Research Journal of Microbiology

ISSN 1816-4935

Academic
Journals Inc.

www.academicjournals.com

OPEN ACCESS Research Journal of Microbiology

ISSN 1816-4935
DOI: 10.3923/jm.2017.243.247

Research Article
Newcastle Antibody Levels in Egg Yolk of Layer Flocks after Chloroform and Ammonium Sulfate Extraction

[1]Abolfazl Ghaniei, [2]SeyedMeysam Abtahi and [1]Milad Hajian

[1]Department of Poultry Diseases, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran
[2]Department of Microbiology, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran

Abstract

Background and Objective: Hemmaglutination Inhibition (HI) is the most common serological assay for detection of immune response in poultry to investigate Newcastle Disease (ND). Also, Egg yolk provides good alternative for blood samples because it is not invasive and also reduce stress in poultry. Nevertheless, there is no standard method for antibody extraction from egg yolk. The objective of this study was to compare two commercial methods (Chloroform and Ammonium Sulfate Extraction) for isolation of antibodies from egg yolk. **Materials and Methods:** In this study, 91 eggs of 7 layer flocks of East Azerbaijan province (Northwest of Iran) included. NDV antibody titers of yolk samples were measured after chloroform and ammonium sulfate extraction by HI. Statistical analysis was carried out by Minitab 17. **Results:** Mean ND titers were significantly higher in chloroform assay (10.8) than ammonium sulfate extraction (3.66) ($p<0.01$). Moreover, unlike chloroform method, there was no correlation between antibody titers of yolk samples evaluated by HI and total protein of the sample in ammonium sulfate method. **Conclusions:** Chloroform extraction is more functional in regard to NDV antibody isolation and immune system evaluation than ammonium sulfate precipitation.

Key words: Ammonium sulfate, chloroform, egg yolk, antibody, Newcastle disease

Received: May 20, 2017 Accepted: July 11, 2017 Published: September 15, 2017

Citation: Abolfazl Ghaniei, SeyedMeysam Abtahi and Milad Hajian, 2017. Newcastle antibody levels in egg yolk of layer flocks after chloroform and ammonium sulfate extraction. Res. J. Microbiol., 12: 243-247.

Corresponding Author: Abolfazl Ghaniei, Department of Poultry Diseases, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran Tel: +984432774737 Fax: +984432777099

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Newcastle Disease (ND) is a viral infection affecting domestic poultry and other bird species[1]. ND is caused by Newcastle Disease Virus (NDV), which belongs to the Paramyxoviridae family[2]. Strains of NDV have been classified into 5 pathotypes known as neurotropic velogenic, viscerotropic velogenic, mesogenic, lentogenic and asymptomatic enteric, based on the clinical signs seen in infected chickens[3]. Eleven serotypes of avian paramyxoviruses have been recognized[4]. Among them, avian paramyxovirus type 1 causes Newcastle disease in poultry[4]. ND causes massive financial loss to global poultry industry. Laboratory tests should be used to confirm clinical suspicion[5]. Molecular assays, viral isolation and presence of specific antibodies are indicative of exposure to NDV. Hemagglutination Inhibition (HI) test is the serologic assay most often used for evaluation of immune response against NDV in affected birds[3].

The IgM, IgA and IgY are three classes of immunoglobulins identified in chicken. Of these, IgY is the major antibody produced by chickens[6]. The IgY is continually synthesized, secreted into the blood and transferred to the egg yolk, where it is accumulated[7]. During egg maturation, the concentrations of IgY in egg yolk are similar to those of IgY in the blood circulation of the hen[8]. To collect blood samples, birds need to be restrained which can cause great stress to them. Egg yolk is a good source of antibody and provides an alternative to serum. Egg yolk samples can be used for evaluation of immune status of layer flocks, efficiently. Egg also represents a good source of immunoglobulins. Production of IgY antibodies has broad application in diagnosis, therapy and prophylaxis[9].

On the other hand, specific antibody has been widely used nowadays for immunodiagnostics subjects and/or for treatment and prevention of infections in humans and animals[10]. The IgY technology has been suggested as a useful strategy to produce a huge amount of specific antibody for the commercial use[11]. Therefore, improvement and standardization in IgY purification from egg yolk are essential. Unfortunately, there is no standard method for antibody extraction from egg yolk.

The main objective of the present study was to evaluate NDV specific antibodies in egg yolk samples of layer flocks of Northwestern Iran by two assays. Chloroform and ammonium sulfate extraction methods were compared to assess which gave more reliable results. To our knowledge, this is the first study evaluating NDV antibodies in egg yolk of layers in this region. Furthermore, efficiency of two extraction methods (Chloroform and Ammonium Sulfate Extraction) was compared.

MATERIAL AND METHODS

Collection of samples: Ninety one eggs from 7 layer flocks of East Azerbaijan province (Northwest of Iran) were collected from January-July 2016. From each flock, 13 eggs were sampled. All flocks were vaccinated against ND during rearing and production several times in a similar program according to guidelines of Iranian Veterinary Organization. The yolk was carefully separated from the white and homogenized. The yolk sample divided into two; one for chloroform extraction and another for ammonium sulfate assay.

Extraction of yolk antibodies by chloroform assay: Two mL of yolk and 2 mL of Phosphate-Buffered Saline (PBS) mixed together. Two volumes of chloroform added to one volume of the mixture[12]. The mixture was then stored 1 h at room temperature. By centrifugation at 383×g for 20 min, the supernatant was separated and kept at -20°C for HI analysis[13].

Extraction of yolk antibodies by ammonium sulfate assay: Egg yolk was diluted with 6 volumes of deionized acidic water (pH = 2) and homogenized. The pH of the egg yolk solution was adjusted to pH 5.0. The mixture stored 12 h at 4°C and then centrifuged 12000×g at 4°C. The supernatant collected and adjusted to pH 7.0 by NaOH 0.1 M.

100 g ammonium sulphate was dissolved in 100 mL distilled water at 50°C, allowed to stand overnight at room temperature and adjusted to pH 7.2. The concentrated sample was first mixed with equal volume of 45% ammonium sulfate and precipitated by centrifugation at 12000×g for 20 min at 4°C. The supernatant discarded. This stage repeated again. Then, the sample was precipitated by 40% saturated ammonium sulfate twice again and the pellet was dissolved in PBS, pH 7.4 and dialyzed against dialysis sacks (12,000 Da; Sigma-Aldrich, USA)[14]. NDV antibodies and total protein of the samples were determined using HI and spectrophotometry (commercial kit; Zist Chemi, Iran)[13]. This kit originally used for measurement of protein in the biological fluid based on pyrogallol red molybdate method. In this survey, this kit was out to measure serial concentrations of ovalbumin protein participated by ammonium sulfate.

Hemagglutination Inhibition (HI): Samples subjected to HI test according to protocol[15]. Briefly, two-fold serial dilutions of the samples were mixed with equal volume (25 µL) of 8HA NDV antigen (Pasouk, Iran). After 45 min incubation at room temperature, 25 µL 1% chicken RBC was added and after 30 min incubation at room temperature, the last well, which had a complete inhibition, was considered as the antibody titer.

Statistical analysis: The non-parametric data (NDV titers) were analyzed using Mann-Whitney U[16]. The parametric data (total protein) was compared by Student's t-test. Values were presented as Means±SD, $p<0.05$ were considered statistically significant.

RESULTS

Mean NDV antibody levels in the yolk samples after chloroform and ammonium sulfate extraction are displayed in Table 1. Mean NDV titers were significantly higher in chloroform assay than ammonium sulfate extraction ($p<0.01$). Mean NDV antibody titers after chloroform and ammonium sulfate extraction was 10.8 and 3.66, respectively. It is interesting to note that both methods showed a similar pattern of NDV antibody titers among flocks. Both methods revealed that flock no.2 and flock no.7 had the highest and the lowest NDV titers, respectively.

In ammonium sulfate extraction, there was no correlation between antibody titers of yolk samples evaluated by HI and total protein of the sample evaluated by spectrophotometry ($R = 0.03$) (Fig. 1).

Assessing frequency distribution of NDV antibody levels after chloroform extraction suggest that the data are normally distributed, with the highest frequencies of 10.0. Whereas, frequency distribution of the titers after ammonium sulfate extraction revealed that they were not normally distributed, with the highest frequency of 3.0.

There was a positive correlation between reduction of NDV antibody titers after chloroform extraction and layer flock age. As the flocks age, we see a decrease in NDV antibody levels (Fig. 2).

Table 1: (Mean±SD) NDV HI antibody titers in egg yolk of flocks after extraction with chloroform and ammonium sulfate

Flock No.	Chloroform (Log2)	Ammonium sulfate (Log2)
1	10.60±1.32	3.15±0.80
2	12.00±1.41	6.38±0.87
3	12.20±1.36	4.69±1.31
4	8.77±1.16	3.46±1.33
5	10.90±1.03	5.77±0.92
6	10.31±0.75	3.38±0.65
7	7.77±1.16	3.00±0.81

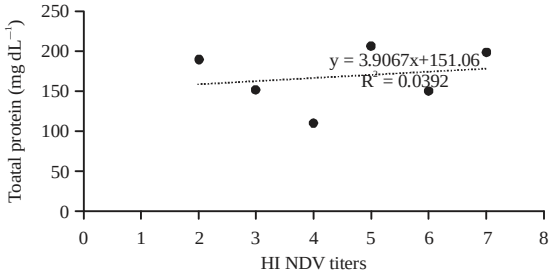

Fig. 1: Total protein and HI NDV titers in egg yolk samples after ammonium sulfate extraction[a]

[a]There is no correlation between them ($R = 0.03$)

DISCUSSION

All egg yolk samples tested positive by two extraction methods (Chloroform and Ammonium Sulfate Extraction). However, chloroform assay tends to yield higher antibodies than ammonium sulfate. In cases where the aim is to extract NDV antibodies from egg yolk, the chloroform extraction is more efficient. Son[17] compared chloroform polyethylene glycol procedure with the polyethylene glycol procedure. They found that the chloroform-polyethylene glycol method yielded 2.57 times more IgY than the other method[17]. Hagan *et al.*[18] also confirmed the suitability of chloroform extraction of yolk for detection of *Mycoplasma synoviae* antibodies. A study was conducted to compare Polyethylene Glycol (PEG) and ammonium sulfate precipitation method, water dilution method, Lithium sulfate precipitation method and the chloroform extraction of anti *Echis carinatus* antivenom antibodies from immunized chicken egg yolk[18]. They noted that PEG and ammonium sulfate was the most efficient method that is in contrast with our findings.

Evaluation of serum antibody levels were not included in this study. Hence, yolk antibody titers with serum antibody titers were not compared. In Iran, layer flocks are vaccinated several times with live and inactivated vaccines during rearing and production. These vaccines can provide a high level of immunity. Such immunity protects flocks against ND losses. It seems that chloroform extraction shows a more realistic picture of flock immunity against ND. It should be noted that HI titer of NDV in yolk samples is higher than serum samples[13,19,20].

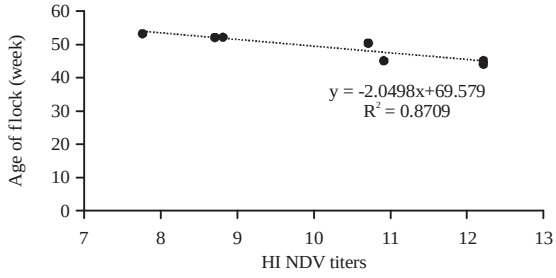

Fig. 2: Relationship between increasing age of flocks and decreasing mean HI NDV titers after chloroform extraction

There were no correlation between yolk antibodies and total protein after ammonium sulfate extraction. This can be explained by this fact that total protein of yolk is influenced greatly by other factors like nutrition of birds other than antibody levels.

Results of this research showed than antibody levels against NDV waned as the flocks get older. Antibody titers will be diminished in older flocks after the first appearance. Such decline of antibodies is a normal phenomenon[13,21].

CONCLUSION

Results of this research revealed that prevalence of NDV antibodies was high in yolk of layers of this region. Chloroform extraction of yolk antibodies is a useful method for monitoring of immune response of layers against ND. These data may generally suggest that chloroform extraction is more reliable and efficient method for IgY isolation than ammonium sulfate precipitation.

SIGNIFICANCE STATEMENTS

This study discovers suitability of chloroform extraction for evaluation of NDV yolk antibodies. This study will help the researcher to uncover the critical areas of NDV yolk antibodies for diagnosis and therapy of infection.

ACKNOWLEDGMENT

This study was financially supported by Urmia University (grant no. 1505).

REFERENCES

1. Alexander, D.J., 2000. Newcastle disease and other avian paramyxoviruses. Rev. Sci. Tech., 19: 443-462.
2. Miller, P.J., E.L. Decanini and C.L. Afonso, 2010. Newcastle disease: Evolution of genotypes and the related diagnostic challenges. Infect. Genet Evol., 10: 26-35.
3. Alexander, D.J. and D.A. Senne, 2008. Newcastle Disease Virus and other Avian Paramyxoviruses. In: A Laboratory Manual for the Isolation, Identification and Characterization of Avian Pathogens, Dufour-Zavala, L. (Ed.). 5th Edn., Chapter 30, American Association of Avian Pathologists, Athens, GA., USA., ISBN-13: 9780978916329, pp: 135-141.
4. Miller, P.J. and G. Koch, 2013. Newcastle Disease. In: Diseases of Poultry, Swayne, D.E. (Eds.). 13th Edn., John Wiley and Sons Inc., New York, USA., pp: 89-107.
5. Cattoli, G., L. Susta, C. Terregino and C. Brown, 2011. Newcastle disease: A review of field recognition and current methods of laboratory detection. J. Vet. Diagn. Invest., 23: 637-656.
6. Da Silva, W.D. and D.V. Tambourgi, 2010. IgY: A promising antibody for use in immunodiagnostic and in immunotherapy. Vet. Immunol. Immunopathol., 135: 173-180.
7. Warr, G.W., K.E. Magor and D.A. Higgins, 1995. IgY: Clues to the origins of modern antibodies. Immunol. Today, 16: 392-398.
8. Bollen, L.S. and J. Hau, 1996. Chicken eggs in polyclonal antibody production. Scand. J. Lab. Anim. Sci., 23: 85-91.
9. Schade, R., E.G. Calzado, R. Sarmiento, P.A. Chacana, J. Porankiewicz-Asplund and H.R. Terzolo, 2005. Chicken egg yolk antibodies (IgY-technology): A review of progress in production and use in research and human and veterinary medicine. Altern. Lab. Anim., 33: 129-154.
10. Nasiri, K., M.R. Nassiri, M. Tahmoorespur, A. Haghparast and S. Zibaee, 2016. Production and characterization of egg yolk antibody (IgY) against recombinant VP8-S2 antigen. Polish J. Vet. Sci., 19: 271-279.
11. Nasiri, K., S. Zibaee, M. Nassiri, M. Tahmoorespur and A. Haghparast, 2016. Production of specific IgY antibody to the recombinant FanC protein produced in *Escherichia coli*. Iran. J. Basic Med. Sci., 19: 883-889.
12. Mohammed, H.O., R. Yamamoto, T.E. Carpenter and H.B. Ortmayer, 1986. Comparison of egg yolk and serum for the detection of *Mycoplasma gallisepticum* and *M. synoviae* antibodies by enzyme-linked immunosorbent assay. Avian Dis., 30: 398-408.
13. Yeo, S., E. Nagy and P.J. Krell, 2003. Indirect method for prediction of hemagglutination inhibition antibody titers to Newcastle disease virus in chickens by titration of antibodies in egg yolk. J. Vet. Diagn. Invest., 15: 184-187.
14. Hey, C.F. and O.M.R. Westwood, 2002. Practical Immunology. 4rd Edn., John Wiley and Sons, New York, USA., ISBN-13: 9780865429611, Pages: 412.
15. Thayer, S.G. and C.W. Beard, 2008. Serologic Procedures. In: A Laboratory Manual for the Isolation, Identification and Characterization of Avian Pathogens, Dufour-Zavala, L. (Ed.). 5th Edn., Chapter 47, American Association of Avian Pathologists, Athens, GA., USA., ISBN-13: 9780978916329, pp: 222-224.
16. Fay, M.P. and M.A Proschan, 2010. Wilcoxon-mann-whitney or t-test? On assumptions for hypothesis tests and multiple interpretations of decision rules. Stat. Surv., 4: 1-39.
17. Son, A.P., 1990. Isolation of IgY from the yolks of eggs by a chloroform polyethylene glycol procedure. Immunol. Invest. J. Mol. Cell. Immunol., 19: 253-258.

18. Hagan, J.C., N.J. Ashton, J.M. Bradbury and K.L. Morgan, 2004. Evaluation of an egg yolk enzyme-linked immunosorbent assay antibody test and its use to assess the prevalence of *Mycoplasma synoviae* in UK laying hens. Avian Pathol., 33: 91-95.
19. Meenatchisundaram, S. and A. Michael, 2010. Comparison of four different purification methods for isolation of anti *Echis carinatus* antivenom antibodies from immunized chicken egg yolk. Iran. J. Biotechnol., 8: 50-55.
20. Raj, N., P. Poulose, P.S. Surya, C. Ravishankar and M. Sebastian, 2009. Comparison of antibody titres of Newcastle disease virus in randomly collected sera and egg yolk of layers. J. Vet. Anim. Sci., 40: 20-21.
21. Yeo, S.G. and W.P. Choi, 1979. Immune status of breeding hens against Newcastle disease. Korean J. Vet. Res., 19: 45-51.