratory, gastrointestinal and central nervous system lesions [3, 27]. Newcastle disease virus (NDV) is designated avian paramyxovirus -1 (APMV-1), which classifies as member of the genus *Avulavirus*, in the family *Paramyxoviridae* [15, 16]. NDV causes a disease that varies in clinical severity and transmissibility depending on the pathotype involved. NDV strains are grouped into five pathotypes based on the clinical signs induced in infected chickens: (1) viscerotropic or (2) neurotropic velogenic with high mortality and intestinal lesions or central-nervous signs; (3) mesogenic with low mortality, respiratory and nervous signs; (4) lentogenic with clinical mild or inapparent infections of the respiratory tract; and (5) asymptomatic enteritic with inapparent intestinal infections [3]. The first outbreaks of ND caused by virulent strains of virus occurred in 1926 in Java, Indonesia and in Newcastle – upon-Tyne, England [6]. Also ND is one of the most important viral diseases of poultry, and it is endemic in poultry industry of Iran and causes economic losses. Also a wide range of avian and non-avian species act as reservoirs of NDV and transmit the disease to susceptible birds [24]. There is long history of NDV recovered from wildlife [12,

20], In Iran, also reported NDV recovered from wildlife [5, 17] and domestic chickens [9, 10, 13, 22], ostriches [8] and Japanese quail [18].

Recently different diagnostic techniques have been developed for detection and differentiation of NDV strains. Proposed by Office International des Epizooties (OIE) new regulations, reverse transcription polymerase chain reaction (RTPCR) are applied in many laboratories of the world as the most reliable methods for the detection and identification [7]. Considerable populations of industrial chicken farms exist in east Azarbaijan province, and there was not any report so far published on the economical losses associated with NDV mortality in the industrial chicken farms.

This study was carried out to detect the NDV infection, using Hemagglutination Inhibition (HI) serological test and to compare mortality rate between infected and healthy broiler chicken farms of east Azarbaijan.

MATERIALS AND METHODS

Samples were collected from 20 broiler farms suspicious to infect with ND, throughout the east Azarbaijan provincial. Totally 360 sera samples were collected from 20 understudy farms. Particular care was taken for the storage and transport of samples.

Serological procedure

The serum samples were tested to determine the antibodies against NDV, using the standard HI method [4]. The antigen used was reconstituted commercial NDV La Sota vaccine. For this purpose, a total of 5 ml of chicken blood was collected aseptically in a disposable syringe containing 1 ml of sodium citrate (4% solution) as an anticoagulant. The blood was centrifuged at 1500 rpm for 15 minutes and the plasma and buffy coat was pipetted off. After washing thrice with phosphate buffer saline (PBS), 1% suspension in PBS was used in HI test.

The test was performed as described by Allan and Gough [4]. Briefly, after making two fold serial dilution of test serum up to 10th well, 4 HA unit of Newcastle disease virus was added upto 11th well and kept at 25-30oC for 25-30 minutes. A 1% chicken RBCs Suspension was added into each well. The samples showing peculiar central button shaped settling of RBCs were recorded as positive and the maximum dilution of each sample causing Hemagglutination inhibition was considered as the end point. The HI titer of each serum sample was expressed as reciprocal of the serum dilution.

RESULTS AND DISCUSSION

Our results indicated six flocks of 20 were infected with Newcastle disease and 14 flocks were negative. In infected flocks mortality rate was 33.2±4.11 and in non-infected flocks it was 12.99±1.12 and statistical analysis revealed that the differences of two groups of flocks was very different ($p<0.01$).

Table1: Mortality rate and HI titer in infected and non-infected flocks

Non-Infected Flocks			Infected Flocks		
Flock No.	Mortality Rate	HI titer	Flock No.	Mortality Rate	HI titer
1	9	5.2	1	21.5	8.10
2	7.5	6.10	2	25.4	9.40
3	9.5	5.10	3	28.6	8.50
4	12	5.10	4	41.5	7.80
5	14.5	4.9	5	34.2	9.50
6	21.5	4.6	6	48	8.50
7	18.1	6.2	-	-	-
8	14.5	6.5	-	-	-
9	19.4	5.1	-	-	-
10	10.5	6.30	-	-	-
11	9.1	4.10	-	-	-
12	13.6	6.20	-	-	-
13	11.9	5.20	-	-	-
14	10.8	4.5	-	-	-
Mean±SE	12.99±1.12	8.63±0.28	Mean±SE	33.20±4.11	5.36±0.20

Serological monitoring of flocks by HI test demonstrated that the antibodies against ND in infected flocks increase very significantly ($p<0.01$), 14 day after disease clinical signs onset. In non-infected flocks HI titers that was obtained from vaccination was normal and sero-conversion was not seen.

In infected flocks greenish diarrhea, depression, reluctant to move was seen and in autopsy green content of gizzard, lesions in intestine was seen, while in healthy flocks there was not any clinical signs or gross lesions.

Several studies indicated that the respiratory diseases in poultry almost frequently due to infections caused by several factors [2, 14]. Newcastle disease virus in broiler chickens is one of the main causes of respiratory diseases and economic losses caused by frequent outbreaks of this disease in poultry farms in recent years, was reported especially in north-west poultry farms of Iran. In most countries, the disease losses, in addition to its prevalence including the principles and controlling and prevention programs, which included costs for permanent control of Newcastle disease. Even in countries free of the disease for international trading screening it has imposition many costs. Newcastle disease is endemic in some countries, and therefore as a limiting factor in the development of industrial poultry production [3]. In Iran most farmers uses vaccines for prevention and control of Newcastle disease in poultry rather than biosecurity, however, a severe form of Newcastle disease in vaccinated flocks also occurred, and causing high mortality rate and reduces growth of poultries [1, 19, 26]. Also, studies have shown that malnutrition, unfavorable weather conditions, levels of maternal antibodies, the challenge virus in the farm, day old chicks and breed quality, and quality of the effects of the vaccine and its administration was effective on Newcastle disease outcomes [3].

Researchers indicated that the vaccination could not prevent disease occurrence in farm conditions and the findings of them are consistent with the results of the present study [19]. Musa and colleagues studied infection in two flocks of broiler chickens in Nigeria was reported 100% mortality despite vaccination, Researchers reported up to 66% mortality during 2002 outbreak of Newcastle disease in vaccinated flocks of California [11]. The results of the present study, indicated despite the vaccination program mortality was 21.5 to 48 percent, (average: 33.20±4.11) in broiler chicken farms and it seems that was because of protection lack against the velogenic strains of Newcastle disease, and this results was in consistent with previous studies [11, 19].

Orsi et al (2010), was reported in Brazil 39.1 percent of flocks were sero positive and from 6.5 to 58.4% of cases the NDV was isolated [21]. Schelling et al., (1999) determined that 5 to 29 percent of small broiler flocks and broiler breeders were seropositive [25]. Researchers was reported in Jordan showed that 41.7% of investigated flocks was infected with Newcastle disease virus, of which 13% of flocks infected only with Newcastle disease virus, while in other cases concurrent infections with infectious bronchitis virus and avian influenza, and Mycoplasma gallisepticum [23].

CONCLUSION

In the present study, in 30% of flocks antibodies titers increases against Newcastle disease (3 logs) and the HI titer in infected flocks was 8.63±0.28 and in non-infected vaccinated flocks it was 5.36±0.2. The results of HI serological test indicated that the increase of antibody titers in non-infected flocks was lower significantly than infected flocks ($p<0.01$).

REFERENCES

[1] P. A. Abdu, A. M. Wakawa, L. Sa'idu, T. M. Joanis, M. Y. Fatihu, *Vet. Clin. Practice Bull.*, **2008**, 1.
[2] S. O. Al-Garib, A. L. J. Gielkens, E. Gruys, G. Kochi, *World's Poultry Science Journal*, **2003**, 59, 185.
[3]D. J. Alexander, D. A. Senne, R. E. Gough, R. C. Jones, *In:* Y. M. Saif (ed.) *Diseases of Poultry*.(Wiley-Blackwell Publishing,Iowa, IA, **2008**) 75.
[4] W. H. Allan, R. E. Gough, *Vet Rec*, **1974**, 95, 120.
[5] M. H. Bozorgmehrifard, H. Keyvanfar, *J Wildl Dis*, **1979**, 51, 335.
[6] T. M. Doyle, *Journal of comparative Pathology and Therapeutics*, **1927**, 48, 1.
[7] O. I. D. Epizootics, *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals.*(OIE,Paris, France, **2011**).
[8] M. Ghiamirad, A. Pourbakhsh, H. Keyvanfar, R. Momayez, S. Charkhkar, A. Ashtari, *African Journal of Microbiology Research*, **2010**, 23, 2492.
[9] M. Hassanzadeh, M. H. Bozorgmerifard, *Int. J. Poult. Sci*, **2004**, 3, 658.
[10] F. Hemmatzadeh, B. Nayeri, E. R. Tofighi, *Int. J. Poult. Sci*, **2006**, 5, 408.
[11] D. R. Kapczynski, D. J. King, *Vaccine*, **2005**, 23, 3424.
[12] M. Kawamura, K. Nagata-Matusbara, K. Nerome, N. Yamane, H. Kida, H. Kodama, H. Izawa, T. Mikami, *Microbiol. Immuno.*, **1987**, 31.
[13] M. Kianizadeh, A. Ideris, M. S. Shahrabadi, R. Kargar, S. A. Pourbakhsh, A. R. Omar, K. Yusoff, *Archives of Razi Institute*, **1999**, 50.
[14] Y. S. Malik, D. P. Patnayak, S. M. Goyal, *J Vet Diagn Investig*, **2004**, 16, 244.
[15] M. A. Mayo, *Arch Virol*, **2002**, 147, 1655.

[16] M. A. Mayo, *Arch Virol*, **2002**, 147, 1071.
[17] M. J. Mehrabanpour, P. D. Fazel, A. Rahimian, M. H. Hosseini, H. Moein, M. A. Shayanfar, *International Journal of Animal and Veterinary Advances*, **2011**, 3, 229.
[18] R. Momayez, R. Gharkhani, P. Pourbakhsh, S. A. Toroghy, R. Shoshtari, A. H. Banani, *Archives of Razi Institute*, **2007**, 62, 39.
[19] I. W. Musa, P. A. Abdu, A. K. B. Sackey, S. B. Oladele, S. Lawal, I. U. Yakubu, *International Journal of Poultry Science*, **2010**, 9, 1116.
[20] C. K. Ojeh, H. O. Okoro, *Trop. Animal. Health Prod.*, **1992**, 24, 211.
[21] M. A. Orsi, L. Doretto Jr, S. C. A. Camillo, D. Reischak, S. a. M. Ribeiro, A. Ramazzoti, A. O. Mendonça, F. R. Spilki, M. G. Buzinaro, H. L. Ferreira, C. W. Arns, *Braz J Microbiol*, **2010**, 41, 349.
[22] G. Rezaeianzadeh, H. Dadras, A. S. Maken Ali, M. H. Nazemshirazi, *Journal of Veterinary Medicine and Animal Health*, **2011**, 3, 105.
[23] D. A. Roussan, R. Haddad, G. Khawaldeh, *Poult Sci*, **2008**, 87, 444.
[24] P. Roy, A. T. Venugopalan, R. Selvarangam, V. Ramaswamy, *Trop. Anim. Health. Prod.*, **1998**, 39, 299.
[25] E. Schelling, B. Thur, C. Griot, L. Audige, *Avian Pathol*, **1999**, 28, 263
[26] M. S. Simon, N. C. Durham, *World Poultry Journal*, **2006**, 22, 48.
[27] D. Swayne, D. A. Halvorson, *In:* Y. M. Saif (ed.) *Diseases of Poultry*.(Wiley-Blackwell Publishing,Iowa, IA, **2008**) 153.