Original Article

TJVR 2020; 4 (1): 17-22

Turkish Journal of Veterinary Research

http://www.dergipark.gov.tr/tjvr

e-ISSN: 2602-3695

The study of some hematologic and biochemical parameters in chickens vaccinated with inactivated dual Newcastle-Influenza vaccine

Mehdi Ezdiyadi[1] Ali Akbar Abolfathi[2] Aref Hoshyari[1]
Abolfazl Jafari-Sales[3] Farhad Farhadi[1]

[1] *Department of Research and Development, Razi Vaccine and Serum Research Institute, Agric. Res. Education and Extension Org, Marand, Iran*
[2] *Department of Biological Science, Ahar Branch, Islamic Azad University, Ahar, Iran.*
[3] *Department of Microbiology School of Basic Sciences, Kazerun Branch, Islamic Azad University, Kazerun, Iran.*

Correspondence: *Mehdi Ezdiyadi (Ezdiyadi.m@Chmail.com)*
Received: 26.09.2019 Accepted: 19.02.2020

ABSTRACT

Objective: Newcastle and Influenza diseases are important viral diseases and its occurrence and virulence in Iran has increased in recent years. The purpose of this study was to evaluate the humoral immune responses of chickens vaccinated with inactivated dual oil emulsion Newcastle disease and avian influenza vaccine in two methods of intramuscular and subcutaneous injection as well as to assess the possible changes in serum biochemical factors.

Materials and Methods: In this study, after subcutaneous and intramuscular inoculation of inactivated dual oil emulsion Newcastle-Influenza vaccine, the serum antibody level and hematobiochemical factors of these avian were analyzed on 7, 14, 21, and 28 days after vaccine injection.

Results: The results showed that the values of biochemical parameters such as albumin, glucose, total protein, triglyceride, cholesterol, ALT, AST, and ALP enzymes, sodium and potassium minerals, and hematocrit and hemoglobin levels in vaccinated and non-vaccinated chickens were not significantly different. Also, in chickens injected with antigen, Newcastle disease and influenza antibody titers were significantly different with those groups containing adjuvant.

Conclusion: The results of this study indicate that the intramuscular and subcutaneous injection of dual combination vaccine is similar in terms of changes in biochemical and hematological factors and Newcastle disease and influenza antibody titers. To ensure safety in inactivated vaccines, the presence of proper adjuvant is essential for immune response.

Keywords: *Avian influenza virus, Newcastle virus, Biochemical factors*

INTRODUCTION

Vaccination is the most effective method to prevent infectious diseases in both humans and animals. Generally, in inactivated vaccines, either whole organisms are killed or the subunit vaccines are effective for their adjuvant addiction (Aguilar and Rodriguez, 2007). Adjuvants are chemical or biological compounds that enhance the function of the immune system. Newcastle is also called "Multiple face disease", due to its several forms. Paramyxoviridae is a family of membranous viruses containing RNA, which have successive single-stranded genomes and negative sense. The disease is caused by a group of very close viruses that form avian paramyxovirus serotype 1 (PMV-1) (Abraham et al., 1986; Adair et al., 1989). The virus is a human pathogen and the most common sign in human infection is conjunctivitis that develops within 24 hours of Newcastle disease virus (NDV)

exposure to the eye (Swayne and King, 2003). The most important way of transmitting Newcastle virus to the flock is through airborne particles. The ELISA method is used to identify antibodies to poultry diseases due to the fact that most of the stages are automated and the results are achieved fast. One of the features of the RT-PCR technique is to be a very rapid detection of the presence of the virus. The oropharynx swab is used as a selective sample. NDV has both haemagglutination and neuraminidase activities. The action of haemagglutination is very important in Newcastle disease and the HI assay is the haemagglutination inhibition assay, which is routinely used to determine the NDV titration. Influenza viruses are classified into three types A, B and C on the basis of their antigen and the presence or absence of common group antigens. All avian influenza viruses are categorized as Type A (EFSA, 2005). This virus is a spherical particle with a diameter of 80-120 nm and a filament form that can be several microns in length. Serologic tests such as haemagglutination inhibition assay and neuraminidase inhibition assay are used to determine the type of influenza. Direct contact with infected birds or with a contaminated discharge and feces is necessary to transmit the infection. The super-acute H7 and H5 influenza viruses and the low-pathogenicity H9 viruses are more important because of their direct transmission to humans and the creation of a novel influenza pandemic. The ability of the virus to diffuse depends on the amount of viruses received by the respiratory or digestive tract of the animal. The National Veterinary Services Laboratory (NVSL) uses a brain-heart infusion broth without any antibiotic to collect the trachea and cloak swabs. Positive allantoic fluid is used for haemagglutination to detect the virus. NA subgroups are detected by micro-NI assay with antiserum prepared against 9 surface antigens (Palmer et al., 1975; Van Deusen et al., 1983; Alizadeh-Arsi et al., 2018). In serological monitoring programs, double immunodiffusion assay is often used to detect anti-NP antigens. There is currently no practical way and specific treatment for the infection caused by avian influenza virus in commercial poultry. It has been experimentally shown that amantadine is effective in reducing mortality (Lang et al., 1970; Beard and Easterday, 1973; Dolin et al., 1982; Webster et al., 1985; Easterday et al., 1997). Newcastle-Influenza Vaccine is an oil-killed vaccine containing inactivated avian influenza (H9N2) serotype with native origin and inactivated Newcastle (V4) serotype produced to prevent Newcastle disease and influenza (H9N2). The purpose of this study was to investigate some hematologic and biochemical parameters in chickens vaccinated with inactivated dual Newcastle-Influenza vaccine.

MATERIALS and METHODS

Chickens hatched from specific pathogen free eggs were used for this study. In this study, eight groups (Five chicks in each group) of 21-day chickens were randomly sampled from a flock of chickens. The sampling steps include: Blood collection from all chickens; blood re-sampling from the wing's vein during the first to fourth weeks, i.e., during 7, 14, 21 and 28 days; and then the HI test to evaluate the response of the chickens to the vaccine in terms of immunogenesis; the assessment of changes in liver enzymes such as Aspartate aminotransferase (AST), Alanine aminotransferase (ALT) and Alkaline phosphatase (ALP); and finally, the measurement of the biochemical parameters such as sodium, potassium, albumin, glucose, cholesterol, triglyceride and total protein. Hemoglobin and hematocrit tests were performed for hematological tests. Viral antigens were prepared according to conventional standard procedures. Auto Analyzer device (Orense, USA) and Pars kits were used to measure the biochemical parameters, and Cell Counter device (Excel, USA) was used to measure hematological parameters, hemoglobin and hematocrit percentage; and Electrolyte Analyzer (Convergent, Italy) device was applied to measure sodium and potassium contents. For the HI test, Newcastle and influenza antigens, Red blood cell 1% and 96 Well microplates were used. Data was put in SPSS software (version 20.0) and ANOVA and Tukey's test were used for analysis. P value of <0.05 was determined as significant.

RESULTS

The humoral immune responses of chickens vaccinated with inactivated dual combination oil Newcastle-influenza vaccine were evaluated in two methods of intramuscular and subcutaneous injection. This study was performed within four weeks after injection and afterwards the results were as follows:

The 7th day results after injection

There was no significant difference between the vaccinated and non-vaccinated (control) chickens in biochemical factors such as albumin, glucose, total

protein, triglyceride, cholesterol, ALP, AST and ALT enzymes, sodium and potassium salts and hematocrit and hemoglobin values after separating the serum of the chickens in each intramuscular and subcutaneous injection group and reading the serum results. Also, in the first week after intramuscular and subcutaneous injection, no significant difference was found in the HI antibody titer.

The 14th day results after injection

According to the isolating serum of chickens by researchers, there was no significant difference between the vaccinated and control chickens. As well as, no significant difference was observed between vaccinated and control chickens in the hematocrit and hemoglobin values in the fourteen days after injection in the blood, in which the anticoagulant substance was added. Although, in the vaccinated chickens, the antibody titer was significantly increased in both Newcastle disease and avian influenza, no antibody titer of Newcastle disease and avian influenza was detected in control chickens.

The 21th day results after injection

Biochemical factors had no significant difference in the results of serum isolated from vaccinated and control chickens within three weeks after subcutaneous and muscular injection. Although, in blood of chickens prepared with anticoagulant, there was no significant difference between vaccinated and non-vaccinated chickens, there was a significant difference between vaccinated and non-vaccinated chickens in terms of the Newcastle disease and avian influenza antibody titer.

The 28th day results after injection

The results of four weeks after subcutaneous and intramuscular injection in chickens showed that there was no significant difference in biochemical parameters between vaccinated and non-vaccinated chickens based on the serum isolated from chickens' blood. As well as, no significant difference was observed in the blood of chickens with anticoagulant substance of vaccinated and non-vaccinated chickens (Figure 1). However, there was a significant difference in Newcastle disease and avian influenza between vaccinated and non-vaccinated chickens (Figures 2 and 3). It should be indicated that subcutaneous and intramuscular injection of dual Newcastle-influenza vaccine produced by Razi Institute has the same results in terms of changes in biochemical factors, hematology and Newcastle disease and influenza titer. The presence of an appropriate adjuvant such as Montanide (ISA-70) oil is essential for immune response in order to create immunity in inactivated vaccines.

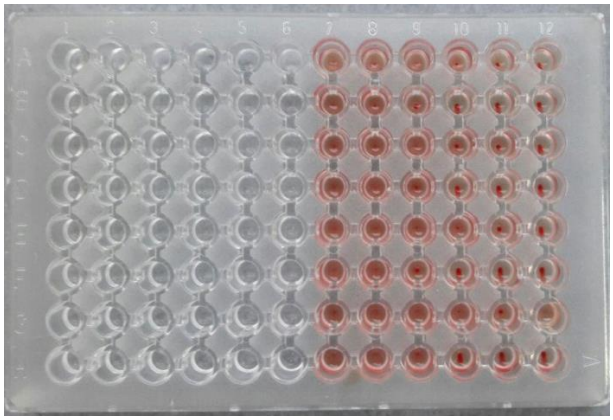

Figure 1. HI Test on the twenty-eighth day

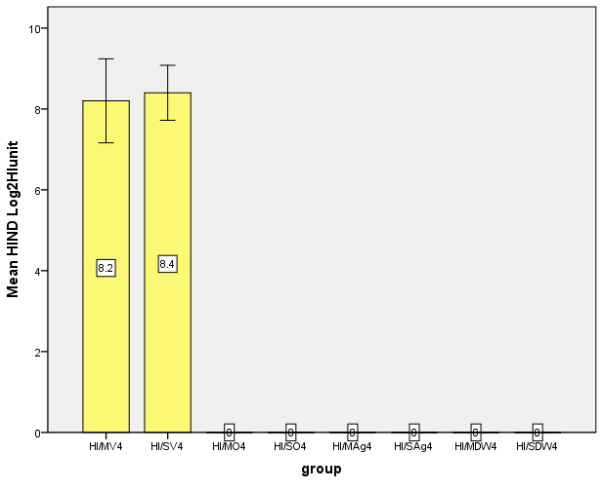

Figure 2. HI Test for Newcastle disease on the twenty-eighth day

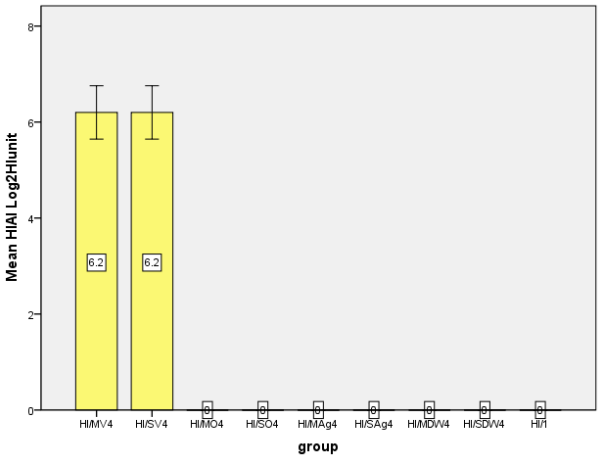

Figure 3. HI Test for avian influenza on the twenty-eighth day

Our results showed that there was no significant difference between vaccinated and non-vaccinated birds in terms of biochemical parameters, and this probably means that injection of inactivated dual combination Newcastle disease and avian influenza vaccines could not change these parameters compared to control birds.

DISCUSSION

We applied the HI assay for Newcastle disease antibody titer in our study and the results showed that Newcastle disease antibody titer can be detected from the second week by this method. Nidhin et al. (2009) in their study, by using HI method to determine the titer showed that the Newcastle disease antibody titer in the egg yolk is significantly more than its amount in serum. We also used the HI method in our study for the influenza antibody titer, and the results showed that the influenza antibody titer could be detected from the second week by this method. Trampel et al. (2006) in a study, used ELISA and AGID to determine the antibody titers. They concluded that antibody levels appear earlier in serum, reach sooner to the peak and remain for a long time; so they preferred antibody monitoring in serum more than that on yolk samples. Alexander et al. (2014) conducted a study, used the HI assay to search and determine the antibody in the egg yolk and showed that by detecting the antibody in the egg yolk, the Newcastle virus turning can be determined in the population of geese. We used the ISA70 oil adjuvant, which could stimulate the immune system and make it to produce antibody by providing the antigen. Iqbal et al. (2008) conducted they prepared the concentrations of 50% and 60% Montanide ISA70 and evaluated the immunogenicity with haemagglutination inhibition (HI) test after injection to layer birds. And they eventually reported that the immune response of the vaccine with 60% oil was better than the vaccine containing 50%. The vaccine was used in our study had 70% adjuvant. Silva et al. (2009) conducted a study, used oil Montanide ISA-70 as an adjuvant in the ratio of 70% to make the vaccine. They concluded that this vaccine protects the vaccinated chickens against the H5N1 virus and provides long-term immunogenicity in these birds. Liu et al. (2011) used three types of adjuvants, including ISA-70, ISA-206 and mineral oil; and they used vaccine injection into SPF chickens to test the constructed vaccines; and after challenging the highly acute influenza virus, they showed that the vaccine produced with the adjuvant ISA-70 and mineral oil could protect 100% of the chickens and the vaccine produced with ISA-206 protected only 40% of the chickens. Finally, they concluded that ISA-70 is the best adjuvant for the construction of an oil-based influenza vaccine that this type of vaccine was also used in our study.

Our results did not show any significant difference in the biochemical parameters between vaccinated and non-vaccinated groups, which was not consistent with other researchers. For example, Talebi (2006) conducted a study, used live Bronchitis, Gumboro and Newcastle vaccines at different ages in the treatment group. Then, blood samples were collected from different groups at different times and the parameters of albumin, calcium, chloride, cholesterol, glucose, magnesium, phosphorus, triglyceride and total protein were examined in the study. He concluded that the physiological values of the biochemical parameters in both groups of one-day chickens are different from its values during breeding period; and cholesterol levels on day zero are close to their values during the breeding period, while the values of other parameters are much lower than their values during the breeding period. Comparison of triglyceride, total protein and albumin values in the vaccinated group showed a significant difference with the recorded values for the control group, but other parameters were not significantly different between the two groups.

The dual Newcastle-Influenza vaccine, with the NewFluRazi brand, was used in the present study that ISA-70 has been used as an adjuvant in its construction. The aim of this study was to evaluate the changes of some hematological and biochemical parameters in the serum of injected chickens in both intramuscular and subcutaneous methods. Silva et al. (2009) assessed the immunogenicity of the inactivated oil emulsion influenza vaccine and stated that the immunity created by the oil-based vaccine in two-month chickens will remain to twelve months; and they announced the maximum visible immunity of the vaccine on the 28th day after injection, and influenza titer began to decline from 150th day. In the present study, our immunogenicity results are consistent with the above results. An effective vaccine is required to have a good antigen as well as a preferred adjuvant. Adjuvant is required to create humoral and cellular immunity, but adjuvants may also have side effects such as inflammation of the tissue, damage and pain (Silva et al., 2009). Kudair and Al-Hussary (2010)

investigated the effects of vaccination on some biochemical parameters in broiler chickens. In their study, they concluded that vaccination against Newcastle disease, infectious bronchitis and Gumboro disease had significant differences in some biochemical parameters between vaccinated and non-vaccinated groups as well as in different ages; and the effect of age was clearer in the vaccinated groups. Their examination also showed that vaccination had no significant effect on the values of glucose, total protein, lipid, triglyceride, lipoprotein, high-density cholesterol, low-density lipoprotein and alanine aminotransferase activity, but the vaccinated chickens showed a significant reduction in albumin values, albumin/globulin ratio and alkaline phosphatase activity compared to control groups. However, the globulin values and the activity of the alkaline aspartate aminotransferase enzyme and lactate dehydrogenase were higher than non-vaccinated groups; no significant difference was observed among different groups in our study.

Although one of the hypotheses was the probability of the impact of Montanide oil on biochemical factors as well as the creating the effective stress on the bird, the results showed that the same results were obtained in the parameters in both subcutaneous and intramuscular injections. As well as, the results of antibody titer showed that Montanide oil was effective in its immunity and durability; and the desired antigens were not able to show the measurable titer by HI assay without that; and the immune system is probably not able to provide a detectable immune response in this way without adjuvant. The similarity of the results of distilled water injections in both subcutaneous and intramuscular injections show that contrary to the assumption, the volume of injections presents the same results, the vaccine, therefore, has the same efficacy in terms of immunogenicity and biochemical and hematological factors in both injection methods. The results of this study did not present any reason for the recommendation of subcutaneous injection. Therefore, despite the use of intramuscular injection in poultry units and vaccine producers, the recommendation for subcutaneous injection seems unnecessary; and according to these results, the vaccine can also be injected into muscle. Of course, it should be noted that this study does not comment about the adjuvant residue in muscle injection, and it certainly requires further investigating and providing documentations that the subcutaneous injection is much safer compared to the intramuscular injection.

CONCLUSION

The results showed that there was no significant difference between vaccinated and non-vaccinated birds in terms of biochemical and hematological parameters. So the vaccine injection failed to change the parameters in comparison with the control birds. Also, the results showed that from the second week, Hi test could detect Newcastle and Influenza Antibody Titer.

ACKNOWLEDGMENTS

Conflict of Interests: The authors declared that there is no conflict of interests.

Financial Disclosure: The authors declared that this study has received no financial support.

REFERENCES

Abraham A, Sivanandan V, Halvorson D.A. and Newman J.A. Standardization of enzyme linked immunosorbent assay for avian influenza virus antibodies in turkeys. *Am J Vet Res*, 1986; 47: 561–566.

Adair BM, McNulty MS, Todd D, Connor TJ, Burns K. Quantitive estimation of Newcastle disease virus antibody levels in chickens and turkeys by ELISA. *Avian Pathol*, 1989; 18:175-192.

Aguilar JC, Rodriguez EG. Vaccine adjuvants revisited. *Vaccine*, 2007; 25(19): 3752-62.

Alexander Y, Alekseee V, Kirill A, Sharsho V, Vasiliy Y, Marchenko ZL. Antibodies to Newcastle disease virus in egg yolks of Great Cormorant (Phalacrocorax carbo) at Qinghai Lake. *Adv Infect Dis*, 2014; 4:194-197.

Alizadeh-Arsi L, Hoshyari A, Ameghi-Roodsary A, Ghaniei A, Jafari B, Jafari SA. Evaluation of antibody titer changes against Newcastle disease and influenza (H9N2) in the egg yolk during incubation period and its impact on the replication of the viruses. *New Findings in Veterinary Microbiology*, 2018; 1(1): 15-27.

Beard CW, Easterday BC. A Turkey Oregon 71, an avirulent influenza isolate with the hemagglutinin of fowl plague virus. *Avian Dis*, 1973; 17: 173–181.

Dolin R, Reichman RC, Madore HP, Maynard R, Linton PN, Webber Jones J. A controlled trial of amantadine and rimantadine in the prophylaxis of influenza A infection. *N Engl J Med*, 1982; 307:580–584.

Easterday BC, Hinshaw VS, Halvorson DA. Influenza. In: Calnek BW, Barnes HJ, Beard CW, McDougald LR, Saif YM, (eds). *Diseases of Poultry*. 10th edition. Iowa State University Press Ltd, 1997;583–605.

Iqbal M, Nisar MA, Noor S, Gill ZJ. Evaluation of oil based avian influenza vaccine (H5NI) prepared with different concentrations of adjuvant. *Pakistan Veterinary Journal*. 2008;28(4).

Kudair IM, Al-Hussary NAJ. Effect of vaccination on some biochemical parameters in broiler chickens. *Iraqi J Vet Sci,* 2010; 24(2):59-64.

Lang G, Narayan O, Rouse BT. Prevention of malignant avian influenza by 1-adamantanamine hydrochloride. *Archiv fur die Gesamte Virusforschung*, 1970; 32:171–184.

Liu CG, Liu M, Liu F, Liu DF, Zhang Y, Pan WQ, et al. Evaluation of several adjuvants in avian influenza vaccine to chickens and ducks. *Virology journal.* 2011;8(1):321.

Nidhin R, Praseena P, Surya PS, Chintu R, Mathew S. Comparison of antibody titres of newcastle disease virus in randomly collected sera and egg yolk of layers. *J Vet Anim Sci*, 2009; 40:20-21.

Palmer DF, Coleman MT, Dowdle WD, Schild GO. Advanced laboratory techniques for influenza diagnosis. Immunology series no. 6. U.S. Department of Health, Education and Welfare, Public Health Service, Centers of Disease Control: Atlanta, GA. 1975.

Silva BC, Martins ML, Jatobá A, et al. Hematological and immunological responses of Nile tilapia after polyvalent vaccine administration by different routes. *Pesquisa Veterinária Brasileira*, 2009; 29(11), 874-880.

Swayne DE, King DJ. Avian influenza and Newcastle disease. *J* Am *Vet Med Assoc,* 2003; 222(11):1534–1540.

Talebi A. Biochemical parameters in broiler chickens vaccinated against ND, IB and IBD. *Int J Poultry Sci*, 2006; 5(12):1151-1155.

Trampel DW, Zhou EM, Yoon KJ, Koehler KJ. Detection of antibodies in serum and egg yolk following infection of chickens with an H6N2 avian influenza virus. Journal of veterinary diagnostic investigation. 2006;18(5):437-42.

Van Deusen RA, Hinshaw VS, Senne DA, Pellacani D. Micro neuraminidase inhibition assay for classification of influenza A virus neuraminidases. *Avian Dis*, 1983; 27:745-750.

Webster RG, Kawaoka Y, Bean WJ, Beard CW, Brugh M. Chemotherapy and vaccination: A possible strategy for the control of highly virulent influenza virus. *J Virol*, 1985; 55:173-176.